# Quantitative Proteomics Analysis of Global Protein Expression in *Campylobacter jejuni* Cultured in Sublethal Concentrations of Bile Acids and Varying Temperatures

**PhD Thesis**

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submitted by

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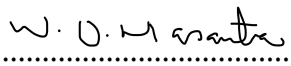
# AFFIDAVIT

I hereby declare that my thesis 'Quantitative Proteomics Analysis of Global Protein Expression in *Campylobacter jejuni* Cultured in Sublethal Concentrations of Bile Acids and varying temperatures' has been written independently. The work is original and has not been submitted in part or full by me for any degree or diploma in any other university. I further declare that materials obtained from other sources have been duly acknowledged.



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Wycliffe Omurwa Masanta, Goettingen, 4th May 2017

# TABLE OF CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| **BHI** | Brain heart infusion broth |
| **CA** | Cholic acid (CA) |
| **CDB** | Campylobacter defined broth |
| **CDCA** | Chenodeoxycholic acid |
| **Cia** | Campylobacter invasion antigen |
| **DCA** | Deoxycholic acid |
| **DMEM** | Dulbecco's Modified Eagle Medium |
| **DIA** | Data-independent acquisition |
| **DDA** | Data-dependent acquisition |
| **GCA** | Glycocholic acid |
| **GPA** | Gentamicin protection assay |
| **HBSS** | Hank's Balanced Salt Soluion |
| **LCA** | Lithocholic acid |
| **LB** | Luria broth |
| **MHB** | Mueller Hinton Broth |
| **MS** | Mass spectrometry |
| **PCA** | Principla component analysis |
| **SILAC** | Stable isotope labeling with amino acids in cell culture |
| **SWATH** | Sequantial window acquisation of all theoretical mass spectra |
| **TCA** | Taurocholic acid |
| **UDCA** | Ursodeoxycholic acid |
| **WT** | Wild type |

# ABSTRACT

*Campylobacter jejuni* is the leading cause of diarrhoea among human beings worldwide. Epidemiological investigations have shown that it affects over 500 million people per year. *C. jejuni* is mainly transmitted to human through consumption of cross contaminated chicken. In most cases, the diarrhoea clears by itself within 3 to 5 days. But it causes a big discomfort in the affected individuals. In addition, it has a huge economic impact due to sick leaves. Because of this, efforts are being put into understanding how *C. jejuni* interacts with human beings and other hosts. An indepth understanding of how this pathogen interacts with its hosts will lead to development of appropriate diagnosis tools and prevention measures.

Bile acids are a major component of the gut fluid in all the hosts of *C. jejuni*. However, the interaction of *C. jejuni* and different types of bile acids at human body temperature of 37$^{o}$C is poorly understood. Consequently, this study was designed to unearth the proteomic response in *C. jejuni* reference strain 81-176 to sublethal concentrations of cholic acid (CA), deoxycholic acid (DCA), lithocholic acid (LCA), taurocholic acid (TCA), chenodeoxycholic acid (CDCA), ursodeoxycholic acid (UDCA) and glycocholic acid (GCA). The specific objectives were: (i) to investigate the response in 81-176 to DCA 0.05% at 37$^{o}$C for 12h and 24h using both stable isotope labeling with amino acids in cell culture (SILAC) and label-free analysis with sequantial window acquisation of all theoretical mass spectra (SWATH); and determine a suitable quantitative method for the study. (ii) To use the method selected quantitative method to investigate global protein expression in 81-176 in response to sublethal concentrations of CA, LCA, TCA, CDCA, UDCA and GCA cultured at 37$^{o}$C for 12h under microaerophilic conditions. (iii) To identify and characterize a currently uncharacterized and widely induced protein (iv) To use label-free analysis with SWATH to investigate protein expression in 81-176 cultured in temperatures of 37$^{o}$C (human) and 42$^{o}$C (chicken) without bile acids..

Intially, the capability of *C. jejuni* to adhere and invade Caco-2 cells in the presence of various concentrations of bile acids was investigated using gentamicin protection assay (GPA). The results showed that DCA, CDCA and GCA promoted adherence and invasion in a dose depandant fashion. LCA and UDCA didn't neither promote nor suppress adherence and invasion. Subsequently, $IC_{50}$ of each bile acid was obtained. Half of this concentration of each bile acid corresponded to the concentrations that are present in the large intestines of human

beings. Hence half $IC_{50}$ concentrations were taken to be sublethal concentration. The concentrations were: CA 0.1%, DCA 0.05%, LCA 0.05%, TCA 0.5%, CDCA 0.05%, UDCA 0.5% and GCA 0.4%. Quantitative proteomic analysis of the response of 81-176 to DCA 0.05% showed that SILAC generated 500 quantifiable proteins and label-free analysis with SWATH generated 957 quantifiable proteins. The difference was attributed to poor incorporation of arginine and lysine in 81-176. As a result, SWATH analysis was used to quantify the response in 81-176 to different bile acids. These analyses found that CA significantly upregulated 19 proteins and downregulated 28 proteins; DCA significantly upregulated 113 proteins and downregulated 79 proteins; LCA significantly upregulated 4 proteins and downregulated 13 proteins; TCA significantly upregulated 51 proteins and downregulated 60 proteins; CDCA significantly upregulated 89 proteins and downregulated 79 proteins; UDCA significantly upregulated 2 proteins and downregulated 4 proteins; GCA significantly upregulated 139 proteins and downregulated 20 proteins. Among the significantly upregulated proteins, MazF was selected for further characterization. The mutant showed significant reduction in adhering onto Caco-2 cells in the presence of CA 0.1% ($p<0.05$). Also, the mutant showed significant reduction in invading Caco-2 cells in the presence of CA 0.1% and TCA 0.5% ($p<0.05$). Similarly, the muatnt showed decline in growth after 20 hr in broth supplemented with CA 0.01%, DCA 0.05%, TCA 0.05%, CDCA 0.05% and GCA 0.4%. Separately, 83 proteins were significantly upregulated and 65 proteins were significantly downregulated between 81-176 that was cultured at $37^oC$ for 12h and 24h. While 83 proteins were significantly upregulated and 50 proteins were significantly downregulated between 81-176 that was cultured at $37^oC$ for 24h and $42^oC$ for 24h. All the differentially expressed proteins belonged to the following biological processes: (i) cell division and cell cycle (ii) maintenance of integrity of outer, periplasmic and inner membranes (iii) DNA replication and transcription (iv) metabolism (v) chemotaxis and motility (vi) stress response and 291 uncharacterized proteins.

In conclusion, SWATH analysis is a more suitable quantitative method for wide scale *Campylobacter* proteomic research. However, other methods such as SILAC should be concurrently included to complement its weaknesses. DCA, CDCA and GCA had the highest number of differentially expressed proteins. Equally, CA differentially expressed a reasonable number of proteins but not as high as DCA, CDCA and GCA. CA, DCA, LCA, TCA, CDCA, UDCA and GCA promote adherence and invasion of epithelial cells. Majority of the proteins which are promoted adherence and invasion are involved in metabolic processes. Also all the

bile acids that were examined in this study are toxic to 81-176. The results show that 81-176 has a well built adaptation system to both bile acid antimicrobial activities and changes in temperatures. This system involves activation and deactivation of a set of genes involved in metabolism, stress response, maintenance of integrity of outer, periplasmic and inner membranes, chemotaxis and motility. Undoubtedly, the findings of this study will enhance the understanding of the biology on the interaction of *C. jejuni* and bile acids.

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# LIST OF PUBLICATIONS

a) Book Chapter

1. Zautner A.E., and **Masanta W.O.** (2016) *Campylobacter*: Health Effects and Toxicity. In: Caballero, B., Finglas, P., and Toldrá, F. (eds.) The Encyclopedia of Food and Health vol. 1, pp. 596-601. Oxford: Academic Press.

b) Publications in peer reviewed journals

1. Mund, N. L.-A. , **Masanta W.O**, Goldschmidt, A.-M., Lugert, R., Groß, U, & Zautner, A.E. (2016) Association of *Campylobacter jejuni* spp. *jejuni* chemotaxis receptor genes with multilocus sequence types and source of isolation. *European Journal of Microbiology and Immunology*. DOI: 10.1556/1886.2015.00041. *In press*.

2. **Masanta, W. O**., Lugert, R., Groß, U., Linsel, G., Heutelbeck, A., & Zautner, A. E. (2016). Seroprevalence of *Campylobacter*-Specific Antibodies in two German Duck Farms – A Prospective Follow-Up Study. *European Journal of Microbiology & Immunology*, *6*(2), 118–123. http://doi.org/10.1556/1886.2016.00007.

3. Zautner, A.E., **Masanta, W**.O., Weig, M., Groß, U., & Bader, O. (2015). Mass Spectrometry-based PhyloProteomics (MSPP): A novel microbial typing Method. *Scientific Reports*, *5*, 13431. http://doi.org/10.1038/srep13431.

4. Zautner, A. E., **Masanta, W. O**., Hinz, R., Hagen, R. M., & Frickmann, H. (2015). Artificially designed pathogens – a diagnostic option for future military deployments. *Military Medical Research*, *2*, 17. http://doi.org/10.1186/s40779-015-0045-2.

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9. Zautner, A. E., **Masanta, W. O**., Tareen, A. M., Weig, M., Lugert, R., Groß, U., & Bader, O. (2013). Discrimination of multilocus sequence typing-based *Campylobacter jejuni* subgroups by MALDI-TOF mass spectrometry. *BMC Microbiology*, *13*, 247. http://doi.org/10.1186/1471-2180-13-247.

# LIST OF TABLES

# LIST OF FIGURES

# 1.0 INTRODUCTION

## 1.1 Brief history of Campylobacter jejuni

The isolation of *Campylobacter* spp. dates back to a period between 1884 and 1940 during which comma and spiral shaped bacteria where constantly isolated from humans and livestock suffering from diarrhea, abortion and death (Butzler, 2004). These bacteria were collectively classified in the genus *Vibrio* which had been created earlier by Otto Friedrich Müller (Vandamme and De Ley, 1991). Improvement in isolation and identification methods between 1920 and 1960 lead to an understanding that *Campylobacter* spp. shared a similar shape with *Vibrio* spp. but had different physiological characteristics and disease outcome (King, 1957). Consequently, two groups of *Vibrios* evolved: the first group contained *Vibrios* with low G+C content growing optimally at 42°C under microaerophilic conditions. The second group was comprised of *Vibrios* that had high G+C content in DNA and grew optimally at 37°C under aerobic conditions (King, 1962). In 1963 Sebald and Veron coined the genus *Campylobacter* to house *Vibrios* in the first group described above (Veron and Chatelain, 1973). Since then, this genus has 25 species and 9 subspecies (Zautner and Masanta, 2016). But only *C. jejuni* and *C. coli* cause gastroenteritis in humans with 95% of the cases attributed to *C. jejuni* (Kaakoush et al., 2015; Sheppard and Maiden, 2015).

The dates when *C. jejuni* was first isolated is unclear due to the absence of suitable diagnostic tools during that period. Unconfirmed historical records suggest that *C. jejuni* could have been one of the bacteria that Dr. Theodor Escherich isolated from infant stool in 1889 (Shulman et al., 2007). However, successful isolation was only reported in 1947 by Vinzent and co-workers, in 1957 by Elizabeth King and in 1968 by Dekeyser and Butzler (Dekeyser et al., 1972; King, 1957). Currently, better diagnostic tools, reliable research models, suitable disease management approaches, improved control mechanisms and epidemiological monitoring programs have been developed. These developments have continued to show that *C. jejuni* is the leading cause of gastroenteritis worldwide beating other food borne bacterial pathogens including *Staphylococcus aureus, Salmonella typhi, Yersinia enterocolitica, Shigella, Clostridium difficle*, Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli* (EHEC), Enteropathogenic *E.coli*, *Bacillus cereus* and *Vibrio cholerae* (Kirk et al., 2015; Marder, 2017). This phenomenon makes in-depth understanding of the biology of *C. jejuni* worthwhile.

## 1.2 Characteristics of *C. jejuni*

*C. jejuni* are Gram-negative bacteria, non-spore formers, have a spiral shape and the length and width of the body is in the ranges of 1.5 – 3.5 µm and 0.2 – 0.4 µm respectively (Pead, 1979). Their distinct stable spiral shape is mediated by its ring shaped flagella and the small amplitude of its helix (Ng et al., 1985). This shape distinguishes it from other *Campylobacter* spp. whose shape is pleomorphic including S-shape spiral, seagull-shaped spiral, ribbon shaped spiral or dimpled and coccus forms. It has a unique corkscrew motility which is propelled by bipolar flagella emerging from a concave depression or crater-like feature on the poles (Müller et al., 2014; Pead, 1979).

Naturally, *C. jejuni* colonizes the intestinal mucosa of mammals and birds (Brown et al., 2004). Other habitats include: water, sewage, beach sand and ground water (Newell et al., 1985). However, chicken is the major host and the main source of transmission to humans (Hermans et al., 2012). In chicken, *C. jejuni* inhabits the crypt mucus of cecum, large intestine and cloacal without attaching onto the surfaces and rarely the spleen and gallbladder (Beery et al., 1988a). Human beings are an accidental host, and it resides in the crypt mucus of duodenum and proximal jejunum (Stahl and Vallance, 2015).

In the laboratory, the following conditions are routinely used to promote wholesome *C. jejuni* cellular growth: (i) a suitable media containing source(s) of carbon, amino acids, metal ions and pH ranging from 6.0 to 7.0, (ii) a microaerophilic environment which consists of 85% $N_2$, 10% $CO_2$, and 5% $O_2$ and (iii) temperatures ranging from 37°C to 42°C, with optimal growth achieved at 42°C (Davis and DiRita, 2008a).

## 1.3. Human disease and epidemiology

### *1.3.1 Clinical presentation of the disease and complications*

*C. jejuni* causes an enteric disease known as campylobacteriosis (Black et al., 1988). The symptoms include: diarrhea, raised body temperature, anorexia, malaise and stomach cramps and occasionally vomiting (Crushell et al., 2004). These symptoms don't kick in at once. It starts with fever which is experienced 2-3 days after exposure to *C. jejuni* gradually followed by mild or severe diarrhea after 3-5 days of exposure combined with anorexia, malaise and stomach cramps and may continue for 7 consecutive days (Black et al., 1988). Diagnosis is mainly through Gram-staining (Gram negative), polymerase chain reaction and cultivation either on blood agar or *Campylobacter* selective agar at 42°C under microaerophilic environment overnight (Hurd et al., 2012). In addition, appropriately equipped diagnostic

laboratories used MALDI-TOF mass spectrometry (Zautner et al., 2013). Normally, campylobacteriosis heals by itself within 3 days of onset. However, in serious cases erythromycin and ciprofloxacin are recommended for adults while in children only erythromycin is recommended (Eiland and Jenkins, 2008; Guerrant et al., 2001).

In some patients, post-infectious sequelae can arise upon recovery from campylobacteriosis. There most common ones are: (i) Guillain–Barré syndrome (GBS) which is a result of the human immune system mistaking its ganglioside GM1 to be *C. jejuni*'s lipopolysaccharide hence attacking itself resulting in limb weaknesses (Willison et al., 2016; Yuki and Hartung, 2012). (ii) Reactive arthritis (ReA) which is characterized by painful joints arising from immune associated inflammation after *C. jejuni* infection (Giovanni Cimminiello et al., 2015). And, (iii) Inflammatory bowel disease (IBD) which is characterized by gut inflammation due to an uncontrolled immune response to non-invasive microbiota species following infection by *C. jejuni* (Kalischuk and Buret, 2010).

### *1.3.2 Epidemiology*

Recent epidemiological data show that campylobacteriosis affects more than 500 million people per year with the majority of the cases going unreported (Kaakoush et al., 2015). These reports show that campylobacteriosis affects all ages, gender and race; but the elderly, children below 5 years and malnourished children are more affected due to weak immune defense systems and unstable composition of microbiota (Kaakoush et al., 2015; Masanta et al., 2013; Platts-Mills and Kosek, 2014). In addition, cases of *Campylobacter* associated post-infectious sequelae are on the rise (Connor and Riddle, 2013; Esan et al., 2017). Equally, resistance to tetracyclines and fluoroquinolones is on the rise (El-Adawy et al., 2015; Nguyen et al., 2016; Wimalarathna et al., 2013). The increased awareness of these situations has been attributed to the availability of reliable diagnostic tools and better epidemiological surveillance schemes (Kaakoush et al., 2015).

The sources of *C. jejuni* transmission to human include: (i) eating contaminated animal and poultry meat, particularly from cross contaminated chicken; (ii) eating contaminated vegetables particularly cross contaminated salads; (iii) drinking contaminated fluids such as milk and water and (iv) association with farm animals, poultry and pets (Hald et al., 2016). Consequently, risk factors include: contaminated foods and fluids, associating with animals, swimming or drinking tap water, traveling, poor sanitation, food production, diverse *C. jejuni* host/environmental adaption strategies and human status such as age, health and feeding habits (Hald et al., 2016; Kaakoush et al., 2015; Strachan et al., 2013).

No particular phylogenetic group of *C. jejuni* is directly linked to a particular type of diarrhea (i.e. mild or severe) or to a particular geographical location or region. The majority of human cases have been linked to serotypes ST-21 and ST-45 but recently ST-257 and ST-677 have been linked to severe hospitalized diarrhea cases in Sweden and Finland respectively (Cody et al., 2012; Harvala et al., 2016; Schönberg-Norio et al., 2006; Zautner et al., 2011). The prevalence of campylobacteriosis has been linked to seasons; it has been shown that its prevalence is higher during summer than winter or during rainy seasons than dry seasons (Nichols et al., 2012; Schielke et al., 2014; Zautner et al., 2011).

### *1.3.3 Pathogenesis process and virulence associated factors*

The *C. jejuni* pathogenesis process is poorly unserstood. As a result, majority of the responsible bacterial virulence factors are not known. However, available literature reveals that the infection process of *C. jejuni* in humans begins when a reasonable amount of cells reach and succeed in settling in the small intestines; for example, Black and co-workers showed that as little as 400 cells of *C. jejuni* can initiate *Campylobacter* associated diarrhea (Black et al., 1988). Since this observation was made, it has been shown that the process leading to diarrhea involves the following intertwined phases: (i) arrival of *C. jejuni* in the stomach, (ii) colonization of small intestines, (iii) adherence to epithelial cells and, (iv) invasion of epithelial cells, damage of tight junctions and evasion of innate immune defense system (Janssen et al., 2008; Konkel et al., 2001; Van Vliet and Ketley, 2001; Young et al., 2007). Below is an overview of each phase:

*(i) Phase 1: entry of C. jejuni in the stomach*

*C. jejuni* is introduced into the human stomach when one ingests contaminated food, water or milk but frequently by eating contaminated chicken meat (Butzler, 2004; Hermans et al., 2012). However, the environment in the stomach is characterized by pH 1.5 to 3.5, high osmolarity, temperature of 37°C, oxidants, poor nutrition and low oxygen levels, which are hostile to *C. jejuni* (Gelberg, 2014; Kararli, 1995). Because of this, *C. jejuni* migrates into the small intestines in search of a favourable environment (Hugdahl et al., 1988). The natural movement of food also helps to transport *C. jejuni* during its migration into the small intestines (Ribet and Cossart, 2015).

*(ii) Phase 2: Colonization of small intestine, mainly, jejunum*

Duodenum, jejunum and ileum make up the small intestine. Its main function is nutrient absorption. The environment in the small intestine is made up of: almost neutral pH

(duodenum pH 5 - 7, jejunum pH 6 - 7 and ileum pH 7), bile acids, limited oxygen, a temperature of 37°C, diverse strains of natural gut microbiota and mucus (Kararli, 1995). Consequently, when *C. jejuni* arrives in the duodenum and jejunum, it encounters a more favourable environment choosing to reside mainly in the crypt mucus of the jejunum (Lecuit et al., 2004; Stahl and Vallance, 2015). Other factors which have been found to encourage *C. jejuni* to prefer residing in the crypt mucus of jejunum include: (a) availability of variety of amino acids which *C. jejuni* utilizes as a source of carbon (Karmali et al., 1986; Leach et al., 1997; Mendz et al., 1997; Westfall et al., 1986); (b) availability of metal ions especially iron which are essential in synthesis of proteins and metabolic processes (Stahl et al., 2012a); (c) availability of various by-products such as SCFAs and vitamins generated by the gut microbiota during fermentation which *C. jejuni* utilizes for growth (Mao et al., 2014; Staib and Fuchs, 2014; Sun and O'Riordan, 2013) and; (d) availability of constantly replenished mucus whose chief component is mucin which has L-fucose as one of its building blocks (Johansson et al., 2011). *C. jejuni* utilizes free L-fucose produced by fucosidases of both the gut microbiota and human small intestine as a carbon source (Stahl et al., 2011).

*(iii) Phase 3: adherence to epithelial cells*

Under normal situations, walls of small intestines are highly guarded against adherence and subsequent invasion by microbial pathogens. Some of these guarding mechanisms include: saliva, acidic pH, microbiota, immunoglobulins, peroxidases, lactoferrins, proteolytic enzymes, phagocytes, catalases, mucus, secretions from paneth cells, innate lymphoid cells, adaptive immune system, among others (Gelberg, 2014). Interference with this norm opens a door for a pathogen to attack the epithelial lining of the small intestines leading to infections (Kamada et al., 2013). For *C. jejuni,* it has been shown that consumption of certain types of foods disrupts the composition of microbiota leading to invasion of epithelia cells (Masanta et al., 2013). For example, fat-rich diet alters the normal composition of microbiota by increasing levels of *E. coli*, *Clostridium* spp. and other *Eubacterium* spp. and reducing the levels *Enterococcus* spp. and *Lactobacillus* spp. (Bereswill et al., 2011). This disruption breaks the colonization resistance mounted by normal composition of microbiota supporting the population of *C. jejuni* to multiply to numbers which overpowers other protective measures leading to its attachment onto the epithelial cells followed by invasion and diarrhea (Stahl and Vallance, 2015).

*(iv) Phase 4: Invasion of intestinal epithelial cells*

In the small intestines, *C. jejuni* is taken up by M-cells (Hu et al., 2008; Kalischuk et al., 2010; Walker et al., 1988). *C. jejuni* avoids engulfment by phagocytes which protect M-cells by increasing synthesis of polysaccharide capsule on its outer coat (Maue et al., 2013; Rose et al., 2012; Stahl et al., 2014). Once inside the cytoplasm, *C. jejuni* is mainly contained in a *Campylobacter* containing vacuole (CCV) which is moulded during uptake (Konkel et al., 2013; Stahl et al., 2014). During its moulding, the vacuole incorporates Lamp-1 of the lysosome which aids the vacuole in evading engulfment by lysosome (Stahl et al., 2014; Watson and Galán, 2008). In addition to Lamp-1, epithelial membrane attached *C. jejuni* injects proteins useful proteins into the cytoplasm, for example, CiaI which also aids CCV in avoiding delivery to lysosomes (Buelow et al., 2011). *C. jejuni* survives inside the CCV by drastically decreasing metabolic activities and utilizing anaerobic respiratory pathway (Liu et al., 2012).

*(v) Phase 5: Intestinal epithelia cell response to invasion by C. jejuni and resulting diarrhea*

It has been shown in a mice model that during invasion by *C. jejuni*, the toll-like receptors, TLR2 and TLR4, of gastrointestinal epithelium sense *C. jejuni* or its effectors and transmit information through MyD88 to NF-kB which recruits innate immune response (Stahl and Vallance, 2015). In addition, the presence of *C. jejuni* or its capsule stimulates gastrointestinal epithelium to recruit cytokines such as interleukin 2, interleukin 4, interferon-γ, tumor necrosis factor-α and a group of antimicrobials which join hands with other players of innate immune response to defend the gastrointestinal epithelium against *C. jejuni* invasion and subsequent clearance of *C. jejuni* (Maue et al., 2013; Shang et al., 2016; Zilbauer et al., 2005).

Ultimately, diarrhea results from a combination of factors: first, the tension imposed on the integrity of epithelia cells alters: (i) the structure and function of tight junction barriers, (ii) normal induction of fluid and (iii) normal electrolyte secretion (Berkes et al., 2003; MacCallum, 2005; Viswanathan et al., 2008). Second, toxin component CdtB which when delivered into the nucleus, unzips the double stranded DNA into single strands leading to termination of cell cycle and subsequently apoptosis (Lai et al., 2016). Lastly, the inflammatory response cascade leads to disruption of blood veins (Martini and Willison, 2016).

## 1.4 Bile acids: A key component of the fluid in the small intestines of human beings

As stated above, *C. jejuni* mainly resides in the small intestines of human beings. Bile acids are one of the major constituents of fluid in the human small intestines. Hence they constantly interact with bacteria that are present in the small intestine including *C. jejuni*. The bile acids are categorized into two groups, namely, primary bile acids and secondary bile acids ( Table 1). Below is a brief description of the synthesis of human bile acids and their secretion to the small intestines.

### *1.4.1 Synthesis of primary bile acids in the liver*

Primary bile acids; cholic acid (CA) and chenodeoxycholic acid (CDCA) are synthesized in the liver from cholesterol (Lefebvre et al., 2009). Two pathways are involved, namely, a classic (neutral) pathway and alternative (acidic) pathway (Li and Chiang, 2015). The classic pathway is the main source of CA and CDCA (Dawson and Karpen, 2015). The first step in this pathway entails enzyme 7α-hydroxylase (CYP7A1) catalyzing the conversation of cholesterol to 7α-hydroxycholesterol. The subsequent steps involve further disintegration of this molecule into: (i) unconjugated CA which is jointly catalyzed by actions of enzymes 12α-hydroxylase (CYP8B1) and 27α-hydroxylase (CYP27A1) and (ii) unconjugated CDCA which is catalyzed by enzyme CYP27A1 (Lorbek et al., 2012). Separately, the alternative pathway yields CDCA only. The pathway progresses in 2 steps: first, cholesterol is oxidized into 27-hydroxycholesterol in a process driven by enzyme CYP27A1; second, hydroxylation of 27-hydroxycholesterol into CDCA in a process that is catalyzed by oxysterol 7α-hydroxylase (CYP7B1) (Chiang, 2004).

Finally, synthesized CA and CDCA undergo N-acylamadation conjugation which is essential for their reabsorption (Dawson and Karpen, 2015; Lorbek et al., 2012). It involves first converting CA and CDCA into their respective acyl-CoA thioester in a process that is catalyzed by cholyl-CoA synthetase (Falany et al., 1994). This is followed by an addition of either glycine or taurine to the respective acyl-CoA thioester in a process involving bile acid-CoA:amino acid *N*-acyltransferase (hBAT). The end results are the following conjugated hydrophobic primary bile acids: CA yields (i) glycocholic (GCA) and (ii) taurocholic (TCA); CDCA yields (i) glycochenodeoxycholic (GCDCA) acid and (ii) taurochenodeoxycholic acid (TCCDA) with the ratio of glycine conjugants being higher than taurine conjugants (Joyce and Gahan, 2016; Kubitz et al., 2012). CA, GCA, TCA, CDCA, GCDCA and TCCDA are

transported through canalicular bile salt export pump (BSEP) for storage in the gallbladder (Lorbek et al., 2012).

### *1.4.2 Release of primary bile acids into the small intestines and subsequent synthesis of secondary bile acids*

The release of CA, GCA, TCA, CDCA, GCDCA and TCCDA from the gallbladder into the small intestines is driven by the hormone cholecystokinin (CCK). The presence of food in the small intestines stimulate endocrine cells to release CCK which contracts the gallbladder releasing CA, GCA, TCA, CDCA, GCDCA and TCCDA into the duodenum (Gomez et al., 1988). In the small intestines, CA, GCA, TCA, CDCA, GCDCA and TCCDA undergo biotransformation by gut bacterial microbiota rendering them soluble and re-absorbable (Canzi et al., 1989; Kim and Lee, 2005). In addition, some of the biotransformed bile acids undergo further modification by sulfation and glucoronidation conjugation (Kirkpatrick et al., 1988). These modifications are briefly described below:

(a) Biotransformation

The small intestine harbours bacterial microbiota which is made up of species from the following phyla: Bacteroidetes, Firmicutes (Tenericutes), Proteobacteria, Verrucomicrobia, Fusobacteria, Actinobacteria and Cyanobacteria (Eckburg et al., 2005). Ridlon et al., 2005 reported the distribution of bacterial microbiota as follows:

(i) the duodenum harbours *Lactobacillus* and *Streptococcus*

(ii) The jejunum harbours *Lactobacillus*, *Streptococcus*, *Staphylococcus* and *Veillonella*

(iii) the ileum harbours *Enterococcus*, *Enterobacteria*, *Clostridium*, *Bacteroides*, *Veillonella* and *Lactobacillus*

(iv) The colon harbours *Bacteroides, Eubacterium, Bifidobacterium, Ruminococcus, Peptostreptococcus, Propionibacterium, Clostridium, Lactobacillus, Streptococcus* and *Methanobrevibacter*.

These bacteria release bile salt hydrolases (BSHs) which degrade bile acid salts for the following reason: (a) nutrition (Huijghebaert et al., 1982; Van Eldere et al., 1996) (b) detoxification (De Boever and Verstraete, 1999; Smet et al., 1995). The actions of these enzymes modify primary bile acids creating secondary bile acids (Hill and Drasar, 1968, 1968; Shindo and Fukushima, 1976). This process is commonly referred to as

biotransformation. Four different types of bile acid biotransformation have been observed in human small intestine:

(i) Oxidation: It involves the removal or addition of $H_2$ at the C-3, C-7 and C-12 of CDCA, CA, DCA and UDCA leading to generation of oxo- and keto- forms. These reactions are catalyzed by enzymes 3α- and 3β-hydroxysteroid dehydrogenase, oxidoreductase and luciferase which are present in *Arthrobacter* spp., *Bacillus* spp., *Bacteroides* spp., *Brevibacterium* spp., *Clostridium* spp. *Corynebacterium* spp., *E. coli*, *Eubacterium* spp., *Lactobacillus* spp., *Micrococcus* spp., *Nocardia* spp., *Peptococcus magnus* and *Pseudomonus* spp. (Baron and Hylemon, 1995; Kang, 2008; Sutherland and Macdonald, 1982; Taiko et al., 1987).

(ii) Epimerization: It involves interchange of α- with β- or vice versa at the C-3, C-7 and C-12 positions of CDCA, CA, DCA and UDCA leading to generation of oxo- and iso- forms. These reactions are catalyzed by hydroxysteroid dehydrogenase (HSDH) that is present in *Bacteroides* spp., *Clostridium* spp. and *Eubacterium* spp. (Edenharder and Schneider, 1985; Hirano et al., 1981; Macdonald and Hutchison, 1982).

(iii) Deamination: It entails breaking the *N*-acyl amide bond which binds taurine and glycine with CA and CDCA leading to generation of unconjugated GCA, TCA, GCDCA and TCDCA. This process is catalyzed by bile salt hydrolases (BSH) that are present in *Bacteroides* spp., *Clostridium* spp., *Lactobacillus* spp., *Bifidobacterium* spp., and *Listeria monocytogenes* (Huijghebaert and Hofmann, 1986). The unconjugated GCA, TCA, GCDCA and TCDCA are either reabsorbed back into the liver for conjugation or further biotransformed into CA and CDCA respectively.

(iv) 7α/β-dehydroxylation: In this reaction CA and CDCA are biotransformed by *Clostridium* spp. and *Eubacterium* spp. into DCA and LCA or UDCA respectively. The process is catalyzed by enzymes 7α- or 7β-HSDH (Lepercq et al., 2004; Macdonald and Roach, 1981).

(b) Sulfonation conjugation

It involves transferring $SO^-_3$ at phosphoadenosine 5'- phosphosulfate (PAPS) to 3-OH position in a process that is catalyzed by sulfotransferase (Glatt, 2000). For example, sulfonation of lithocholic acid yields gylcolithocholic and taurolithocholic bile acids (PALMER and BOLT, 1971).

(c) Glucuronidation conjugation

It involves the addition of a glucuronide molecule to a biotransformed bile acid in a process that is catalyzed by UDP-glucuronosyltransferases (UGT) enzymes (Matern et al., 1984). For example, glucoronidation conjugation of chenodeoxycholic in the liver into acyl CDCA-24glucuronide (CDCA-24G) in a process that is catalyzed by UDP-glucuronosyltransferases-1A3 (UGT1A3) (Erichsen et al., 2010; Trottier et al., 2006).

**Table 1**: Human bile acid pool and their sources

| **Class** | **Metabolic conversations** | **Bile Acids** |
|---|---|---|
| **Primary bile acids** | Breakdown of cholesterol by classic and alternative pathways | (a) Cholic acid (CA); glyco and tauro conjugation leads to: (i) Glycocholic (GCA) and (ii) Taurocholic (TCA)<br><br>(b) Chenodeoxycholic acid (CDCA); glyco and tauro conjugation leads to: (i) Glycochenodeoxycholic (GCDCA) acid (ii) Taurochenodeoxycholic acid (TCCDA)<br><br>(Lefebvre et al., 2009) |
| **Secondary bile acids** | | |
| | (i) From primary bile acids through gut microbial 7α-dehydroxylation | CA biotransforms into deoxycholic acid (DCA) and CDCA biotransforms into lithocholic acid (LCA) (Masuda and Oda, 1983; Mitropoulos and Myant, 1967; Norman and Donia, 1962) |
| | (ii) From primary or secondary bile acids: | |
| | (a) through gut microbial 7α/β-epimerization | Oxo-lithocholic biotransforms into ursodeoxycholic acid (UDCA) (Odermatt et al., 2011) |
| | (b) through gut microbial 3α/β-epimerization | Iso-bile acids (isoLCA, isoIDCA and isoIUDCA) (Nagengast et al., 1993). But rare. |
| | (c) through gut microbial 5α/β-epimerization | Allo-bile acids (allo-CA, allo-DCA, allo-LCA and allo-UDCA) (Monte, 2009). But rare. |
| | (d) through gut microbial oxidation | Oxo-(keto-) bile acids (7-ketolithocholic acid and 12-lithocholic acid) (Odermatt et al., 2011). But rare. |

## 1.5 Quantitative proteomics and its application to bacteria-bile acid research

The goal of quantitative proteomics study is the detection, identification and quantification of the whole protein complement of a biological system, and the global quantitative characterization of its changes when its normal status is perturbed (Patterson and Aebersold, 2003). Relative and absolute changes of protein and peptide concentrations in a perturbed system are usually measured by high resolution mass spectrometry (MS) (Bantscheff et al., 2007). In a nutshell, MS-based quantitative measurements are grouped into two: quantitative labeling quantification and label-free labeling quantification (Boja and Rodriguez, 2012; Washburn, 2011).

### *1.5.1 Quantitative labeling quantification*

In a labeling-based quantitative approach, differential expression of proteins is analysed by comparing the LC-MS or LC-MS/MS spectral differences between endogenous peptides and their stable isotope-labeled analogues (Sap and Demmers, 2012). Three major labeling methods have been established:

a) Metabolic labeling:

In this type of labeling, cells are cultured in media which are supplemented with amino acids or nutrients carrying stable heavy isotopes (Gouw et al., 2010). These are incorporated into the synthesized proteins, and the corresponding mass shifts and associated signal intensities provide information on the differential concentrations of peptides, and therefore proteins. At the beginning, $^{15}$N-enriched media were successfully used in metabolic labeling (Conrads et al., 2001; Oda et al., 1999). This success lead to the development of a superior and currently frequently used metabolic labeling method called stable isotope labeling in cell culture (SILAC) (Ong et al., 2002). In this method, heavy stable amino acids (most commonly arginine and lysine) are used because this corresponds with the enzyme specificity of trypsin as the most frequently used endoproteinase (Zhang and Neubert, 2009). In a two-plexed experiment, two sets of bacterial cultures are prepared; the first set - bacteria are cultured in media with light Arg and Lys. Second set - bacteria are cultured in media containing heavy Arg and Lys with several sub cultivation steps until ≥95% incorporation rate is achieved. Subsequently, 1:1 mixtures of the light and heavy protein samples are prepared and separated e.g. by SDS-PAGE. The resulting bands are sliced into small pieces, digested with trypsin and analyzed by LC-MS/MS. The ratios of the generated spectra of both light and heavy peptides

are used to calculate differential expression of proteins (Ong et al., 2002; Zhang and Neubert, 2009).

b) Chemical or enzymatic labeling:

In this labeling method, proteins are chemically or enzymatically labeled after extraction and purification (Sap and Demmers, 2012). Three main techniques which use this labeling method include:

(i) isotope-coded affinity tags (ICAT) which utilizes cysteine labeling to measure differential protein expression (Shiio and Aebersold, 2006). ICAT labeling reagents are made up of three parts: a cysteine reactive group, a linker containing light and heavy isotopes which can be differentiated by MS and an affinity tag (biotin) (Chan et al., 2015). Experimentally, two protein samples are labeled with light and heavy ICAT reagent. The two mixtures are combined and digested with trypsin. The cysteine rich peptides are affinity tagged, purified and measured by MS (Shiio and Aebersold, 2006).

(ii) Dimethyl labeling: In this technique, N-termini and ε-amino groups of lysine residue are labeled through reductive amination with formaldehyde and cyanoborohydride (Hsu and Chen, 2016). Initially, peptides are generated by digestion with trypsin. A Schiff base is formed via reductive amination when formaldehyde reacts with the N-terminus or an ε-amino group of a Lys residue. This base is reduced to a reactive secondary amine by cyanoborohydride. The secondary amine reacts with formaldehyde to form dimethylated peptides which are measured by MS/MS (Hsu et al., 2003).

(iii) $^{18}O$ labeling: This technique uses trypsin digestion to label carboxyl termini of peptides with two atoms of $^{18}O$ (Stewart et al., 2001). The labeling procedure involves digesting proteins with trypsin (or a proteases enzyme) in $^{18}O$ and $^{16}O$ labeled water. The ratio of $^{18}O$ and $^{16}O$ in the resulting peptides is analyzed by MS and MS/MS (Miyagi and Rao, 2007).

c) Isobaric tags labeling:

In this technique, isobaric tags employ the principle of carboxylic acid active ester chemistry to label free primary or secondary amino groups in either proteins or peptides (Gygi et al., 1999). Reagents incorporate an isotopic balancer group which links an amin-reactive group with an isotopic reporter group (Christoforou and Lilley, 2012). LC-MS/MS analysis of the tryptically digested samples after mixing produce a cumulative MS signal, and MS/MS spectra containing a set of reporter mass signals whose intensity corresponds to the initial

protein concentration in the samples (Rauniyar and Yates, 2014). This labeling approach is employed by both 'isobaric tags for relative and absolute quantification' (iTRAQ) (Ross, 2004) and 'tandem mass tags' (TMT) (Rauniyar et al., 2013).

### *1.5.2 Label-Free Quantification*

In this approach to protein quantification, proteins or peptides are not labeled (Griffin et al., 2010). The experimental approach involves digestion of protein replicates with trypsin, separation of peptides by LC, and quantification from either the MS or MS/MS spectra (Neubert et al., 2008). In addition to absence of labeling, another important distinct feature of label-free quantification approach is the LC-MS/MS spectra quantification approach (Wang et al., 2008; Zhu et al., 2010). Four different LC-MS/MS spectra quantification approaches are available whose usage depends on the equipment. They include: First, Spectral counting: In this approach, protein quantification of a given protein is directly related to the average sum of the corresponding LC-MS/MS peptide spectra in the sample (Milac et al., 2012; Zhang et al., 2006). Second, MS1 label-free analysis in which a concentration of a given protein is calculated from the peak area value of corresponding peptides (Aoshima et al., 2014). Third, $MS^E$ where both the precursor and fragment ion information of a protein are simulatenously extracted (Plumb et al., 2006). This results in the generation of both the molecular mass and fragment ion information of the protein under consideration which are used to identify it. Fourth, data-independent acquisition (DIA) with sequential window acquisition of all theoretical mass spectra (SWATH). This approach employs a data-dependant acquisition (DDA) generated ion library to identify data-independent acquisition (DIA) generated m/z windows ion spectra (Gillet et al., 2012; Huang et al., 2015).

In general, the following advantages make both label and label-free quantitative proteomics approaches very attractive: enhanced simplicity, specificity, accuracy and reproducibility of results, rapid availability of results, analysis of multiple samples concurrently, and analysis of both post-translational modifications and protein complexes (Wasinger et al., 2013).

## 1.6 Physiological response of intestinal bacteria to bile acids and quantitative proteomics

Transcriptional analysis has been widely used to gain an in-depth understanding into the physiological response of a few intestinal bacteria to bile acids. Through these studies, it has been found that bile acids impacts biological processes including: DNA replication and transcription (Kristoffersen et al., 2007), DNA damage and repair (Kandell and Bernstein, 1991), cell wall and cell membrane biogenesis (Merritt and Donaldson, 2009), fatty acid and phospholipid metabolism (Taranto et al., 2003), amino acid biosynthesis (Sanchez et al., 2005), efflux systems (Lin et al., 2005), energy metabolism (Leverrier et al., 2004), protein synthesis (Prouty et al., 2004) and stress defense mechanisms (Bernstein et al., 1999).

Of greatest interest is the recent application of quantitative proteomics in two studies which investigated the tolerance of bile acid stress in *Lactobacillus* spp. In the first study, Hamon and colleagues used 2D-LC-MS to conclude that 6 out of 15 genes previously identified via transcriptional analysis were responsible for bile acid tolerance in three *Lactobacillus plantarum* strains (LC56, LC 804 and 299V) (Hamon et al., 2011). In the second study, Lee and colleagues used iTRAQ to investigate the global bile stress response in *Lactobacillus johnsonii* PF01 (Lee et al., 2013). The study revealed numerous previously unknown bile tolerance proteins in *Lactobacillus* spp. In addition, the findings of this study generated the first detailed proposal on bile stress response in *Lactobacillus* spp. Evidently, the findings of these two studies showed that quantitative proteomics can point to hitherto unknown proteins, and lead to a better understanding of the physiological response of bacteria to bile acids.

# 2.0 HYPOTHEIS, AIM AND OBJECTIVES OF THE STUDY

## 2.1 Hypothesis of this study

As explained earlier, *C. jejuni* resides in the human small intestines where it continuously interacts with different bile acids. However, literature search shows that the response of different biological processes in *C. jejuni* to the different bile acids remains uninvestigated. Available results from few studies on physiological response of *Bifidobacterium* spp., *Lactobacillus* spp. and *Helicobacter pylori* to bile reveal a picture of re-arrangement of various biological systems such as transcriptional regulators, chaperones, membrane transporters, enzymes, stress mitigating proteins, energy metabolism and outer membrane proteins (Ruiz et al., 2013a). This thesis therefore hypothesized that a similar picture of rearrangement of biological processes was true for *C. jejuni*. In addition, some of the proteins which were significantly differentiated in *C. jejuni* in response to bile acids promoted its adherence on and invasion of epithelia lining of the human small intestine. The information that this study has generated will increase the current understanding of the biology of *C. jejuni.*

## 2.2 Aim of the thesis

The first aim of this thesis was to use a suitable quantitative proteomic approach to investigate the proteomic response of *C. jejuni* to sublethal concentrations of seven dominant human bile acids and identify previously uncharacterized proteins. These bile acids are: CA, DCA, LCA, TCA, CDCA, UDCA and GCA. The second aim was to characterize the adherence and invasion of at least one of the unknown widely expressed *C. jejuni* protein in Caco-2 cells.

## 2.3 Objectives of the thesis

(i) To investigate the response in 81-176 to DCA 0.05% at 37$^{o}$C for 12h and 24h using both stable isotope labelling with amino acids in cell culture (SILAC) and label-free analysis with sequential window acquisition of all theoretical mass spectra (SWATH); and determine a suitable quantitative method for the study.

(ii) To use the method selected quantitative method to investigate global protein expression in 81-176 in response to sublethal concentrations of CA, LCA, TCA, CDCA, UDCA and GCA cultured at 37$^{o}$C for 12h under microaerophilic conditions.

(iii) To identify and characterize a currently uncharacterized and widely induced protein.

(iv) To use label-free analysis with SWATH and investigate protein expression in 81-176 cultured in temperatures of 37$^{o}$C (human) and 42$^{o}$C (chicken) without bile acids.

# 3.0 MATERIALS AND METHODS

## 3.1 Investigating the influence of bile acids on the ability of 81-176 to adhere and invade Caco-2 cells

Gentamicin protection assay (GPA) was used to investigate the influence of bile acids on the ability of 81-176 to adhere and invade Caco-2 cells. Briefly, GPA is an assay that is used to determine the ability of eukaryotic cells to internalize bacteria (Friis et al., 2005). Experimentally, bacteria and eukaryotic cells are co-incubated to allow internalization to take place. In order to increase the number of internalized bacteria, a low number of bacteria in the inoculum or multiplicity of infection (MOI) is recommended (Hu and Kopecko, 1999). Consequently, an antibiotic called gentamicin is added to kill the non-internalized bacteria. Finally, the internalized bacteria are retrieved, cultured in appropriate media and their numbers are determined.

In this study, a concentration of 2 x$10^4$/mL Caco-2 cells was seeded in each well of a 24-well plate containing 1ml Dulbecco's minimal essential medium (DMEM) supplemented with 1% fetal calf serum (FCS) and 1% non essential amino acids without antibiotics and incubated at 37$^{\circ}$C under 5% $CO_2$-95% air atmosphere for 24h to 72h until a confluence of 90% was observed. These semi-confluent cells were washed three times with warm Hank's Balanced Salt Solution (HBSS), and to each well was added 1mL DMEM media supplemented with 1% fetal calf serum (FCS) and 1% non essential amino acid without antibiotics and 5µL of appropriate concentration of bile acid. 3 wells on each plate contained DMEM media lacking a corresponding bile acid to act as a control. All *C. jejuni* isolates were cultured for 16h to 18h at 42$^{\circ}$C under microarophilic conditions to achieve an optical density at $A_{540}$ of 0.2 (OD $A_{540}$ of 0.2 corresponds to 5 x $10^8$ CFU/ml) (Khanna et al., 2006). *C. jejuni* inoculums were washed twice in warm HBSS to centrifuging at 4000 rpm for 10 minutes and diluting with HBSS to multiplicity of infection (MOI) of 1:10 using the formula below:

$$\text{MOI} = \frac{\text{Number of 81-176 } (5 \times 10^8)}{\text{Number of Caco-2 cells}} = \text{Y + HBSS to achieve MOI of 1:10}$$

10µL of diluted *C. jejuni* suspension was inoculated into each well followed by centrifugation of each plate at a low speed of 1000 x g for 2 min to bring all the *C. jejuni* isolates directly in

contact with Caco-2 cells. From this point onwards adherence and invasion were performed separately.

### *3.1.1 Adherence assays*

Plates that were allocated for adherence assays were incubated at 37°C under 5% $CO_2$-95% air atmosphere for 30 minutes, after which the media was removed and the cells were washed 3 times with warm HBSS. The cells were then overlaid with 100µL of 1% Triton X-100 and left to incubate for 10 minutes to lyse and detach. Consequently, 900µL LB medium was added to the arising suspension and homogenously mixed by pipetting. 20µL of each diluted suspension was inoculated on Columbia blood agar and incubated at 42°C under microaerophilic conditions for 24h. Finally, the number of colonies on each plate was counted and the number of adherent *C. jejuni* isolates was recorded. Only plates with 10 or more colonies were counted. STATISTICA software was used to analyze the differences by two-way ANOVA. Bile acid concentrations were taken to be dependable variables and 81-176 to be an independent variable.

### *3.1.2 Invasion assays*

Plates that were allocated for invasion assays were incubated at 37°C under 5% $CO_2$-95% air atmosphere for 2h, after which the media was removed and the cells were washed 3 times with warm HBSS. 1mL DMEM supplemented with 1% FCS, 1% non essential amino acid and 100 ug/mL gentamicin was added to each well and the plates incubated at 37°C under 5% $CO_2$-95% air atmosphere for 2h. The cells were then washed 3 times with warm HBSS and overlaid with 100µL of 1% Triton X-100 and left to incubate for 10 minutes to lyse and detach. Consequently, 900µl LB medium was added to the arising suspension and homogenously mixed by pipetting. 20µl of each suspension was inoculated on Columbia blood agar and incubated at 42°C under microaerophilic conditions for 24h. Finally, the number of colonies on each plate was counted and the number of invade *C. jejuni* was recorded. Only plates with 10 or more colonies were counted. STATISTICA software was used to analyze the differences by two-way ANOVA. Bile acid concentrations were taken to be dependable variables and 81-176 to be an independent variable.

## 3.2 Determination of *C. jejuni* $IC_{50}$ of each bile acid and evaluation of 81-176 growth in half $IC_{50}$ concentrations

CDB containing different concentrations of each bile acid was prepared (Table 3). Bile acid concentrations were derived from the range of 2mM to 30mM (0.2 to 2%) that is present in the human small intestine (Begley et al., 2005a). The $OD_{600}$ of *C. jejuni* 81-176 growing for

16h at 42$^{o}$C under microaerophilic conditions while shaking at 150rpm was measured and diluted to an $OD_{600}$ of 1.0 using neutral CDB (lacking bile acids). 1.5ml of CDB containing various concentrations of each bile acid and 1.5ml of diluted suspension of *C. jejuni* 81-176 were transferred into test tubes with cocks. The inoculum was incubated for 16h at 42$^{\circ}$C under microaerophilic conditions with shaking at 150rpm. An inoculum of 1.5ml neutral CDB and 1.5ml diluted suspension of *C. jejuni* 81-176 was included as a control. Lastly, $OD_{600}$ of each inoculum was measured, recorded; and finally a graph of $OD_{600}$ vs. concentration (mM/l) of *C. jejini*'s growth in response to each bile acid was drawn from which the *C. jejuni* $IC_{50}$ of each bile acid was determined (Bailey et al., n.d.; Kusano-Kitazume et al., 2012; Soothill et al., 1992). Thereafter, the growth of 81-176 in Mueller Hinton Broth (MHB) supplimented with half $IC_{50}$ concentrations of each bile acid. The growth evaluations were carried out as described before (Davis and DiRita, 2008b). STATISTICA software was used to analyze the differences by one-way ANOVA.

**Table 2**: Concentrations of bile acids which were used in determining *C. jejuni* $IC_{50}$

| **%age** | **Concentrations in mM/L** | | | | | | |
|---|---|---|---|---|---|---|---|
| | CA | CDCA | TCA | GCA | DCA | LCA | UDCA |
| 1.5% | 38.21mM | 38.21mM | 29.09mM | 197.24mM | 38.21mM | 39.83mM | 38.21mM |
| 0.75% | 19.11mM | 19.10mM | 14.55mM | 98.62mM | 19.10mM | 19.91mM | 19.10mM |
| 0.38% | 9.55mM | 9.68mM | 7.27mM | 49.31mM | 9.55mM | 10.09mM | 9.68mM |
| 0.19% | 4.78mM | 4.84mM | 3.64mM | 24.65mM | 4.78mM | 5.05mM | 4.84mM |
| 0.09% | 2.39mM | 2.29mM | 1.82mM | 12.32mM | 2.39mM | 2.39mM | 2.29mM |
| 0.05% | 1.19mM | 1.27mM | 0.91mM | 6.16mM | 1.20mM | 1.33mM | 1.27mM |
| 0.02% | 0.60mM | 0.51mM | 0.45mM | 3.08mM | 0.48mM | 0.53mM | 0.51mM |
| 0.01% | 0.30mM | 0.25mM | 0.23mM | 1.54mM | 0.24mM | 0.27mM | 0.25mM |
| 0% | 0mM | 0mM | 0mM | 0mM | 0mM | 0mM | 0mM |

## 3.3 Quantitative proteomics

### *3.3.1 Establishment of SILAC for C. jejuni*

(i) *C. jejuni* isolates and culture conditions

Suitable isolates for SILAC analysis were selected from 303 *C. jejuni* strains which had been isolated from different sources, namely, cattle (52), chicken (73), turkey (28) and humans (150). Chicken, turkey and cattle isolates were generously provided by the German *Campylobacter* reference center of the Bundesinstitut für Risikobewertung (BfR, Federal Institute for Risk Assessment) in Berlin, Germany. Reference strains NCTC 11168, NCTC 11828 (81116), 81-176, and 84-25 were obtained from Leibniz Institute - DSMZ German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany. Human isolates were isolated from stool samples of campylobacteriosis patients treated at the University Medical Center Göttingen (Germany) from 2000-2004. These isolates had been stored as cryobank stocks (Mast Diagnostica, Reinfeld, Germany) at -80°C. Prior to auxotyping, they were thawed then cultured in on Columbia agar base (Merck, Darmstadt, Germany) supplemented with 5% sheep blood (Oxoid Deutschland GmbH, Wesel, Germany) and incubated overnight at 42°C under microaerophilic conditions (5% $O_2$, 10% $CO_2$, 85% $N_2$).

(ii) Selection of suitable *C. jejuni* isolate: Auxotyping

Auxotyping was performed using a defined broth. The components of this medium were grouped into 5 solutions and individual components (Table 4; all chemicals were obtained from Sigma-Aldrich, Germany). Prior to auxotyping, the growth of C. jejuni reference strain 81-176 in the define broth (CDM), Luria-Bertani (LB) broth, Muller Hinton (MH) broth and Barin-heart infusion (BHI) broth was investigated. The $OD_{600}$ reading was taken after every 4h for a period of 20h. STATISTICA software was used to analyze the growth differences by one-way ANOVA. In the auxotyping exeperiment, all 291 isolates were tested for their ability to grow in the absence of arginine, lysine, serine, leucine, isoleucine, valine, cysteine hydrochloride, cystine, proline, methionine, and a combination of leucine, isoleucine and valine. Isolates which demonstrated growth on the typing media were designated as prototrophs and those that demonstrated no growth on the typing media were designated auxotrophs.

*(iii) Comparison of C. jejuni growth in defined campylobacter broth with and without labelled arginine and lysine*

The prototrophic isolate 81-176 and the arginine-auxotrophic isolate av4258 (Table 5) were cultured in MCD broth containing both heavy L-arginine ($^{13}C_6{}^{15}N_4$-Arg, Silantes, Munich, Germany) and L-lysine ($^{13}C_6{}^{15}N_2$-Lys, Silantes, Munich, Germany) and MCD broth containing both unlabeled arginine (Sigma-Aldrich) and unlabeled lysine (Sigma-Aldrich) at 42°C under microaerophilic conditions (5% $O_2$, 10% $CO_2$, 85% $N_2$) while shaking at 150rpm over a period of 48h. During this period, the OD readings were taken after every 4h and growth compared. In addition, at 16h, live-dead-staining using the LIVE/DEAD *Bac*Light Bacterial Viability kit L13152 (Invitrogen detection technologies) was performed on each of the cultures and examined under fluorescence microscope as in accordance to the manufacturer's instructions.

(iv) Labeling

The isolates av4258 and 81-176 were cultured over 6 passages in broth containing unlabeled lysine and unlabeled arginine. Incubation time for each passage was 32h which corresponds to 2X generation time under microaerophilic conditions (5% $O_2$, 10% $CO_2$, 85% $N_2$). 81-176 was cultured on Columbia Blood Agar for 16h at 42 °C under microaerophilic conditions and harvested with MCD broth with and without labeled arginine/lysine. The $OD_{600}$ was measured and adjusted to $OD_{600}$ 1 by adding appropriate quantities of corresponding broth. The adjusted labeled and unlabeled CDM broth cultures were incubated for 32h at 42 °C under microaerophilic conditions becoming the first passage. At the end of each 2X generation time and before passaging the next passage, samples for protein extraction were collected from both and agar cultures for protein extraction.

(v) Protein extraction and acetone precipitation

Cells were harvested by centrifuging at 4000 rpm for 10 minutes. The resulting pellets in both situations were resuspended in 1 ml of 0.9% aqueous sodium chloride solution. The suspensions were sonicated on ice using 5 bursts at a setting of 3 and 30% duty cycles (Branson Model 250) for 30s with 30s intervals until the suspension became clear. Cell debris were discarded by centrifuging at 12000 rpm at 4°C for 15 min. Protein concentration in the supernatant was quantified using either Bradford assay (Bio-Rad, Munich, Germany) or NanoDrop2000-pedestal model (Thermo Scientific, Germany). This was followed by visualization on 12% SDS-PAGE gel electrophoresis.

The crude protein extract was concentrated using the acetone precipitation technique. Acetone and ethanol (80%) were cooled to -20°C. 400µl of the each protein supernatant was transferred into a 2ml eppendorf tube and centrifuged for 10 min at 4°C and 13300 rpm. 1600 µl of -20°C cooled acetone was added, vortexed thoroughly and incubated at -20°C overnight. The samples were centrifuged for 30 mins at 4°C and 13300 rpm. As much supernatant (acetone) as possible was carefully removed from the tubes by pipetting without damaging the protein pellets. The tubes were then left open to air-dry under clean bench for 1 hour to completely remove acetone from the pellets. Subsequently, -20°C cooled 400 µl ethanol (80%) was added into the tubes and the pellets washed by centrifugation for 30 min at 4°C and 13300rpm. As much supernatant (ethanol) as possible was carefully removed from the tubes by pipetting without damaging the protein pellets. The tubes were further left to air-dry under a clean bench for 1h to completely remove ethanol from the pellets. This was followed by analyzing the incorporation efficiency of heavy arginine and lysine.

(vi) Determination of Incorporation Efficiency

Sample preparation: protein pellets were reconstituted in 1× NuPAGE LDS Sample Buffer (Invitrogen) and separated on 4-12 % NuPAGE Novex Bis-Tris Mini Gels (Invitrogen). The gels were stained with Coomassie Blue for visualization. Each band was cut and sliced into small pieces (app. 1 $mm^3$ cubes) which were destained and dehydated in 50µL acetonitrile/25mM $NH_4HCO_3$ (2:1) for 15 minutes. The cubes were rehydrated in 25mM $NH_4HCO_3$ for 10 to 20 minutes. The rehydration solution was discarded and the pieces dried in SpeedVac for 30 min. The pieces were further reduced by incubation at 60°C for 1h in 25µL solution of 10mM dithiothreitol (DTT) and 25mM $NH_4HCO_3$. DTT was discarded and the pieces alkylated in 25µL of 55mM 2-iodoacetamide in the dark at room temperature for 45min. The pieces were washed in 25mM $NH_4HCO_3$ and dried in a SpeedVac for 30 min. Finally, the pieces were digested with 70µL trypsin (sequencing grade, promega) in 25mM $NH_4HCO_3$ and incubated at 37°C overnight. The peptides were extracted by socking the mixture in 2% acetonitrile for 10 min and sonication for 10min. They were concentrated by a drying in SpeedVac and reconstituted in a solution of 2% acetonitrile and 0.1% formic acid for nanoLC-MS/MS analysis as previously described (Gillet et al., 2012).

NanoLC-MS/MS analysis: Mass spectrometry was performed on a hybrid quadrupole-orbitrap mass spectrometer (Q Exactive, Thermo Fisher Scientific, Bremen, Germany) equipped with a Flexion nanospray ionization source, operated under Excalibur 2.4 software and coupled to a nanoflow chromatography system (Easy nanoLC-II, Thermo Fisher Scientific).

Experimentally, the samples were enriched on a self-packed reversed phase-C18 precolumn (0.15 mm ID x 20 mm, Reprosil-Pur 120 C18-AQ 5 µm, Dr. Maisch, Ammerbuch-Entringen, Germany) and separated on an analytical reversed phase-C18 column (0.075 mm ID x 200 mm, Reprosil-Pur 120 C18-AQ, 3 µm, Dr. Maisch) using a 37 min linear gradient of 5-35 % acetonitrile/0.1% formic acid (v:v) at 300 nl/min. For data dependent acquisition (DDA) the following experimental cycle was used: one full MS scan across the 350-1600 *m/z* range was acquired at a resolution setting of 70,000 FWHM, an AGC target of 1*10e6 and a maximum fill time of 60 msec. Up to the 12 most abundant peptide precursors of charge states 2 to 5 above a 2*10e4 intensity threshold were then sequentially isolated at 2.0 FWHM isolation width, fragmented with nitrogen at a normalized collision energy setting of 25%, and the resulting product ion spectra recorded at a resolution setting of 17,500 FWHM, an AGC target of 2*10e5 and a maximum fill time of 60 ms. Selected precursor *m/z* values were then excluded for the following 15 s. Two technical replicates per sample were acquired.

Data processing: Raw data were processed using MaxQuant Software version 1.5.2.8 (Max Planck Institute for Biochemistry, Martinsried, Germany). Peak lists were searched against the *C. jejuni* subsp. *jejuni* strain 81-176 (serotype O:23/36 ) proteome (UniProt v08.2016, 1748 proteins) with common contaminants added. The search included carbamidomethlyation of cysteine as a fixed modification and methionine oxidation and *N*-terminal acetylation as variable modifications. The maximum allowed mass deviation was 6 ppm for MS peaks and 20 ppm for MS/MS peaks. The maximum number of missed cleavages was two. The false discovery rate was determined by searching a reverse database. The maximum false discovery rate was 1%. The minimum required peptide length was six residues. The peptide list was filtered for lysine and arginine containing peptides with a valid heavy/light ratio. For each peptide, the incorporation was calculated as 1 _ (1/(ratio H/L _ 1)). The maximum of a density distribution of all peptides represents the estimated incorporation level. All calculations and plots were done with the R software package.

### *3.3.2 SILAC analysis of proteomic response in 81-176 to DCA 0.05%*

This experiment was performed as described above (in section on establishment of SILAC for *C. jejuni*) with the following modifications:

(i) Labeling, media composition and culture conditions: Following incorporation results, 81-176 was cultured in *Campylobacter* defined broth supplemented with both labeled and unlabelled arginine and 1.20mM deoxycholic acid for 12h while shaking at 150rpm. Protein

samples were harvested, mixed in a 1:1 ratio (w/w) and processed for nanoLC-MS/MS analysis as described above.

(ii) Preparation of samples for nanoLC-MS/MS analysis: After alkylation, samples were digested with Arg-C-endopeptidase (V1881, sequencing grade, Promega) instead of trypsin.

(iii) Data analysis: Raw data were processed using MaxQuant Software version 1.5.2.8 (Max Planck Institute for Biochemistry, Martinsried, Germany). Peak lists were searched against a UniProtKB-derived *C. jejuni* strain 81-176 protein sequence database (v2016.07, 1748 protein entries) along with a set of common lab contaminants. The search included carbamidomethlyation of cysteine as a fixed modification; methionine oxidation and acetylation of protein *N*-terminal ArgC/P cleavage with a maximum of 2 missed cleavages as variable modifications. The maximum allowed mass deviation was 6 ppm for MS peaks and 20 ppm for MS/MS peaks. The false discovery rate was determined by searching a reverse database. The maximum false discovery rate for both peptides and proteins was 1%. Perseus Software version 1.5.0.15 (Max Planck Institute for Biochemistry, Martinsried, Germany) was used to obtain relative protein quantitation values from the MaxQuant Software results and perform statistical evaluation.

### *3.3.4 Label-free analysis of proteomic response in 81-176 to sublethal concentrations of different bile acid*

(i) Sample preparation: Strain 81-176 was cultured in *Campylobacter* defined broth for 12h while shaking at 150rpm supplemented with the following bile acid concentrations: CA 0.1%, DCA 0.05%, LCA 0.5%, TCA 0.5%, CDCA 0.05%, UDCA 0.5% and GCA 0.4%. At the same time, 81-176was cultured at 37$^{o}$C and 42$^{o}$C for 12h and 24h without bile acids. Protein samples were harvested and described in section (v) above. Proteins were purified by precipitation using a standard acetone precipitation protocol (acetone: sample 4:1, v/v, -20°C, overnight). Protein preparations were dissolved using sodium 3-[(2-methyl-2-undecyl-1, 3-dioxolan-4-yl) methoxy]-1-propanesulfonate (Rapigest, Waters) cleavable surfactant (Yu et al., 2003). After reduction and alkylation of cysteine residues with dithiothreitol and iodoacetamide, proteins were digested using sequencing grade porcine trypsin (Promega) at a 1:50 enzyme-to-substrate ratio (w:w). Following acidic cleavage of the surfactant, the resulting fatty acids were pelleted and removed by centrifugation. The resulting peptide mixtures were dried in a SpeedVac centrifuge and stored at -20 °C prior to analysis.

(ii) LC/MS/MS acquisition: Protein digests were analyzed on a nanoflow chromatography system (Eksigent nanoLC425) hyphenated to a hybrid triple quadrupole-time of flight mass spectrometer (TripleTOF 5600+) equipped with a Nanospray III ion source (Ionspray Voltage 2200 V, Interface Heater Temperature 150°C, Sheath Gas Setting 10) and controlled by Analyst TF 1.6 software build 6211 (all AB Sciex). In brief, peptides from each digest were dissolved in loading buffer (2% aqueous acetonitrile vs. 0.1% formic acid) to a concentration of 0.5µg/µl, desalted on a trap column (Dr. Maisch RP-C18aq, particle size 5 µm, 30 x 0.150 mm, 60 µL loading buffer) and separated by reversed phase-C18 nanoflow chromatography (Dr. Maisch RP-C18aq, particle size 3 µm, 250 x 0.075 mm, linear gradient 90 min 5%>35% acetonitrile vs. 0.1% formic acid, 300 nL/min, 50°C).

Qualitative LC-MS/MS analysis was performed using a Top25 data-dependent acquisition (DDA) method with an MS survey scan of *m/z* 380-1250 accumulated for 250 ms at a resolution of 35.000 FWHM. MS/MS scans of *m/z* 180-1750 were accumulated for 100 ms at a resolution of 17.500 FWHM and a precursor isolation width of 0.7 FWHM, resulting in a total cycle time of 3.4 s. Precursors above a threshold MS intensity of 200 cps with charge states 2+, 3+ and 4+ were selected for MS/MS, the dynamic exclusion time was set to 15 s. Two technical replicates of 1.5µg protein equivalent of each sample were acquired for qualitative analysis, for protein identification and generation of a spectral library for targeted data extraction.

For data-independent acquisition (DIA) SWATH analysis, MS/MS data were acquired for 100 precursor segments of variable size (5-40 *m/z* each), resulting in a precursor m/z range of 400-1250. Fragments were produced using Rolling Collision Energy Settings and fragments acquired over an *m/z* range of 380-1600 for an accumulation time of 40ms per segment. Including a 250 ms survey scan this resulted in an overall cycle time of 4.5 s. Three technical replicates of 2.0µg protein equivalent of each sample were acquired for quantitative analysis.

(iii) LC/MS/MS data processing: Protein identification was achieved using ProteinPilot Software version 5.0 build 4304 (AB Sciex) at "thorough" settings. A total of 551.443 MS/MS spectra from the combined qualitative analyses were searched against the *C. jejuni* strain 81-176 proteome from UniProtKB (revision 07-2016, 1804 protein entries) supplemented with 51 commonly observed lab and workflow contaminants. Global false discovery rates (FDR) were adjusted to 1% at both the protein and peptide level using a forward/reverse decoy database approach.

SWATH peak extraction was achieved in PeakView Software version 2.1 build 11041 (AB Sciex) using the SWATH quantitation microApp version 2.0 build 2003. Following retention time alignment on a set of 12 endogenous peptides, peak areas were extracted for up to the eight highest scoring peptides per protein group at 6 transitions per peptide, an extracting ion current (XIC) width of 75 ppm and an XIC window of 8 min, and filtered to an estimated FDR of 1% (Lambert *et al*., 2013). The resulting peak areas were then exported at the fragment, peptide and protein level for further statistical analysis with Perseus Software version 1.5.0.15 (Max Planck Institute for Biochemistry, Martinsried, Germany). The Empirical Bayes Analysis for Mixed Models in R package limma was used to determine proteins that were significantly upregulated and downregulated in 81-176 by each bile acid (Smyth, 2004). Proteins which showed a twofold log change higher than 1 and an FDR-adjusted p-value less that 0.05 were considered to be significantly expressed.

**Table 3:** Components of *Campylobacter* defined broth used in this study

**Solution 1**

| Compound | stock solution (mg/ml) | Pre-dilution | stock solution (500 ml) | Volume of stock solution for 1 L | Final Concentration (mM) |
|---|---|---|---|---|---|
| Aqua dest. | - | - | 495 ml | 100 mL | - |
| L - Aspartate | 5.0 | - | 2.5 g | | 3.76 |
| L - Glutamate | 13.0 | - | 6.5 g | | 8.83 |
| NaCl | 58.0 | - | 29.0 g | | 100mM |
| $K_2SO_4$ | 10.0 | - | 5.0 g | | 5.74 |
| $MgCl_2 . 6H_2O$ | 4.1 | - | 2.05 g | | 2.02 |
| $NH_4Cl$ | 2.2 | - | 1.1 g | | 4.11 |
| EDTA | 0.037 | 1.85 g in 100 ml water | 1 ml | | 0.013 |

Stored in 50ml Red Cups; EDTA promotes solubility and maintains metal bondages.

**Solution 2**

| Compound | stock solution (mg/ml) | Pre-dilution | stock solution (500 ml) | Volume of stock solution for 1 L | Final Concentration (mM) |
|---|---|---|---|---|---|
| Aqua dest. | - | - | 500 | 10 mL | - |
| L - Arginine hydrochloride | 15.0 | - | 7.5 g | | 0.71 |
| Serine | 5.0 | - | 2.5 g | | 0.48 |

Stored in 50ml Red Cups

**Solution 3**

| Compound | stock solution (mg/ml) | Pre-dilution | stock solution (500 ml) | Volume of stock solution for 1 L | Final Concentration (mM) |
|---|---|---|---|---|---|
| Aqua dest. | - | - | 500 | 10 mL | - |
| L - Leucine | 9.0 | - | 4.5 | | 0.69 |
| L - Isoleucine | 3.0 | - | 1.5 | | 0.23 |
| L - Valine | 6.0 | - | 3.0 | | 0.51 |

Stored in 50ml Red Cups

**Solution 4**

| Compound | stock solution (mg/ml) | Pre-dilution | stock solution (1000 ml) | Volume of stock solution for 1 L | Final Concentration (mM) |
|---|---|---|---|---|---|
| Aqua dest. | - | - | 1000 | 200 mL | - |
| $K_2HPO_4$ | 17.4 | - | 17.4 g | | 20.0 |
| $KH_2PO_4$ | 13.6 | - | 13.6 g | | 20.0 |

Stored in 1L Bottle

**Solution 5**

| Compound | stock solution (mg/ml) | Pre-dilution | stock solution (100 ml) | Volume of stock solution for 1 L | Final Concentration (mM) |
|---|---|---|---|---|---|
| Aqua dest. | - | - | 100 | 0.2 mL | - |
| NAD | 10.0 | - | 1g | | 0.003 |
| Thiamine hydrochloride | 10.0 | - | 1g | | 0.006 |
| Calcium pantothenate | 10.0 | - | 1g | | 0.004 |

Stored in Eppendorff Cups and 50ml Red Cup

**Amino acid mix**

| Compound | stock solution (mg/ml) | Pre-dilution | stock solution (500 ml) | Volume of stock solution for 1 L | Final Concentration (mM) |
|---|---|---|---|---|---|
| Aqua dest. | - | - | 500 ml | 10 mL | - |
| L-phenylalanine | 5.0 | - | 2.5g | | 0.30 |
| L - Alanine | 10.0 | - | 5.0g | | 1.12 |
| L – Histidine | 5.0 | - | 2.5g | | 0.32 |
| L – Threonine | 5.0 | - | 2.5g | | 0.42 |
| L – Lysine | 5.0 | - | 2.5g | | 0.30 |
| L – Glycine | 2.5 | - | 1.25g | | 0.33 |
| L - Trypthophan | 8.0 | - | 4.0g | | 0.39 |

Stored in 50 ml Red Cups

**Individual Components**

| Compound | stock solution (mg/ml) | stock solution | Storage vessel | Volume of stock solution for 1 L | Final Concentration (mM) |
|---|---|---|---|---|---|
| L - Cysteine hydrochloride* | 17,5 | 1,75 g in 100 mL | 50 mL Red Cup | 3,5 mL | 0,35 |
| L - Cystine* | 12,0 | 1,2 g in 100 mL | 250 ml Bottle | 3,0 mL | 0,15 |
| Oxaloacetate | 2,0 | 2,0 g in 1000 mL | 1 L Bottle | 100 mL | 1,52 |
| $NaHCO_3$ | 84,0 | 1,26 g in 15 mL | 15 ml Blue Cup | 0,5 mL | 0,5 |
| Biotin M=244,31 g/mol | Saturated solution | 500 mg in 5mL | 2 ml Eppendorf | 7,3 µL 0,73293 mg | 0,003 |
| Thiamine pyrophosphate hydrochloride | 4,6 | 0,46 g in 100 mL | 250 ml Bottle | 100 µL | 0,001 |
| L - Proline | 5,0 | 2,5 g 500 mL | 15 mL Blue Cups and 500 mL Bottle | 10 mL | 0,43 |
| L - Methionine | 14,9 | 1,49 g in 100 mL | 50 mL Red Cup | 1,0 mL | 0,1 |
| $CaCl_2$ . $1H_2O$ | 37,0 | 18,5 in 500 mL | 500 mL Bottle | 1,0 mL | 0,25 |
| $Fe(NO_3)_3$ . $9H_2O$ | 4,0 | 2g in 500 mL | 500 mL Bottle | 1,0 mL | 0,01 |

*was freshly prepared before usages

## 3.4 Characterization of *cjp47 (cjj81176_pVir0047)*

### *3.4.1 Bioinformatics analysis*

A search was carried out in UniProt to identify the name and other characteristics of protein Q8GJA8_CAMJJ. It was found to be *cjp47 (cjj81176_pVir0047)*. In addition, multiple sequence analysis was carried out to understand the genetic relationship of *cjp47 (cjj81176_pVir0047)* and other related genes.

### *3.4.2 Construction of mutant*

Construction of mutant was chronologically done as described below:

(i) Amplification of *cjp47 (cjj81176_pVir0047)*.

PCR primers containing an *Xba*I restriction site (underlined) Forward: (nnnnnn<u>tctaga</u>gggttttaaaagcttaaggtttgataaaccc); and Reverse: (nnnnnn<u>tctaga</u>ggcttatcttttagataggttgccccgtc) were used. Each 50 µl of PCR mixture contained 40 ng genomic DNA, 10 mM TRIS-HCl pH8.3, 50 mM KCl, 1.5 mM $MgCl_2$, all four dNTPs (each 0.2 mM) and 2.5 U Taq DNA polymerase. After initial incubation at 95C for 1 min, 35 cycles at 95°C for 1min, 54°C for 1 min and 72°C for 1 min were carried out with a final incubation at 72°C for 5 min. PCR products were analyzed on 1% agarose gels stained with midori green (Nippon Genetics Co. Ltd., Japan).

(ii) Restriction of *cjp47 (cjj81176_pVir0047)* PCR product and pBluescript vector (pBSK)

PCR product with the right band size was purified and its concentration measured with NanoDrop2000 spectrophotometer - pedestal mode (Thermo Scientific). Similarly, the concentration of pBSK vector was measured. Both purified PCR product and pBSK vector were digested with enzyme *Xba*I (#R0145L, New England BioLabs, NEB) as recommended by the manufacturer to generate  ends. Hence, the reaction mixture was prepared as follows: 2µl purified PCR product or pBSK vector, 1µl *Xba*I (NEB), 1µl Cutsmart buffer #B72045 (NEB) and 6µl $ddH_2O$. The mixture was incubated at 37°C for 2h. The restricted products were purified (Qiagen QIAquick PCR Purification Kit) and their concentration measured with NanoDrop2000. Restricted pBSK_*Xba*I vector was subsequently dephosphorylated using antartic phosphatase #M0289 as recommended by the manufacturer (NEB). In this study the reaction contained 2µl antarctic phosphatase reaction buffer (10x), 2µl antarctic phosphatase, and 16µl pBSK_*Xba*I. Both restricted and purified *cjp47 (cjj81176_pVir0047)_Xba*I PCR product and dephosphorylated pBSK_*Xba*I vector were stored at -20°C.

(iii) Ligation of digested *cjp47 (cjj81176_pVir0047)* PCR product with pBSK vector (constructing *cjp47 (cjj81176_pVir0047)*-pBSK vector)

Quick Ligation Protocol (NEB# M2200) was used to ligate *cjp47 (cjj81176_pVir0047)*_XbaI PCR product with pBSK_*Xba*I vector. Both products were appropriately diluted to concentrations recommended. The mixtures were incubated at room temperature for 20 minutes.

High Efficiency Transformation Protocol C2992 (NEB) was followed to transform *E. coli* cells with 5µl of the ligation mixture. The transformants were cultured overnight at 37°C in LB agar supplemented with 100µg/mL ampicillin. Each resulting colony was picked and subcultured overnight at 37°C in LB broth supplemented with 100µg/mL ampicillin for plasmid extraction. GenElute Plasmid Miniprep Kit (Sigma-Aldrich, Germany) was used to extract the plasmids in accordance with the manufacturer's instructions. Successful ligated CJJ81176_pVir0047_pBSK vectors were identified by digesting the extracted plasmids with *Xba1* and analyzing the results on 1% agarose gel electrophoresis.

(iv) Construction of *cjp47 (cjj81176_pVir0047)*-pBSK $Kan_r$ knockout vector

Primers: mazFinv1_Forward: (cttcattccattcatcaaatttcaaatc) and mazFinv2_Reverse: (gataataagagaaaaataacatttgaaagc) were used to construct CJJ81176_pVir0047-pBSK $kan_r$ knockout vector. 50µl reaction mix was prepared as follows: 25µl Master Mix, 1µl forward inverse primer, 1µl reverse inverse primer, 10µl plasmid and 13µl ddH2O. The inverse PCR was performed under initial denaturation 98°C 30sec, followed by 34 cycles of 98°C 10sec, 57°C 30sec and 72°C 2 min (T100 Thermal Cycler, Bio-Rad). Successful results were confirmed by gel electrophoresis. The inverse PCR product with the correct band size was gel-extracted and purified (Qiagen QIAquick PCR Purification Kit) and its concentration measured with NanoDrop2000. Quick Ligation Protocol M2200 (NEB) was used to ligate successful inverse PCR product with kanamycin cassette as recommended by the manufacturer.

High Efficiency Transformation Protocol C2992 (NEB) was followed to introduce 5µl of the ligation mixture into competent *E. coli* cells. The transformants were cultured overnight at 37°C in LB agar supplemented with 50µg/mL kanamycin. Each resulting colony was picked and subcultured overnight at 37°C in LB broth supplemented with 50µg/mL kanamycin for plasmid extraction. GenElute Plasmid Miniprep Kit (Sigma-Aldrich, Germany) was used to extract the plasmids in accordance with the manufacturer's instructions. Successful ligated

*cjp47 (cjj81176_pVir0047)_Xba*I_pBSK vectors were identified by digesting the extracted plasmids with *Xba*I and analyzing the results on a 1% agarose gel. Plasmids with expected right band size were sequenced in both directions with M13 primers.

(v) Transformation of of 81-176 with *cjp47 (cjj81176_pVir0047)*-pBSK $kan_r$

Prior to transformation, competent 81-176 WT cells were prepared as follows: 81-176 was cultured on Columbia Blood Agar (CBA) for 16h at 42°C under microaerophilic conditions. Cells were harvested using ice-cold 272 mM sucrose and 15% glycerol buffer and centrifuged at 5000g at 4°C for 10 min. The pellet was resuspended in 1ml ice-cold buffer and washed two more times by centrifuging at 5000g at 4°C for 10 min. The resulting pellet (competent cells) was resuspended in 400µl ice-cold buffer from where aliquots of 50µl were transferred into vials and stored at -80°C.

Transformation of 81-176 with *cjp47 (cjj81176_pVir0047)*_pBSK_$kan_r$ vector into was performed using electroporation as described elsewhere (Tareen *et al*., 2010). In a nut shell, 1µl of a 500ng/µl *cjp47 (cjj81176_pVir0047)*-pBSK $kan^r$ vector (diluted in $ddH_2O$ where required) was transferred and gently mixed with 50 µl competent 81-176 WT. The mixture was incubated in ice for 1 minute and transferred to ice-cold 0.2-cm electroporation cuvettes. Electroporation was performed at 2.5kV, 25µF and 200Ω using a BTX Electro Cell Manipulator, Model ECM 600, 120V (BTX, Germany). Immediately after the pulse, 100µl SOC medium was added into the cuvette and the bacteria suspension was transferred onto non-selective CBA and incubated overnight at 37°C under microaerophillic conditions. Then, cells were harvested in 300µl Mueller-Hinton and cultured at 42°C in blood agar supplemented with 50µg/mL kanamycin under microaerophilic conditions for 3 to 4 days. Resulting colonies were analyzed for homologous recombination (*cjp47 (cjj81176_pVir0047)*mutants, Δ).

(vi) Analysis of homologous recombination (Δ *cjp47 (cjj81176_pVir0047)*

PCR was performed using primers and conditions described in section (i) above to analyze successful homologous recombination. Prior to the reaction, DNA of colonies resulting from transformation experiment above were extracted using Qiagen QIAamp DNA Extraction Kit as recommended by the manufacturer. PCR products were analysed on a 1% agarose gel.

### *3.4.3 Comparison of invasion of Caco-2 cells by Δ cjp47 (cjj81176_pVir0047) and wild type*

Gentamicin Protection Assay were used to compare the ability of Δ *cjp47 (cjj81176_pVir0047)* and wild type to invade Caco-2 cells in DMEM medium that is supplemented with 0mM, 25mM, 50mM and 100mM CA, LCA, TCA and GCA as described in section 3.1. In addition, their growth in MHB was compared as described before (Davis and DiRita, 2008b).

### *3.5 ANOVA statistical analyses*

Analysis of varience (ANOVA) was widely used in this study. This analysis determines if there is a significant difference between means of the factors under consideration (Kim, 2014). Both one-way and two-way ANOVA were used in this study where appropriate. One-way ANOVA was used in cases where the statistical difference of one factor in different independent experimental groups was being investigated; for example, comparison of the growth of 81-176 in different types of broths. In this example, growth was the main factor under consideration. Hence, one-way ANOVA was used. On the other hand, two-way ANOVA was used in situations which involved two independent variables and a dependent variable. For example, it was applied in adherence and invasion assays. In these assays, type of bile acid and chosen concentrations were treated as independent variables while both adherence and invasion were treated as dependent variables. In addition, whenever statistical differences were found, a post hoc test was performed to determine the groups which were statistically different.

# 4.0 RESULTS

## 4.1 Stable isotope labeling of *C. jejuni* proteins

### *4.1.1 CDB is suitable for SILAC*

The first step towards establishing SILAC for analyzing the response of *C. jejuni* to sublethal concentrations of CA, DCA, LCA, TCA, CDCA, UDCA and GCA was to check the suitability of CDB. In this regard, the growth of *C. jejuni* 81-176 in CDB, LB broth, MH broth and BHI broth was compared at 12h, 16h and 20h. The results showed that the growth of 81-176 in CDB was similar to its growth in LB broth, MH broth and BHI broth (fig 1). This finding informed the decision to use CDB in SILAC experiments.

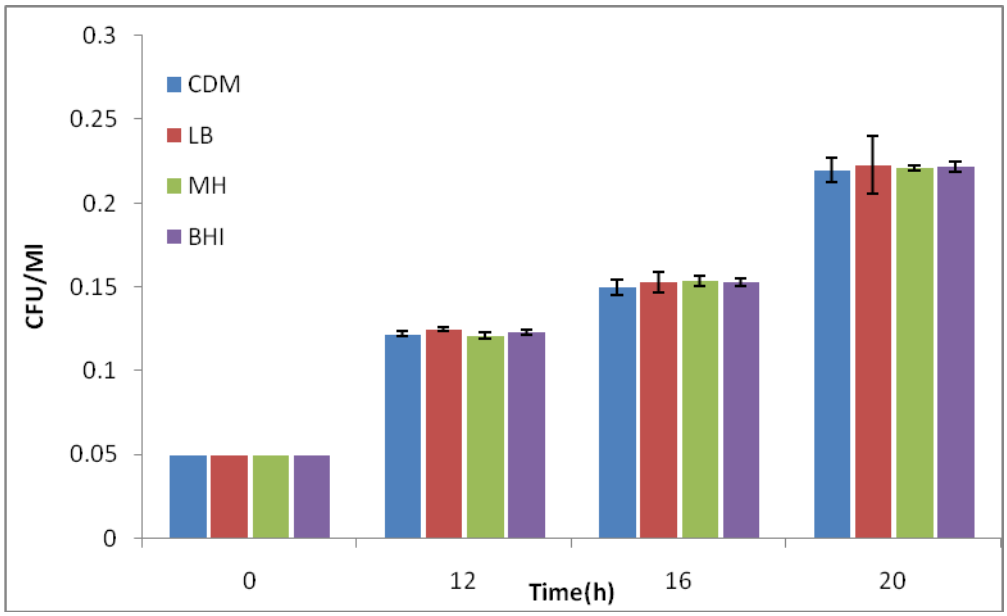



**Fig. 1. Comparison of growth of 81-176 in CDM, LB, MH and BHI at 12h, 16h and 20h**. One-way ANOVA revealed no significant differences in growth of 81-176 between these broths at each time point. $p<0.05$. This finding showed that the growth of 81-176 in CDM was comparable to LB, MH and BHI. The experiment was done in three biological replicates.

### *4.1.2 C. jejuni av4258 is an arginine auxotroph*

The next step was to identify a suitable strain for SILAC experiments. A suitable strain meant one that could strictly feed on heavy labelled $^{13}C^{15}N$-arginine and 4, 4, 5, 5 – $^{2}H$–lysine from CDB and efficiently incorporate them into its proteome (Zanivan et al., 2013). To identify this suitable strain, amino acid nutritional requirements analysis of 304 previously characterized *C. jejuni* strains were tested as described in Materials and Methods. This auxotyping analysis revealed that only 1 strain (av4258) in the collection strictly required either arginine or serine for growth; 17 strains strictly required methionine for growth and majority of the strains were

prototrophic (Table 4). These results are in agreement with those that were found in two previous studies (Tenover et al., 1985; Tenover and Patton, 1987).

**Table 4**: Auxotrophism in 304 *C. jejuni* strains

| **Nutrition requirement** | **Number** |
|---|---|
| Prototrophs | 285 |
| Methionine auxotrophs | 17 |
| Arginine auxotroph (av4258) | 1 |
| Serine auxotroph (av4258) | 1 |

### *4.1.3 Same percentage of heavy $^{13}C^{15}N$-arginine incorporation efficiency in auxotroph and prototroph strains*

Having identified av4258 to be an arginine auxotrophic *C. jejuni* isolate, the next step was to confirm the efficiency at which both heavy isotope labeled $^{13}C^{15}N$-arginine and 4, 4, 5, 5 – $^{2}H$–lysine in CDB were incorporated into its protein pool. Consequently, auxotroph av4258 and prototroph gal4116 (acting as a control that belongs to the same MLST ST) were cultured in CDB containing $^{13}C^{15}N$-arginine and 4, 4, 5, 5 – $^{2}H$–lysine for 6 passages of 32h each at 42$^{o}$C under microaerophilic conditions. The passage period of 32h was selected based on the findings of a previous *in vitro* study which showed that *C. jejuni* continues to actively grow up to 40h (Wright et al., 2009a). Therefore, it was reasoned that after 32h all essential *C. jejuni* proteins will have been synthesized. Hence an appropriate $^{13}C^{15}N$-arginine and 4, 4, 5, 5 – $^{2}H$–lysine incorporation efficiency percentage could be obtained. Protein samples were processed as described in Material and Methods and the mass spectrometry results revealed that both, av4258 and gal4116 strains achieved acceptable $^{13}C^{15}N$-arginine incorporation efficiency standards of >95% at the third passage (Table 5). On the other hand, 4, 4, 5, 5 – $^{2}H$–lysine incorporation efficiency in both strains did not achieve the required standard of >95% with the highest being 80% after passage 6 (Table 5). LIVE/DEAD BacLight Bacterial Viability staining (ThermoFisher Scientific, Germany) was performed on the samples to determine if the failure to achieve acceptable 4, 5, 5 – $^{2}H$–lysine incorporation efficiency was due to toxicity effects. The results which are displayed in Fig.2 show that $^{13}C^{15}N$-arginine and 4, 5, 5 – $^{2}H$–lysines do not affect the growth of both av4812 and gal4116. Hence, toxicity was not the reason responsible for poor 4, 5, 5 – $^{2}H$–lysine incorporation efficiency.

**Table 5**: Incorporation of labeled arginine and lysine in *C. jejuni* strains av4258 and gal4116

| | Strain av4258 (auxotroph) | | Strain gal4116 (prototroph) | |
|---|---|---|---|---|
| Generation | Arginine | Lysine | Arginine | Lysine |
| P1 | 86.9% | 63.0% | 75.3% | 57.1% |
| P2 | 98.1% | 76.5% | 94.6% | 74.4% |
| P3 | 99.3% | 80.9% | 98.3% | 79.5% |
| P4 | 98.6% | 76.3% | 98.9% | 81.1% |
| P5 | 98.6% | 77.2% | 99.2% | 80.3% |
| P6 | 99.3% | 79.9% | 99.4% | 80.0% |

T-test analysis showed a significant difference between $^{13}C^{15}N$-arginine incorporation efficiency in P1, P2 andd P3 of av4258 and gal4116 ($p<0.05$); there was no a significant difference between $^{13}C^{15}N$-arginine incorporation efficiency in P3, P4, P5 and P6 of each strain ($p>0.05$); finally, there was a significant difference between 4, 4, 5, 5 – $^{2}H$–lysine incorporation efficiency in P1, P2, P3, P4, P5 and P6 of each strain ($p<0.05$). (n = 3).

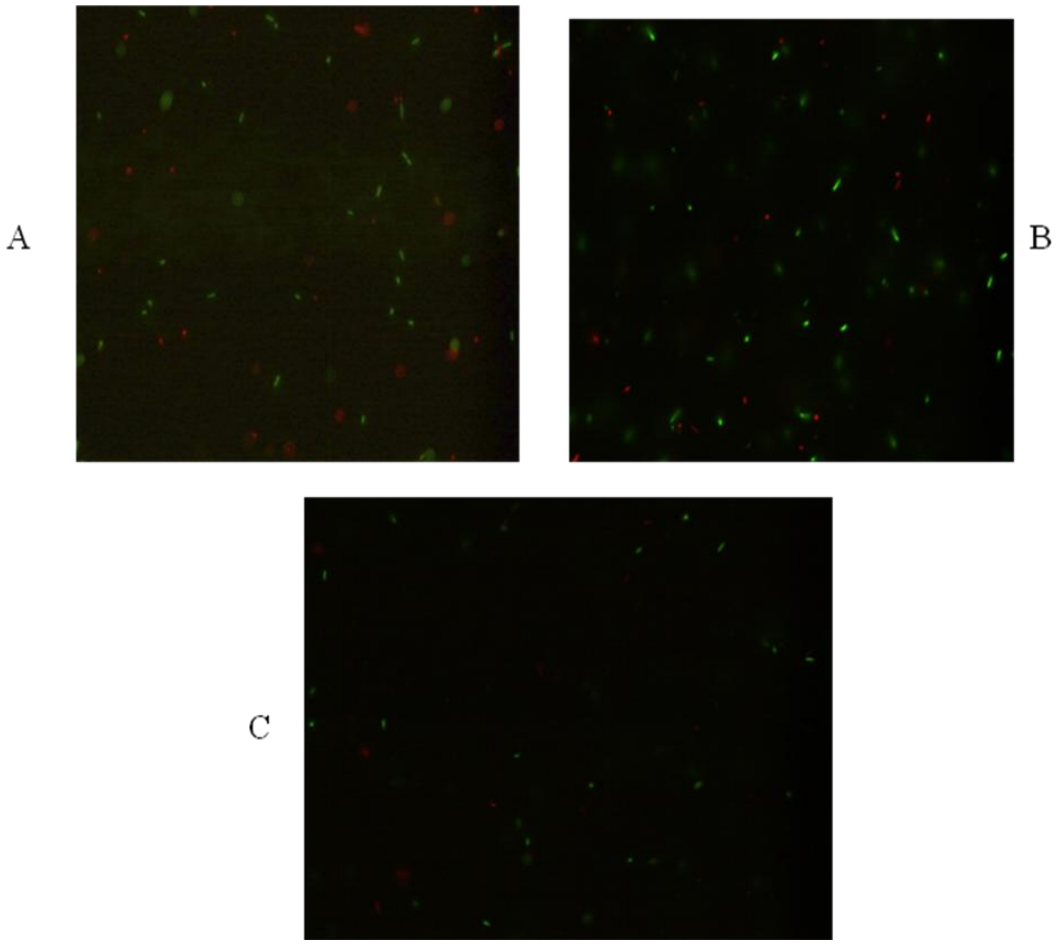



**Fig.2. Testing toxicity of $^{13}C^{15}N$-arginine and 4, 5, 5 – $^{2}H$–lysine on gal4116 and av4258.** LIVE/DEAD BacLight Bacterial Viability staining showing that $^{13}C^{15}N$-arginine and 4, 5, 5 – $^{2}H$–lysine do not affect the growth of both gal4116 (A) and av4258 (B) as compared to the control (C) that was cultured in normal amino acids. These pictures represent results observed from three independent experiments.

### *4.1.4 Heavy $^{13}C^{15}N$-arginine incorporation efficiency in other prototrophs*

$^{13}C^{15}N$-arginine incorporation efficiency in *C. jejuni* prototrophic strains B17, 81-176, 11168 and av518 was also determined. The decision for this analysis was based on the following three reasons. First, the experiment above showed similar incorporation efficiency of $^{13}C^{15}N$-arginine in av4258 (auxotroph) and gal4116 (prototroph). Second, biological and clinical information about av4258 was unavailable hence; it could be difficult to correctly interpret the proteomics results. Therefore, it was reasoned that well known strains B17, 81-176, 11168 or av518 should be used for SILAC experiment and subsequent investigations. However, this decision could be adopted if their $^{13}C^{15}N$-arginine incorporation efficiency was comparable to that found in av4258 and gal4116. Consequently, analysis of $^{13}C^{15}N$-arginine incorporation efficiency in B17, 81-176, 11168 and av518 was carried out as described in the section on Materials and Methods. Strains av4258 and gal4116 were employed as controls. Mass spectrometry results showed that all the strains achieved acceptable $^{13}C^{15}N$-arginine incorporation efficiency (>95%) in passage 3 (Table 6). These findings lead to the selection of *C. jejuni* 81-176 to be used in this study. In addition, proteomics data and other useful biological information on 81-176 were freely available hence the results of this study could be correctly interpreted.

**Table 6**: Comparison of $^{13}C^{15}N$-arginine incorporation efficiency in 5 prototrophic strains (n = 3)

| Passage/Strain | av4258 | B17 | 81-176 | 11168 | gal4116 | av518 |
|---|---|---|---|---|---|---|
| P1 | 86.8% | 83.4% | 89.1% | 84.4% | 82.8% | 87.4% |
| P2 | 95.2% | 96.2% | 97.8% | 95.6% | 96.4% | 95.8% |
| P3 | 99.0% | 98.7% | 99.4% | 98.8% | 98.6% | 98.6% |
| P4 | 99.7% | 99.5% | 99.7% | 99.5% | 99.2% | 98.0% |
| P5 | 99.9% | 99.6% | 99.8% | 99.5% | 99.3% | 98.4% |
| P6 | 99.5% | 99.7% | 99.4% | 99.7% | 99.3% | 98.7% |

T-test analysis showed a significant difference between $^{13}C^{15}N$-arginine incorporation efficiency in P1 and P2 of each strain ($p<0.05$); there was a significant difference among $^{13}C^{15}N$-arginine incorporation efficiency in P1 of each strain ($p<0.05$); there was a significant difference among $^{13}C^{15}N$-arginine incorporation efficiency in P2 of each strain ($p<0.05$); there was no a significant difference between $^{13}C^{15}N$-arginine incorporation efficiency in P3, P4, P5 and P6 of each strain ($p>0.05$); finally, there was no a significant difference among $^{13}C^{15}N$-arginine incorporation efficiency in P3, P4, P5 and P6 of each strain ($p>0.05$).

## 4.2 81-176 invasion into Caco-2 cells depends on the type of bile acid and its concentration

This experiment was performed to test the assumption that bile acids influence the ability of 81-176 to adhere and invade Caco-2 cells. The findings showed that CA, DCA, TCA, CDCA and GCA influenced 81-176 adherence and invasion of Caco-2 cells (Fig. 3a and b). Further, their influence increased with increase in concentration of these bile acids. At the individual level, CA, LCA and GCA had the greatest influence on adherence. On the other hand, DCA,

TCA, CDCA and GCA had the biggest influence on the invasion of Caco-2 cells. In both cases, the influnce was dose-dependent. However, the influnce of UDCA on the adherence and invasion of Caco-2 cells was not clear. These observations were essential in setting up experiments for evaluating the role of bile acids in promoting the pathogenesis of *C. jejuni*.

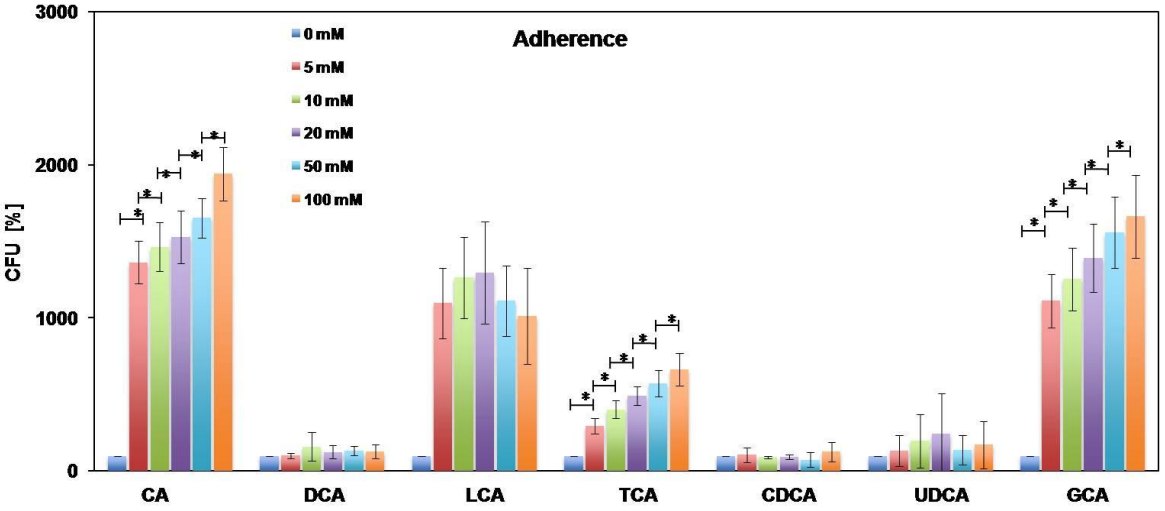



**Fig. 3a**.GPA showing the influence of different concentrations of CA, DCA, LCA, TCA, CDCA, UDCA and GCA on adherence of 81-176 on Caco-2 cells. CA, TCA and GCA had a significant influence on the capability of 81-176 to adhere on Caco-2 cells, $p<0.05$. The experiment was done in three independent replicates.

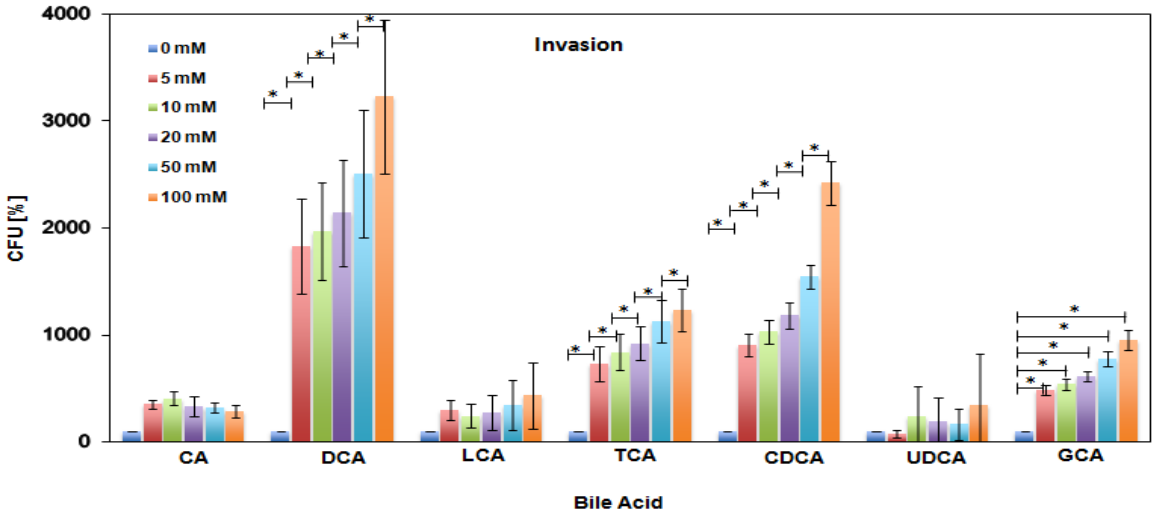



**Fig. 3b**. GPA showing the influence of different concentrations of CA, DCA, LCA, TCA, CDCA, UDCA and GCA on invasion of 81-176 on Caco-2 cells. DCA, TCA, CDCA and GCA had a significant influence on the capability of 81-176 to invade Caco-2 cells, $p<0.05$. The experiment was done in three independent replicates.

## 4.3 CA, DCA, LCA, TCA, CDCA, UDCA and GCA have different $IC_{50}$ values

### *4.3.1 CA, DCA, LCA, TCA, CDCA, UDCA and GCA have different $IC_{50}$ concentrations*

One of the broad objective of this study was to investigate the physiological response of 81-176 to low concentrations of bile acids. Hence, it was reasoned that a concentration of half of the IC50 of each bile acids was appropriate. To obtain the $IC_{50}$ of each bile acid, samples were collected after cultivation for 18h in CDB with different concentrations and $IC_{50}$ were determined as follows: Initially, minimum inhibition concentration of each bile acid was determined using the formula [(*AveCtr–AveB*)/*AveCtr*] × 100; In this formula: *AveCtrl* is the average OD600 readings of control in each test sample and *AveB* is the average OD600 readings of three culture samples per bile acid (da Silva Gomes et al., 2014; Wang et al., 2010). Subsequently, a linear regression analysis was done to establish a relationship between the MIC and concentration of each bile acid as described in probit analysis (Sakuma, 1998). Finally, an inhibition curve of colonies/ml (Y-axis) and concentration (mM) was drawn and the intercept of the two taken as the IC50 value of each bile acid shown in Table 7 (Sakuma, 1998; Soothill et al., 1992).

**Table 7**: IC50 values of bile acids used in this study

| Bile acid | MIC | $IC_{50}$ | Half $IC_{50}$ |
|---|---|---|---|
| **CA** | 0.4% | 0.2% | 0.1% |
| **DCA** | 0.2% | 0.1% | 0.05% |
| **LCA** | 2% | 1.00% | 0.5% |
| **TCA** | 0.2% | 0.1% | 0.5% |
| **CDCA** | 0.2% | 0.1% | 0.05% |
| **UDCA** | 2% | 0.1% | 0.5% |
| **GCA** | 1.4% | 0.74% | 0.4% |

The figures shown in this table are an average of three independent experiments and rounded off to one decimal place.
Mean value n = 3, $p<0.05$.

### *4.3.2 81-176 has different growth behaviour in sublethal concentrations of CA, DCA, LCA, TCA, CDCA, UDCA and GCA*

Growth of 81-176 in MHB that was supplemented with half $IC_{50}$ concentration of each bile acid was evaluated over a period of 48h. OD 600 readings of each bile acid were taken after every 4h. The results revealed an interesting growth behaviour of 81-176 in these bile acids (fig 4). Briefly, the growth curves of all bile acids displayed an element of well defined lag and exponential phases between 0h and 16h. The lines of the growth curves of UDCA and control shared a similar path between 0h and 16h. Similarly, the lines of growth curves of

LCA, control and UDCA shared a similar path between 0h and 8h. Interestingly, LCA displayed a similar growth pattern as UDCA. Both display a unique growth pattern between 16h and 32h and unexpected exponential growth after 36h. The lines of their growth curves travelled a distance apart from 8h but converged at 24h; and shared a similar path and growth trend between 36h to 48h.

On the other hand, between 0h and 16h, the lines of the growth curves of CA, DCA, TCA, CDCA and GCA shared a similar pattern like the control and UDCA. But the lines of the growth curves of CA, DCA, TCA, CDCA and GCA were a distant from those of both the control and UDCA. Interestingly, after 16h CA initiated a well defined stationary phase while DCA, TCA, CDCA and GCA initiated unique growth patterns. First, the growth curves of TCA and GCA portrayed a similar pattern from 16h and 48h with growth curve lines running parallel to each other and characterized by a short distance between them. A look at this pattern, reveals that: (i) they both displayed a unique growth pattern between 16h and 36h; (ii) short stationery phase between 36h and 40h and; (iii) unexpected exponential growth after 40h. Second, the growth curve of DCA displayed (i) a V-shaped growth pattern between 16h and 24h; (ii) a brief stationary phase between 24h and 40h and; (iii) an exponential growth from 40h to 48h. Third, the growth curve of CDCA displayed (i) a decline in growth between 16h and 20h; (ii) unexpected exponential growth between 20h and 36h; (iii) a brief stationary phase between 36h and 40h and (iv) a decline in growth from 40h to 48h.

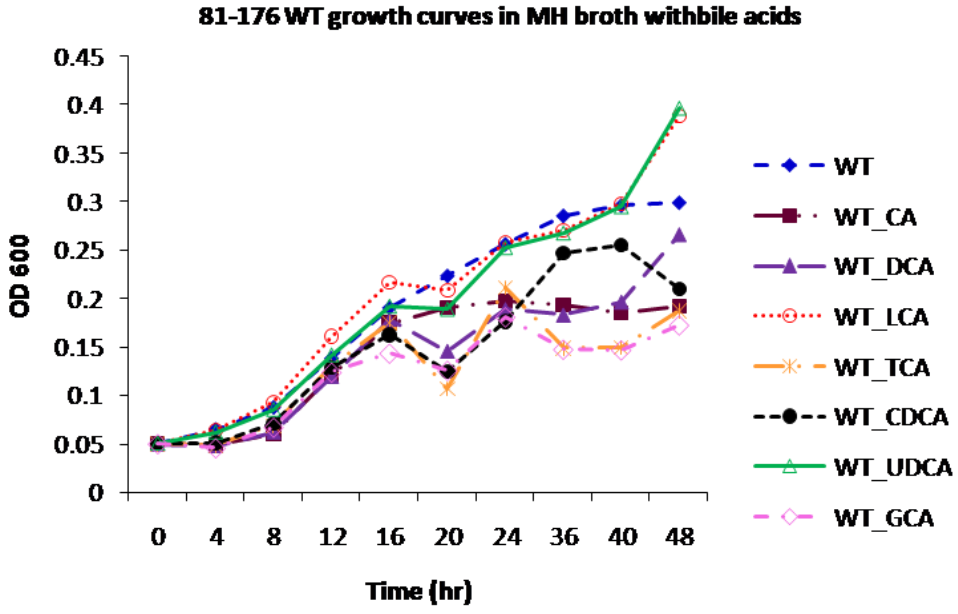



**Fig. 4**. A growth curve showing the comparison of the growth of 81-176 in MHB without bile acids (control_WT) and 81-176 in MHB supplemented with various bile acids at 37$^{o}$C for a period of 48h. OD measurements were done after every 4h. The graph shows the average results of three independent experiments. However, due to a small standard deviation between the independent experiments, no error bars are visible.

## 4.4 Quantification of 81-176 proteomic expression in response to DCA 0.05% using SILAC

In order to gain a deeper insight into proteomic response of 81-176 to low concentration of DCA at 37°C, the following factors were employed: (i) $^{13}C^{15}N$-arginine containing CDB was supplemented with DCA concentration of 0.05% (half $IC_{50}$) was used for the investigation, (ii) samples for protein analysis were collected at 12h (mid-exponential phase) and (iii) culture temperature of 37°C was used (fig 5). Consequently, *C. jejuni* 81-176 was cultured in 3ml CDB supplemented with DCA 0.05% for 12h at 37°C under microaerophilic conditions. To quantify the proteins, three replicates of heavy isotope labeled and unlabelled protein samples were independently purified and separated on SDS-PAGE (50µg per lane). The bands were sliced into small pieces and digested with ArgC. The resulting peptides were measured using Quadrupole-orbitrap mass spectrometry as described in Materials and Methods. The arising raw data were analyzed using MaxQuant 1.5.3.8 and UniProtKB CAMJJ 2016-09 and identified 857 proteins. Of these proteins, 500 proteins were accurately quantified (fig. 5; scatter plot).

A total of 128 proteins were significantly differentiated (Appendix 1). These proteins were categorized into the following biological functional groups: cell wall organization, chemotaxis, DNA transcription, DNA replication, metabolism, motility, pathogenesis, protein synthesis, stress response, transport, two-component regulatory system and uncharacterized (fig. 6). Examples of significantly upregulated proteins included: transcription termination factor Rho (Rho), aspartate aminotransferase (aspC), GTP cyclohydrolase-2 (ribA), dCTP deaminase (dcd), methionine aminopeptidase (map), succinate dehydrogenase, C subunit (sdhC), fibronectin-binding protein (cadF) and 60 kDa chaperonin (groL). Interestingly, common proteins which are known to promote invasion of epithelium cells were not significantly upregulated (Malik-Kale et al., 2008a). They include: *Campylobacter* invasion antigen B (ciaB), flagellar motor switch protein FliG (FliG), paralyzed flagella protein PflA (PflA), co-chaperone protein DnaJ (Dnaj), capsular polysaccharide ABC transporter and periplasmic polysaccharide-binding protein (kpsD). Lastly, other known DCA-induced proteins including: CmeABC efflux pump proteins, catalase A (katA) and flagellum protein FlaA (FlaA), Flagellar protein FlaG (flaG), Flagellar hook protein FlgE were significantly downregulated. These findings show that DCA 0.05% is not toxic to 81-176 and induces virulence associated proteins. Importantly, these results coupled with the observation in fig.3, insinuate that there are other yet to be known invasion proteins beyond the commonly known invasion proteins.

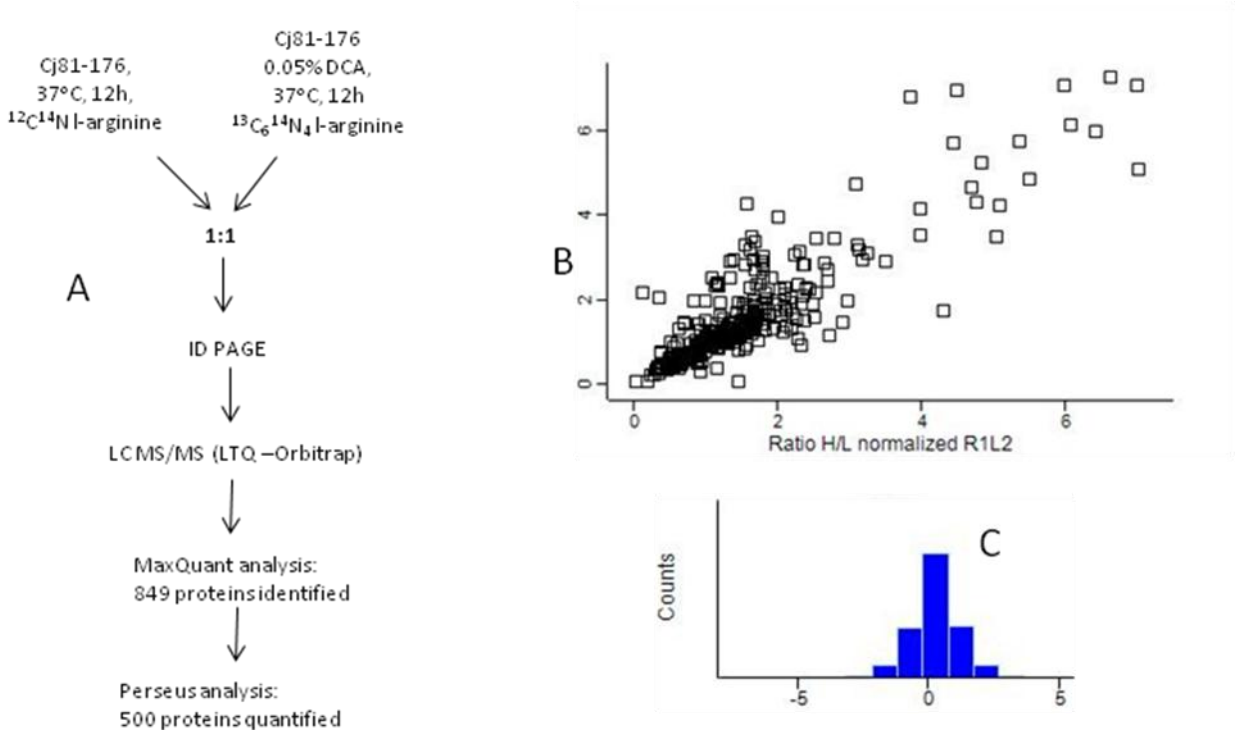



**Fig. 5**. **SILAC of 81-176 quantitative proteomic response to DCA 0.05%**. A: Shows the SILAC scheme that was developed. A total number of 500 proteins were quantified using persues (log2>1). B: Scatter plot showing the correlation of 81-176 protein expression between response to DCA 0.05% and control. C: Histogram showing the distribution of the measured proteins in experiment B was homogenously distributed.

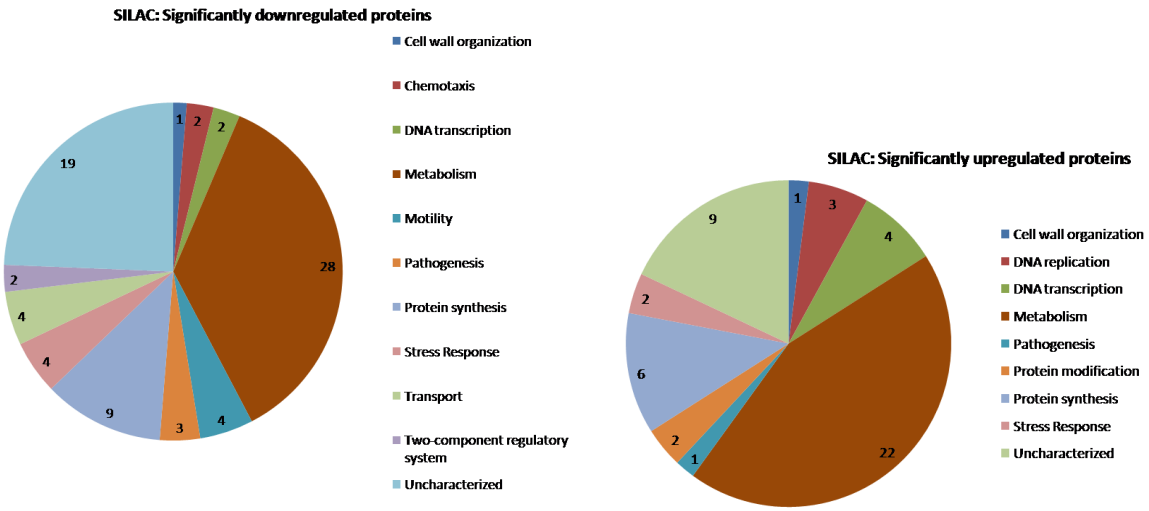



**Fig. 6**. **Functional categorization of SILAC quantified proteins**. These proteins were extracted from 81-176 which had been cultured in CDB supplemented with DCA 0.05% for 12h at 37°C. These proteins were quantified using persues where log2>1 was interpreted as significantly upregulated and log2<1 was interpreted as significantly downregulated.

## 4.5 Label-Free analysis with SWATH yields more quantifiable proteins than SILAC

### *4.5.1 Comparison of SILAC and Label-Free analysis with SWATH*

This investigation was performed to determine a suitable quantitative method between SILAC and label-free analysis with SWATH for this study. An initial comparison of proteomic response in 81-176 to DCA 0.05% using SILAC and label-free analysis with SWATH showed that the latter yielded more quantifiable proteins; SILAC yielded 500 proteins while label-free analysis with SWATH yielded 957 proteins. In addition, label-free analysis with SWATH had the following advantages over SILAC: it was financially cheaper, faster to get results (6 days), required less technical expertise and was easy to perform. Due to these advantages, label-free analysis with SWATH was mainly used to analyze proteomic response of 81-176 to CA 0.1%, DCA 0.05%, LCA 0.5%, TCA 0.5%, CDCA 0.05%, UDCA 0.5% and GCA 0.4%. Fig. 7 and Fig. 8 show how the analysis was performed. As a result, a SWATH-MS spectral reference library containing 1079 proteins (14644 peptides) at 1% FDR was generated by data dependant acquisition (DDA) analysis of all 13 samples by injecting nanoLC/MS/MS (fig.9).

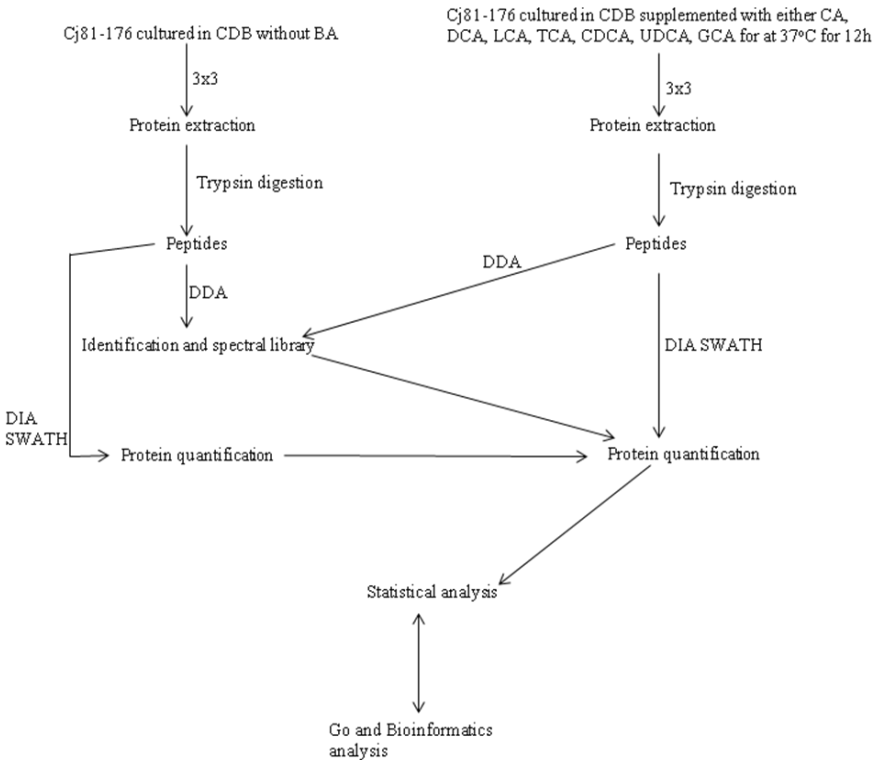



**Fig.7**. **Label-free analysis with SWATH analysis scheme used in the study**. A DDA library of 1079 proteins was developed and a total number of 957 were quantified and identified.

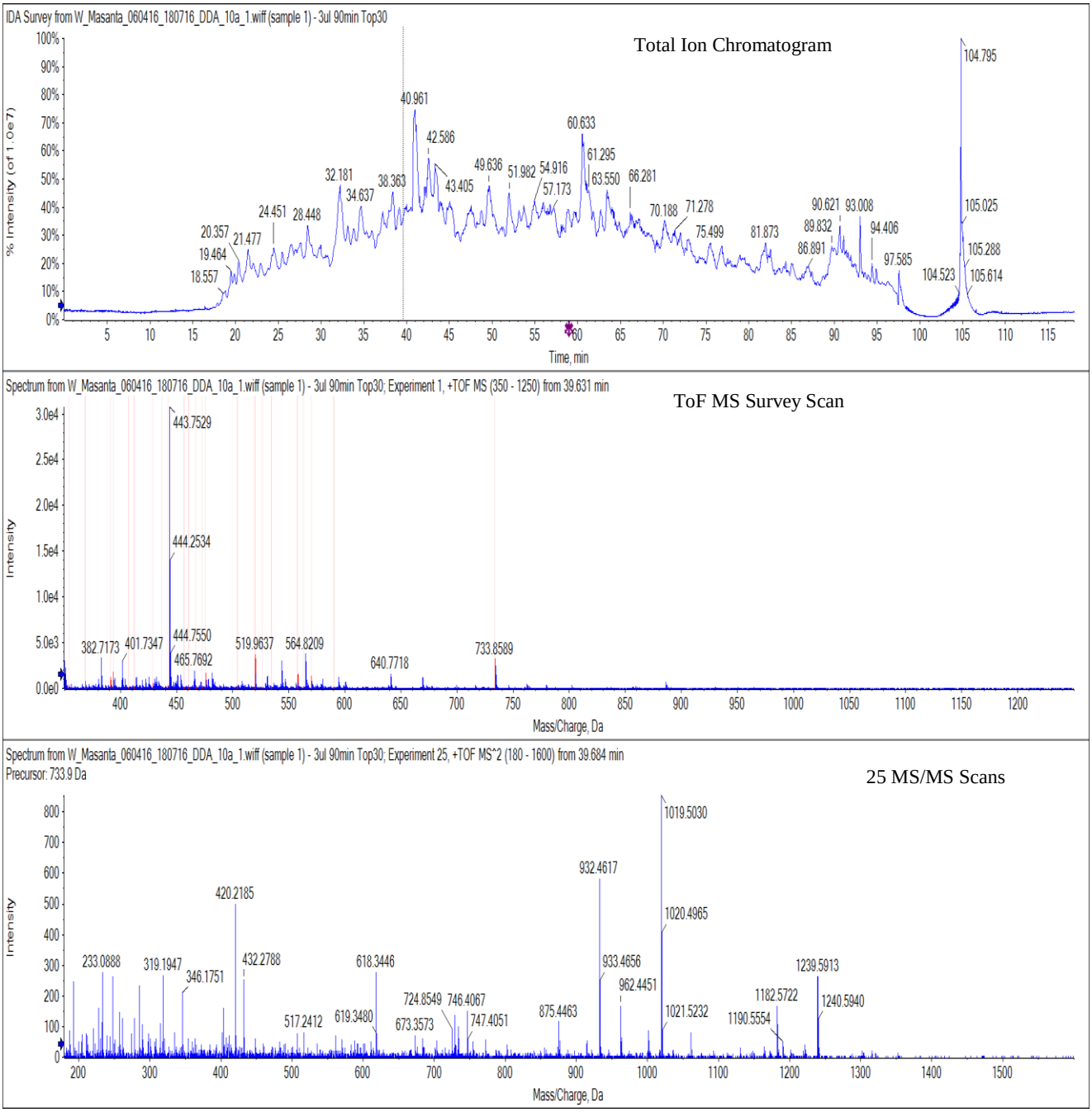
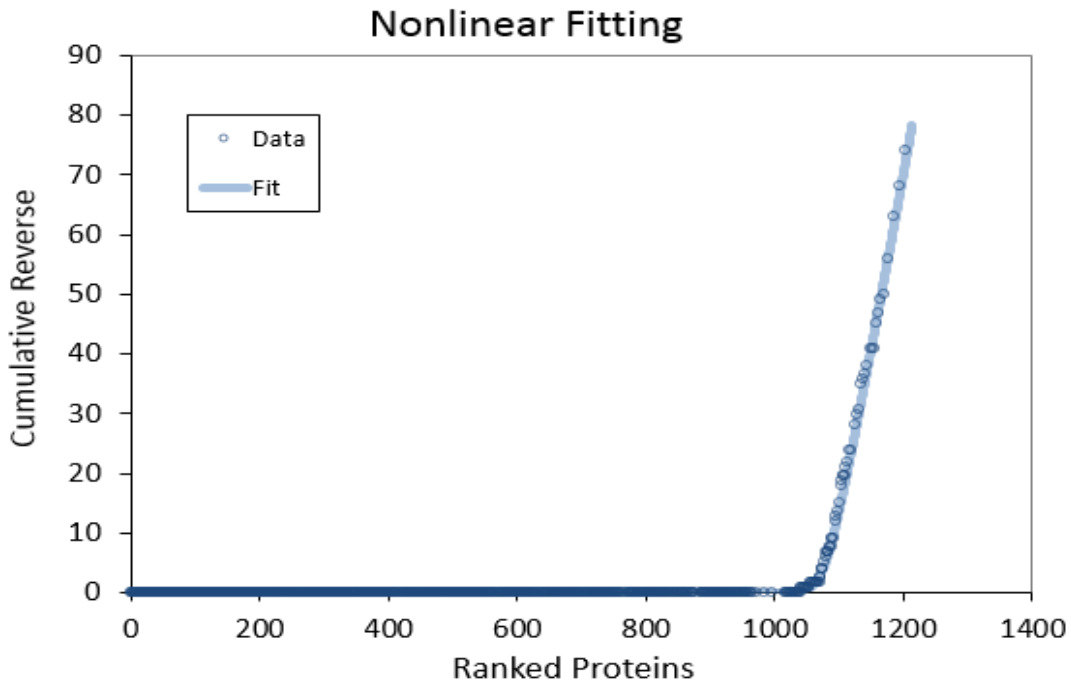



**Fig.8 Screenshots of DDA-nanoLC/MS/MS runs.** The runs were displayed by the instrument control software MassLynx (Waters Corporation). Experimental conditions were: nominal 1.5µg digest on TT5600 for 90 min gradient, 13 samples with 2 technical replicates resulting in 26 injections

**Fig 9**: Protein, Peptide and Spectral level False Discovery Rates Analysis results from Protein Pilot 5.0

Identification Yield at FDR Threshold

| Data Level | FDR Type | FDR | ID Yield |
|---|---|---|---|
| Protein | Local | 1% | 1021 |
| | | 5% | 1041 |
| | | 10% | 1049 |
| | Global | 1% | 1079 |
| | | 5% | 1127 |
| | | 10% | 1180 |
| Distinct peptide | Local | 1% | 11842 |
| | | 5% | 13579 |
| | | 10% | 14314 |
| | Global | 1% | 14644 |
| | | 5% | 16834 |
| | | 10% | 18445 |
| Spectral | Local | 1% | 298243 |
| | | 5% | 342637 |
| | | 10% | 363805 |
| | Global | 1% | 371071 |
| | | 5% | 424621 |
| | | 10% | 424621 |



### *4.5.2 Classification of proteomic response in 81-176 to sublethal concentrations of different bile acids SWATH and Principal Component Analysis (PCA)*

PCA was performed to analyse the correlation between proteins of each sample replica and correlation between proteins that were induced by different bile acids. Subsequent to DDA analysis, 6 protein replicates of each 81-176 sample which had been cultured in CDB supplemented in CA 0.1%, DCA 0.05%, LCA 0.5%, TCA 0.5%, CDCA 0.05%, UDCA 0.5%, GCA 0.4% and 0% (control) respectively were subjected to SWATH through Data Independent Acquisition (DIA) method. Peptides/proteins present in each sample were quantified by generating a spectral library from the DDA data using PeakView 2.2 software with the SWATH microApp 2.0 (SCIEX). These analyses lead to the reliable quantification of 957 proteins across all samples. MarkerView 1.2.1 software (SCIEX) was used to perform principal component analysis (PCA). The results displayed three distinguishable protein groups in the experimental samples: first group comprised DCA and CDCA proteins indicating that they are correlated; second group comprised CA, LCA, TCA and UDCA indicating correlation; and the third group comprised GCA proteins (fig.10). In addition, PCA showed that proteins of each sample replicates were closely positioned to each other indicating that the samples were reproducibly prepared. Hence, the mass spectrometry results were reliable and reproducible.

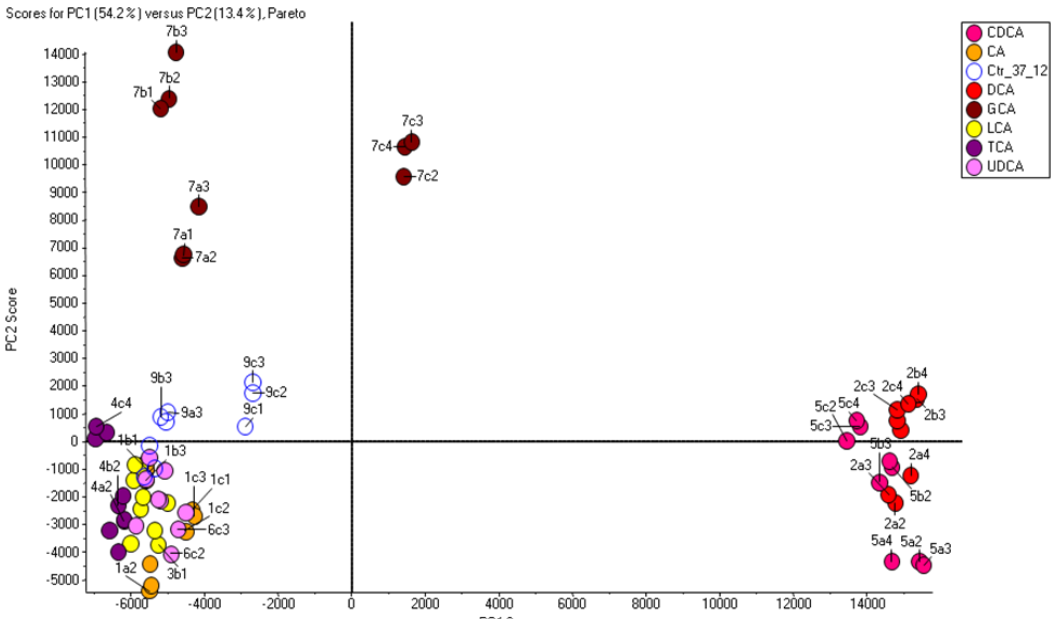



**Fig. 10**. **PCA analysis displaying the correlation between different protein biological replicates of *C. jejuni* 81-176 cultured in CBD supplemented with low concentrations of different bile acids for 12h at 37°C**. Numbers are the following bile acids: 1a,b,c are replicates of CA 0.1%, 2a,b,c are replicates of DCA 0.05%, 3a,b,c are replicates of LCA 0.5%, 4a,b,c are replicates TCA 0.5%, 5a,b,c are replicates of CDCA 0.05%, 6a,b,c are replicates of UDCA 0.05% and 7a,b,c are replicates of GCA 0.4%.

### *4.5.3 Plausibility check*

PCA having revealed that the samples were well prepared hence the data is reliable and reproducible. The next step was to check the plausibility of the expected results. A previous study showed that multidrug efflux transporter CmeABC plays an important role in bile resistance (Lin et al., 2002, 2003). Consequently, CmeABC was selected to check the plausibility of the proteomic response of 81-176 to CA, DCA, LCA, TCA, CDAC, UDCA and GCA. Using Markerview, the following observations were made in relation to the control: first, LCA, and UDCA had almost same activation signal of CmeA, CmeB and CmeC to that of the control. Second, CA, TCA and GCA activated CmeA, CmeB and CmeC with an almost equal signal and higher than the control. Lastly, DCA and CDCA activated CmeA, CmeB and CmeC with the highest signal. The findings are shown in figure 11 below. This plausibility check implied that each protein in 81-176 responded appropriately to each bile acid used in this study. Hence the results of the rest of the genes were reliable.

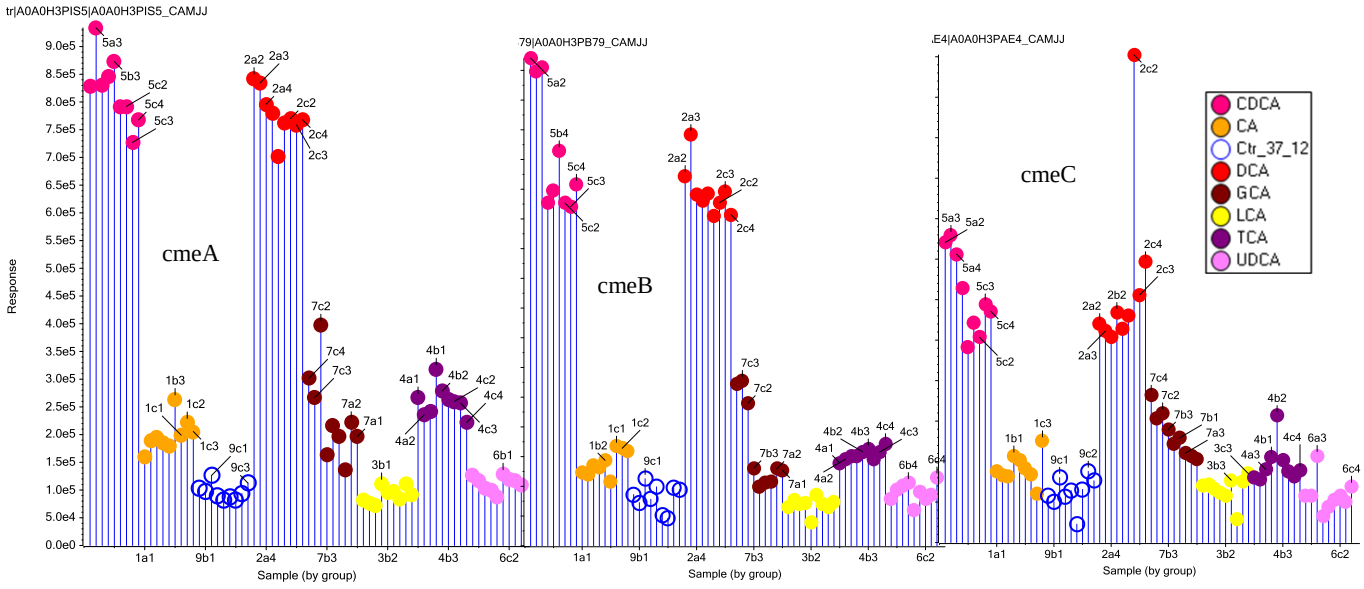



**Fig 11. 81-176 proteomic response plausibility check using CmeABC proteins**. LCA and UDCA produced similar activation signal to the control. CA, TCA and GCA produced an almost equal activation signal but higher than the control. DCA and CDCA produced the highest activation signal.

### *4.5.4 Biological processes in 81-176 influenced by sublethal concentration of each bile acid*

DAVID GO analysis revealed that the 957 quantified proteins belonged to the following nineteen biological processes: cell cycle, cell division and septation (8 proteins), cell morphogenesis (1 protein), cell wall organization (4 proteins), chaperone (11 proteins), chemotaxis (14 proteins), DNA modification (2 proteins), DNA replication (19 proteins), DNA transcription (11 proteins), metabolism (327 proteins), motility (17 proteins), pathogenesis (31 proteins), peptidoglycan biosynthesis (5 proteins), protein modification (3 proteins), protein synthesis (103 proteins), Protein synthesis regulation (1 protein), Ribosome biogenesis (3 proteins), stress response (49 proteins), transport (49 proteins), two-component regulatory system (8 proteins) and uncharacterized (291 proteins). Importantly, 700 of the 957 proteins were significantly differentiated (459 known proteins and 241 uncharacterized proteins). But as shown in fig.12A and fig.12B, the significantly differentiated proteins belonged to fifteen biological processes in 81-176. These include: cell cycle and cell division, protein folding (chaperones), chemotaxis, DNA replication, DNA transcription, metabolism, motility, cell wall organization, protein modification, protein synthesis, pathogenesis, stress response, transport and two-component regulatory system. Interestingly, proteins which belonged to metabolism, protein synthesis and transport were the highest and were significantly regulated by DCA, CDCA, TCA and GCA. For example, DCA significantly upregulated 57 proteins in metabolism, followed by CDCA (39 proteins) and GCA (27 proteins). Similarly, GCA significantly upregulated 17 proteins in protein synthesis, followed by DCA (13 proteins) and TCA and CDCA (5 proteins each). In transport, GCA had the highest number of upregulated proteins (8) and CA, DCA, TCA and CDCA upregulated similar number of proteins. On the other hand, LCA and UDCA had the least number of proteins that were differentiated in the these biological processes. DCA, TCA, CDCA and GCA produced a similar trend of dominancy among the 241 uncharacterized proteins (134 were significantly upregulated and 107 were significantly down regulated). In this group of proteins, GCA scored the highest number of upregulated proteins and TCA scored the highest number of downregulated proteins.

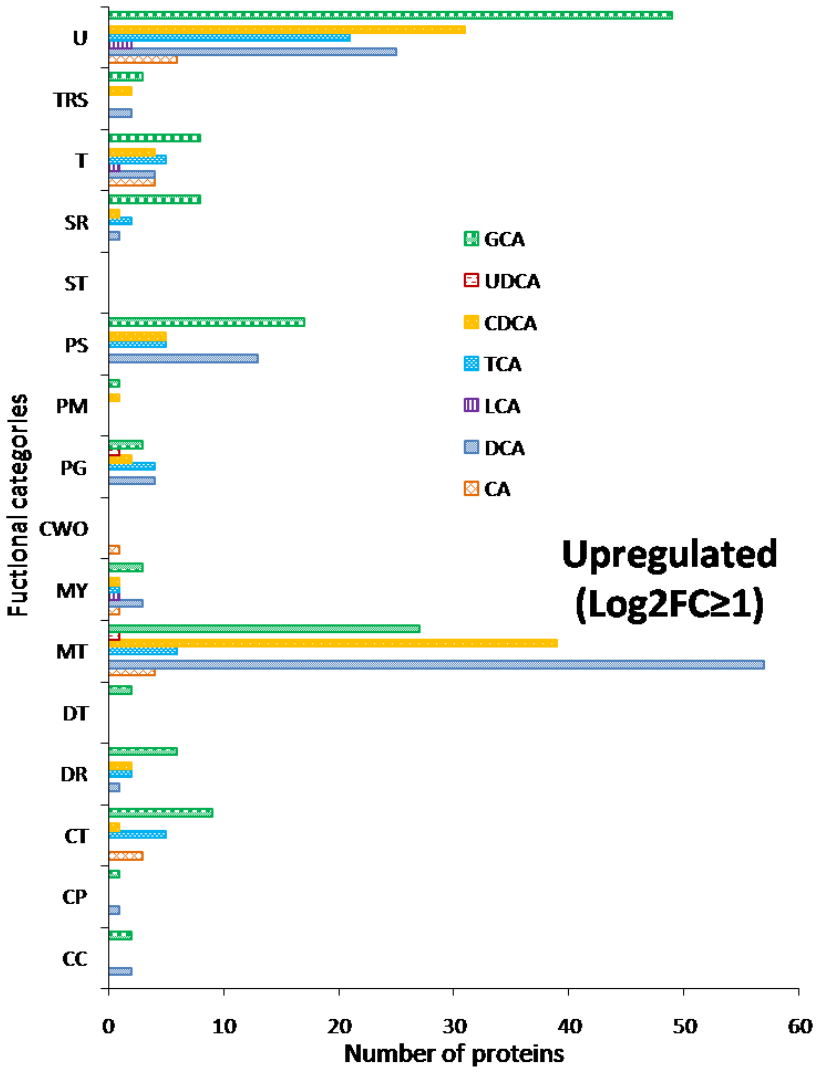



**Fig 12A**. **SWATH:** Number of significantly upregulated proteins (Log2FC≥1). The functional categories identified include: cell cycle and cell division (CC), chaperone (C), Chemotaxis (CT), DNA replication (DR), DNA transcription (DT), metabolism (MT), motility (MY), cell wall organization (CWO), pathogenesis (PG), protein modification (PM), protein synthesis (PS), signal transduction (ST), stress response (SR), transport (T), two-component regulatory system (TRS) and uncharacterized (U). Majority of the significantly upregulated proteins belonged to metabolism, protein synthesis and uncharacterized functional categories. DCA, TCA, CDCA and GCA had the greatest influence on each functional group while LCA and UDCA had the least influence.

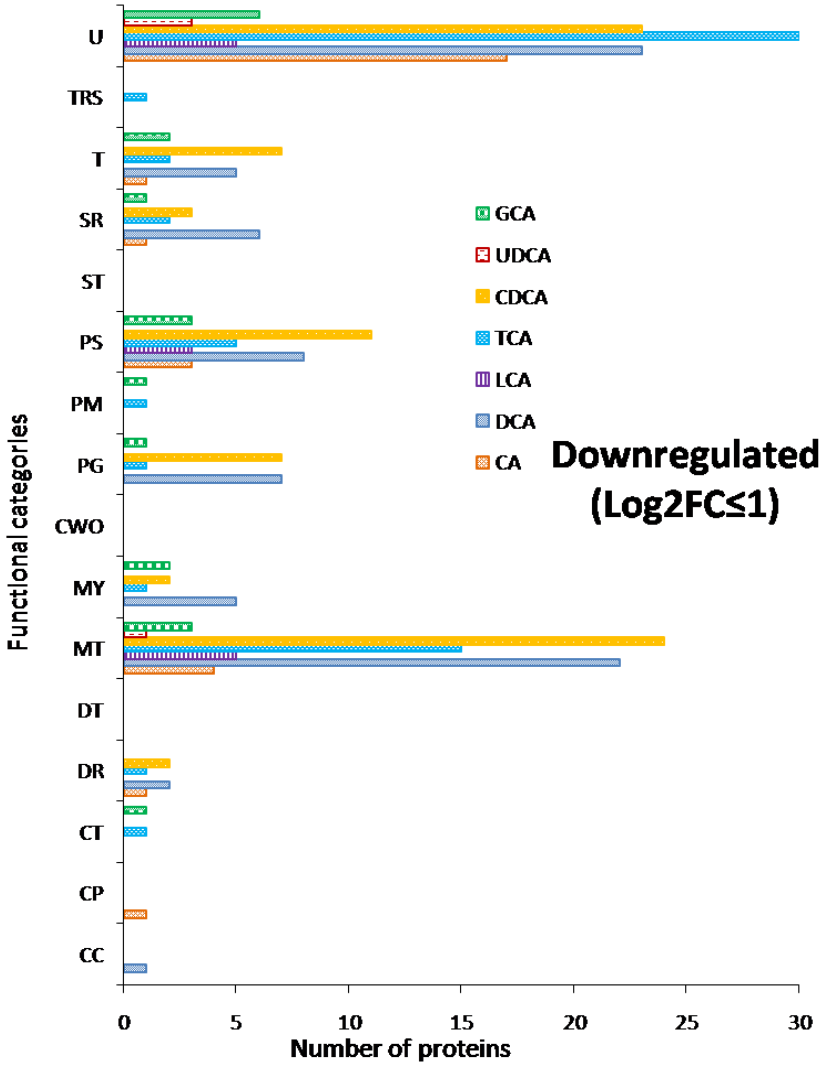



**Fig 12B**. **SWATH:** Number of sgnificantly downregulated proteins (Log2FC≤1). The functional categories identified include: cell cycle and cell division (CC), chaperone (C), Chemotaxis (CT), DNA replication (DR), DNA transcription (DT), metabolism (MT), motility (MY), cell wall organization (CWO), pathogenesis (PG), protein modification (PM), protein synthesis (PS), signal transduction (ST), stress response (SR), transport (T), two-component regulatory system (TRS) and uncharacterized (U). Majority of the significantly upregulated proteins belonged to metabolism, protein synthesis and uncharacterized functional categories. DCA, LCA, TCA, CDCA and GCA had the greatest influence on each functional group while LCA and UDCA had the least influence.

### *4.5.5 Significantly differentiated proteins in 81-176 in response to sublethal concentration bile acids*

Empirical Bayes Analysis for Mixed Models in R package limma was used to determine proteins that were significantly upregulated and downregulated in 81-176 by each bile acid (Smyth, 2004). In mathematical terms, the problem was to evaluate the influence of each bile acid on each of the 957 proteins that had been recovered from 3 biological and 3 technical replicate protein test samples of 81-176. These samples were collected from cultures of seven different bile acids that had been grown for 12h at 37$^{o}$C (biological samples, N = 24 and technical replicates, n = 72). For calculation purposes, the control was assigned a working number 9 while CA, DCA, LCA, TCA, CDCA, UDCA and GCA were assigned 1, 2, 3, 4, 5, 6 and 7 respectively. Before the analysis, each protein was substituted by its gene using Uniprot. Consequently, mixed model analysis was performed in two stages: in the first stage, regression coefficient of the influence of each bile acid on the expression of each gene was determined independently; and in the second stage, the regression coefficients of each bile acid were compared in a single equation to create a relationship on influence of expression on genes between the bile acids. Finally, moderated t-statistics was used to measure protein expression between the bile acids. Proteins which showed a log2 fold change higher than 1 and an FDR-adjusted p-value less that 0.05 were considered to be significantly expressed (Table 8; Appendix 2).

**Table 8**: Number of significantly differentiated proteins in 81-176 by each bile acid

| Bile acid | **Number of significantly expressed proteins** | | |
|---|---|---|---|
| | Significantly upregulatated (log2 Fold Change ≥1) | Significantly downregulated (log2 Fold Change ≤1) | Total |
| 0.1% CA | 19 | 28 | 47 |
| 0.05% DCA | 113 | 79 | 192 |
| 0.5% LCA | 4 | 13 | 17 |
| 0.5% TCA | 51 | 60 | 111 |
| 0.05% CDCA | 89 | 80 | 169 |
| 0.05% UDCA | 2 | 4 | 6 |
| 0.35% GCA | 139 | 20 | 159 |

VennPainter program was used to generate a spherical 7-Venn diagram (Lin et al., 2016). This diagram was useful in distinguishing proteins that were significantly induced by each individual bile acid and not the others (fig. 13 and Table 9). This analysis generated interesting results: in overall, GCA had the highest number of significantly upregulated proteins (77) that were not significantly upregulated by other bile acids. It was distantly followed by DCA (35), CDCA (24), TCA (14) and CA (4) respectively. LCA and UDCA did

not significantly upregulate proteins that other bile acids didn't. On the other hand, TCA had the highest number of downregulated proteins (39) that are not downregulated by other bile acids. This was followed by CDCA (19), DCA (17), GCA (4), CA (4), LCA (1) and UDCA (1).

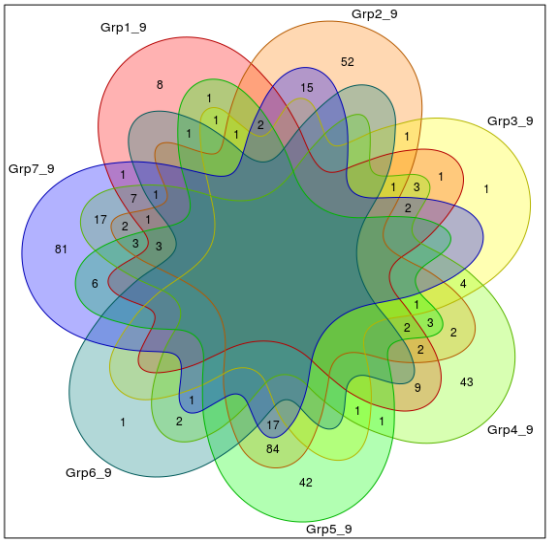



**Fig. 13. A spherical 7-Venn diagram showing significantly expressed protein in 81-176 cultured in CDB which was supplemented with low concentrations of 7 different bile acids**. 1_CA significantly expressed 8 proteins, 2_DCA significantly expressed 52 proteins, 3_LCA significantly expressed 1 protein, 4_TCA significantly expressed 43 proteins, 5_CDCA significantly expressed 42 proteins, 6_UDCA significantly expressed 1 protein and 7_GCA significantly expressed 81 proteins. See table A for details on number of upregulated and downregulated by each bile acid.

**Table 9**: Number of significantly expressed unique proteins by each bile acid

| | **Significantly upregulated** (log2 Fold Change ≥1) | **Significantly downregulated** (log2 Fold Change ≤1) | **Tota1** |
|---|---|---|---|
| 0.1% CA | 4 | 4 | 8 |
| 0.05% DCA | 35 | 17 | 52 |
| 0.5% LCA | 0 | 1 | 1 |
| 0.5% TCA | 14 | 39 | 53 |
| 0.05% CDCA | 24 | 19 | 43 |
| 0.05% UDCA | 0 | 1 | 1 |
| 0.35% GCA | 77 | 4 | 81 |

These proteins were further classified into biological process which they participate (fig 14A and fig 14B). Interestingly, majority of the upregulated proteins belonged to metabolism, protein synthesis, transport, stress response, chemotaxis and DNA replication in descending order. Only GCA upregulated a two-component regulatory system that was not expressed by other bile acids. Equally, GCA and TCA upregulated known pathogenesis factors that were not expressed by other proteins; GCA over expressed CJJ81176_pVir0047 (sialic synthesis)

and TCA over expressed TatA (protein transport). GCA significantly expressed 32 uncharacterized proteins, UDCA (10), TCA (6), and CA (4). It is worthwhile to note that UDCA and LCA had not significantly expressed proteins which were not significantly expressed by other bile acids.

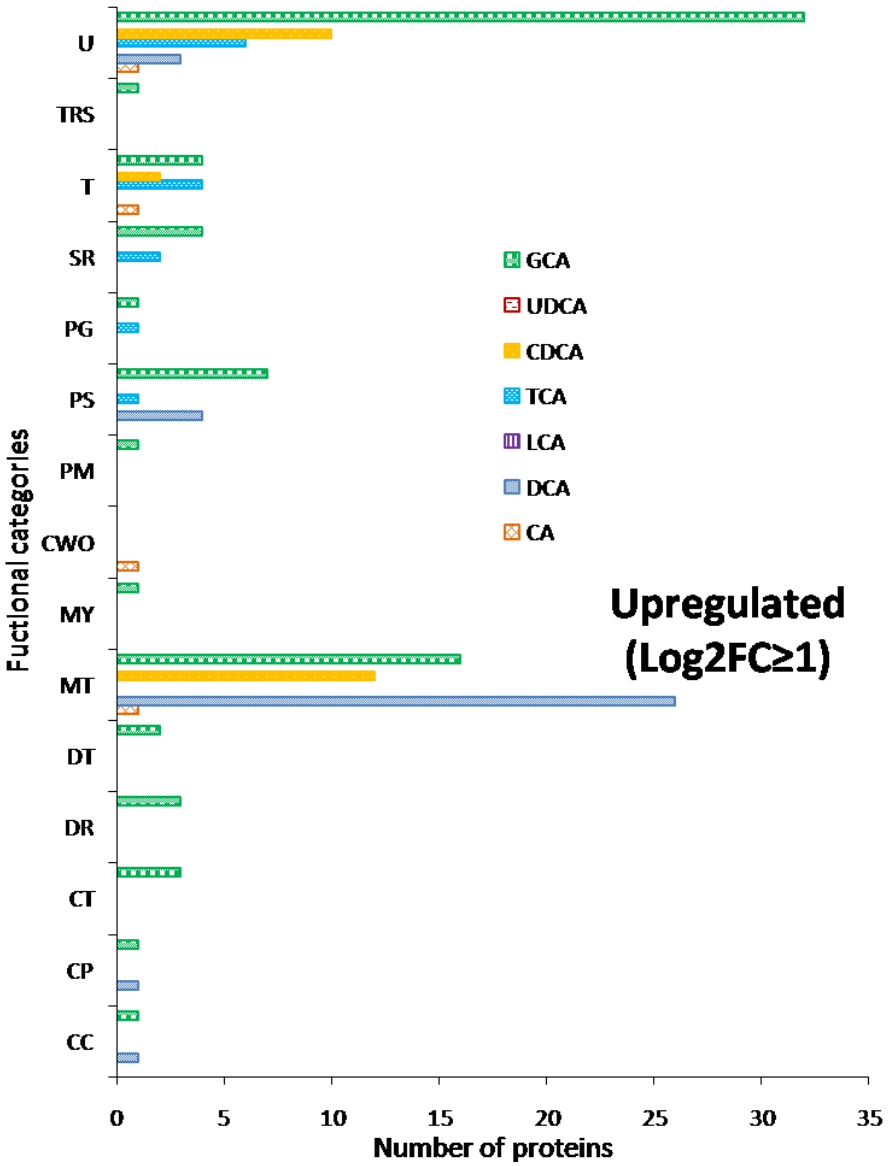



**Fig 14A**. **SWATH:** Significantly upregulated unshared proteins by each bile acid (Log2FC≥1). The functional categories identified include: cell cycle and cell division (CC), chaperone (C), Chemotaxis (CT), DNA replication (DR), DNA transcription (DT), metabolism (MT), motility (MY), cell wall organization (CWO), pathogenesis (PG), protein modification (PM), protein synthesis (PS), signal transduction (ST), stress response (SR), transport (T), two-component regulatory system (TRS) and uncharacterized (U). The figure shows that TCA and GCA induced the highest number of unique proteins.

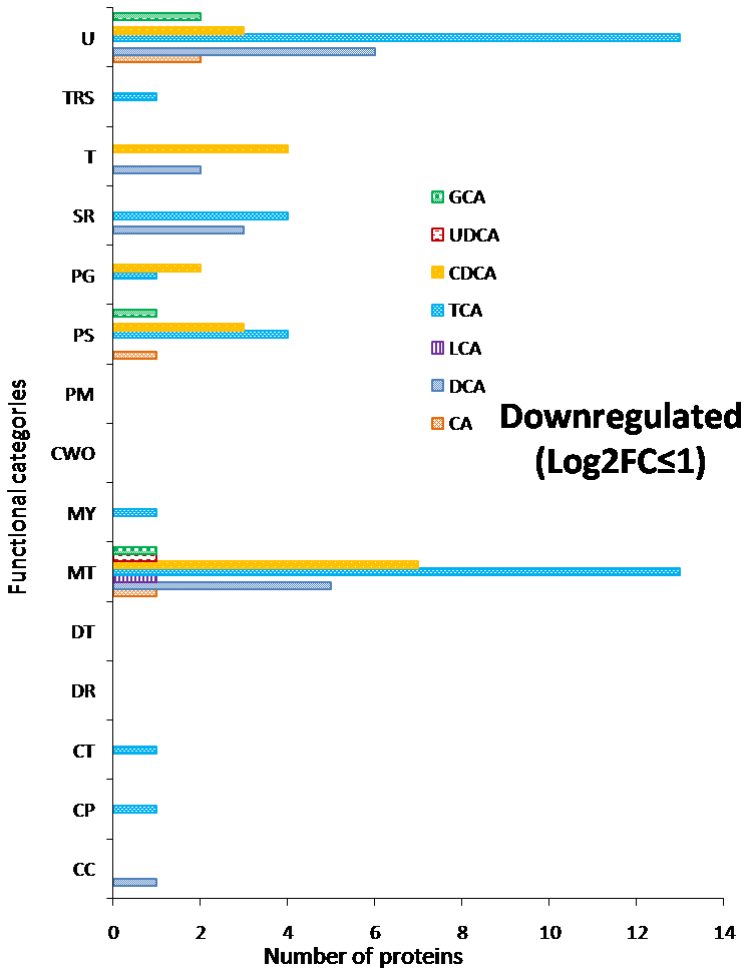
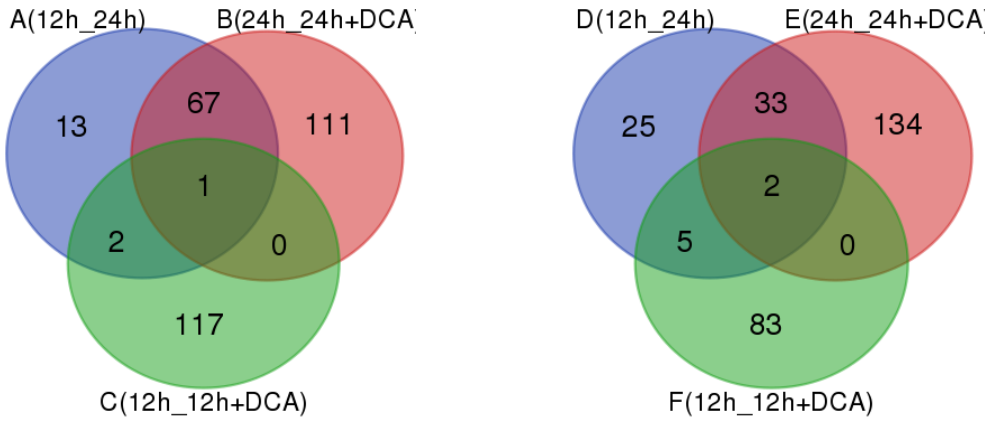



**Fig 14B**. **SWATH:** Significantly downregulated unshared proteins by each bile acid (Log2FC≥1). The functional categories identified include: cell cycle and cell division (CC), chaperone (C), Chemotaxis (CT), DNA replication (DR), DNA transcription (DT), metabolism (MT), motility (MY), cell wall organization (CWO), pathogenesis (PG), protein modification (PM), protein synthesis (PS), signal transduction (ST), stress response (SR), transport (T), two-component regulatory system (TRS) and uncharacterized (U). Majority of the unique and significantly induced proteins belonged to metabolism, protein synthesis, pathogenesis, signal response, transport and uncharacterized functional categories. However, there was no bile acid that downregulated unique proteins in the following categories: protein modification, cell wall organization, DNA transcription and DNA replication.

### *4.5.6 Comparison of differentially expressed proteins in 81-176 at 12h and 24h cultured in CDB supplemented with DCA 0.05% at 37°C*

Fig 4. demonstrated diverse growth patterns of 81-176 in bile acids under examination in this study. DCA was selected to provide an insight into this phenomenon. Consequently, the proteomic expression at 12h and 24h in 81-176 cultured in CDB supplemented with 0.05% at 37°C was evaluated. The results of significantly differentiated proteins at 24h indicated an active 81-176 but fighting various types of stresses (Appendix 3). Key examples to illustrate this observation include: (i) significantly upregulated BamA, YidC and PorA were (ii) significantly upregulated stress response factors: FtsH, PbpA, KatA, DnaJ, ClpX, GroL, and Cj81176_0717 (iii) CmeABC multidrug efflux system was significantly upregulated (vi) significantly upregulated: AtpA, AtpC, AtpD, AtpF, AtpG and AtpH (vi) significantly upregulated UbiE, UbiX and IlvC. (v) significantly upregulated SecD, SecF and SecG. Finally, PseC and PseI were significantly upregulated at 12h and PseD,E,F,J were significantly upregulated at 24h. In addition, significantly upregulated 34 uncharacterized proteins. As shown is figure 15 below, at 24h, 81-176 had 111 significantly upregulated distinct proteins and 134 significantly downregulated distinct proteins. These group of proteins could be responsible for V shaped growth pattern that was observed in figure 4.




` **Fig 15. Comparison of significantly differentiated proteins in 81-176 cultured in CDB supplemented with DCA 0.05%**. A: control, 84 proteins were significantly upregulated; B: 178 distinct were significantly upregulated at 24h; C: 119 distinct proteins were significantly upregulated in 81-176 at 12h. D: control, 65 proteins were significantly downregulated; E: 167 proteins were significantly downregulated at 24h; F: 88 distinct proteins were significantly downregulated in 81-176 at 12h.

## 4.6 Unexpected quantification strength and weakness of SILAC when compared to label-free analysis with SWATH

Two very interesting observations arose from the quantification results of SILAC and label-free analysis with SWATH. First, as mentioned in section 4.5.1, SILAC unexpectedly yielded

500 quantifiable proteins. Table 10 lists uniprot codes of the proteins that were quantified by label-free analysis with SWATH but not SILAC. Second, SILAC lead to the identification and quantification of 23 proteins which were not identified by label-free analysis with SWATH (table 11). Lastly, both SILAC and SWATH analyses produced 13 inconsistent quantification results (table 12).

**Table 10**. List of uniprot codes of proteins quantified by label-free analysis with SWATH but not SILAC

| UniProt_Accession | UniProt_Accession | UniProt_Accession | UniProt_Accession | UniProt_Accession |
|---|---|---|---|---|
| A1VYE9 | A0A0H3PC31 | A1VXN9 | A0A0H3PDW4 | A0A0H3P9C2 |
| A0A0H3P9Z7 | A1VXL7 | A1VYI7 | A0A0H3PAV1 | A0A0H3PIC7 |
| A0A0H3PDA2 | A0A0H3P9S3 | A1W1J3 | A0A0H3P9B0 | A0A0H3PHT3 |
| A0A0H3P9C8 | A1W0U9 | A1VYJ3 | A0A0H3P9M8 | A0A0H3P9N8 |
| A1W0K3 | A0A0H3PAK3 | A1VZ23 | Q2TJD3 | A0A0H3PA18 |
| A0A0H3PH13 | A1VYK3 | A1VYB8 | A0A0H3P9B9 | A0A0H3PAL8 |
| A0A0H3PC09 | A0A0H3PEH9 | A1VYR0 | A0A0H3P9J8 | A0A0H3P991 |
| A0A0H3P9J1 | A0A0H3PHX0 | A0A0H3PID1 | A0A0H3PAV3 | A0A0H3P9M6 |
| A0A0H3P9H6 | A0A0H3PEG0 | A0A0H3PAZ6 | A0A0H3PD19 | A0A0H3PGL6 |
| A0A0H3PBE4 | A0A0H3PAW0 | A0A0H3P9K7 | A0A0H3PBZ1 | A0A0H3PCJ6 |
| A0A0H3P9J9 | A0A0H3PBR0 | A1VXI1 | A0A0H3PBE2 | A0A0H3PAU3 |
| A0A0H3PEF7 | A0A0H3PB04 | A1VY31 | A0A0H3PAH4 | A0A0H3PBM5 |
| A0A0H3P9C4 | A0A0H3PHU2 | A0A0H3PDX5 | A0A0H3PJ52 | A0A0H3PIU3 |
| A0A0H3PB49 | A1W0W6 | A1W162 | A0A0H3PAS3 | A0A0H3PAF3 |
| A0A0H3PA34 | A0A0H3PAG4 | A0A0H3PAE1 | A0A0H3P986 | A0A0H3PJ65 |
| A0A0H3PIF4 | A0A0H3PHM5 | A0A0H3PAL9 | A0A0H3P9S8 | A0A0H3PEL5 |
| A0A0H3PHS4 | A0A0H3PJK7 | A1VXM1 | A0A0H3PCR9 | A0A0H3PEP7 |
| A1VX79 | A0A0H3PD90 | A0A0H3PBB3 | A0A0H3P973 | A0A0H3PAA2 |
| A0A0H3PA99 | A0A0H3PAQ1 | A1VXV6 | A0A0H3PGE8 | A0A0H3PJ75 |
| A0A0H3PED7 | A1VXU6 | A0A0H3PDV7 | A0A0H3P968 | A0A0H3PAX9 |
| A0A0H3PBJ8 | A1VZ01 | A1W0R3 | A0A0H3P9G9 | A0A0H3PC13 |
| A0A0H3PH67 | A0A0H3PAC7 | A1VZU7 | A0A0H3P971 | A0A0H3PBJ6 |
| A0A0H3PGG1 | A0A0H3PB10 | A1W165 | A0A0H3PGV9 | A0A0H3PC06 |
| A0A0H3P989 | A0A0H3PB89 | A1VZW5 | A0A0H3PCE6 | A0A0H3PBR7 |
| A0A0H3PGQ1 | A0A0H3P9Z1 | A1VZH5 | Q2A947 | A0A0H3PF31 |
| A0A0H3P9Q7 | A0A0H3PA24 | A0A0H3PB64 | A0A0H3PJE6 | A0A0H3PC19 |
| A0A0H3PEX3 | A0A0H3PHN4 | A0A0H3PHQ7 | A0A0H3PE72 | A0A0H3PBN8 |
| A1W0W2 | A0A0H3P9T0 | A1VYA6 | A0A0H3PAZ8 | Q29W30 |
| A1VY40 | Q29VW1 | A1VZ20 | A0A0H3PJA6 | A0A0H3PB55 |
| A1W1X0 | A0A0H3PH15 | A1VXH9 | A0A0H3PEH5 | A0A0H3PBJ9 |
| Q29W37 | A1VZB5 | A1VXQ2 | A0A0H3PAH9 | A0A0H3PIW6 |
| A0A0H3PBS3 | A0A0H3PHG1 | A0A0H3PBY8 | A0A0H3P9M3 | A0A0H3PIY1 |
| A1W091 | A0A0H3PBB5 | A0A0H3P9Q3 | A0A0H3PBW5 | A0A0H3PAB4 |
| A1VZM8 | Q0Q7I1 | A0A0H3PA75 | A0A0H3P9L3 | A0A0H3PB96 |
| A0A0H3PAP1 | A0A0H3PC48 | A0A0H3PAG5 | A0A0H3PJL7 | A0A0H3PAI8 |
| A0A0H3PAM5 | A0A0H3PBK5 | A0A0H3PJI4 | A0A0H3PJK4 | A0A0H3PAP9 |
| A0A0H3PEB1 | A0A0H3PBG9 | A0A0H3P9V7 | A0A0H3PBP0 | A0A0H3PA27 |
| A1VX95 | A0A0H3PIU0 | A0A0H3PEB4 | A0A0H3PJA2 | A0A0H3PHF9 |
| Q5QKR5 | A1W0M1 | A0A0H3P9D2 | A0A0H3PHF5 | A0A0H3PHJ5 |
| A0A0H3PBU8 | A0A0H3PCU6 | A0A0H3PEV1 | A0A0H3P9Z9 | A0A0H3P9M2 |
| A1VXU8 | A0A0H3PBF9 | A1W0X9 | A0A0H3P9T5 | A0A0H3PAS8 |
| A0A0H3PJ70 | A1VYU1 | A0A0H3PED2 | A0A0H3PBU4 | Q0Q7K3 |
| A0A0H3P9A3 | A1VYT7 | A0A0H3PCT3 | A0A0H3PAS6 | A0A0H3PHH2 |
| A0A0H3PAM7 | A0A0H3PDD6 | A0A0H3PBG2 | A0A0H3P9I3 | A0A0H3PAS5 |
| A0A0H3PCZ7 | A0A0H3PIY4 | A0A0H3P9M1 | A0A0H3PA44 | A0A0H3PHG6 |
| A1VYF9 | A0A0H3PAN1 | A1VYZ7 | A0A0H3PAA5 | A0A0H3PDH2 |
| A1VZR0 | A0A0H3PAY1 | A0A0H3PAZ2 | A0A0H3PBE5 | A0A0H3PHP5 |
| A0A0H3PBD0 | A0A0H3P9B6 | A0A0H3PCA8 | A0A0H3PJC9 | A0A0H3PHH8 |
| A0A0H3PB78 | A1W068 | A0A0H3PA35 | A0A0H3PH37 | A0A0H3P9N1 |
| A0A0H3PD50 | A1W035 | A0A0H3PBJ5 | A0A0H3PI86 | A0A0H3PDB4 |

| | | | | |
|---|---|---|---|---|
| A1VZN9 | A1VZB4 | A0A0H3PAP0 | A0A0H3PAT8 | A0A0H3P981 |
| A0A0H3P9T9 | A1VY70 | A0A0H3PAE7 | A1VYL9 | A0A0H3PGX2 |
| A0A0H3PBH6 | A0A0H3PJF7 | A0A0H3PIA1 | A0A0H3PBF8 | A0A0H3PCX6 |
| A0A0H3PHN8 | A0A0H3PHB9 | A1VXG9 | A0A0H3PB69 | A0A0H3PAC0 |
| A0A0H3P9E4 | A0A0H3P9E8 | A0A0H3PIG5 | A0A0H3P994 | A0A0H3P9D1 |
| A0A0H3PET1 | A1VY43 | A0A0H3PDE7 | A0A0H3PAR1 | A0A0H3PH34 |
| A0A0H3P9A4 | A0A0H3P9P3 | A0A0H3PE25 | A0A0H3PDU8 | A0A0H3PAL5 |
| A0A0H3PHA3 | A1W0S0 | A0A0H3PAQ2 | A0A0H3PBB8 | A0A0H3PAL1 |
| A0A0H3PJ78 | A1VYQ4 | A0A0H3PA42 | A0A0H3PBE0 | A0A0H3P9D8 |
| A0A0H3PB53 | A0A0H3PAH1 | A0A0H3P9J0 | A0A0H3PBM4 | A0A0H3PAJ5 |
| A1W1K9 | A1VY44 | A0A0H3PA60 | A0A0H3PEH2 | A0A0H3PGW3 |
| A0A0H3PJ97 | A1W062 | A0A0H3PA66 | A0A0H3PJB0 | A0A0H3PAI2 |
| A1VZJ8 | A0A0H3PIU8 | A1VXJ1 | Q8GJC7 | A0A0H3PCX2 |
| A1W0D6 | A0A0H3PIZ8 | A0A0H3P9J7 | A0A0H3PA26 | Q2M5Q0 |
| A0A0H3PBH7 | A0A0H3PA78 | A0A0H3P9L8 | A0A0H3PIS1 | Q2M5Q9 |
| A1W0R9 | A0A0H3PIF6 | A0A0H3PBJ1 | A0A0H3PES2 | Q2M5Q7 |
| A1VXP5 | A0A0H3PEY5 | A0A0H3PBD1 | Q6QNL7 | A0A0H3PII9 |
| A0A0H3P9S5 | A0A0H3PDD9 | A0A0H3PAC4 | Q0Q7J3 | Q2M5R0 |
| A0A0H3PAJ2 | A0A0H3PAR2 | A0A0H3PA51 | A1VZ10 | A0A0H3PDS7 |
| A0A0H3PCG8 | A0A0H3P9C5 | A0A0H3PBL7 | A0A0H3PAX0 | A0A0H3PAF1 |
| A0A0H3PI21 | A0A0H3PAC3 | Q0Q7H5 | A0A0H3PEN1 | A0A0H3PBG0 |
| A0A0H3PA38 | A0A0H3PCP5 | A0A0H3PA76 | Q29VV2 | A0A0H3PB67 |
| A0A0H3P9L0 | A0A0H3PAD9 | A0A0H3PGP1 | Q29VV3 | A0A0H3PAA3 |
| A0A0H3PH05 | A0A0H3PH73 | A0A0H3PEA5 | A1W0U8 | A0A0H3PAM9 |
| A0A0H3PAU6 | A0A0H3P9F0 | A0A0H3PJB3 | Q6QNL8 | A0A0H3P9U1 |
| A0A0H3PA89 | A0A0H3PB07 | A0A0H3PHE3 | Q29VV4 | A0A0H3P9W6 |
| A0A0H3PCI0 | A0A0H3PEE2 | A0A0H3PJ16 | A1VYV6 | A0A0H3PAA1 |
| A0A0H3PIT1 | A1VZQ5 | Q0Q7I0 | A0A0H3P9V0 | A0A0H3PIL0 |
| A0A0H3PHL1 | A0A0H3PAN7 | A0A0H3P9B1 | A0A0H3PAB7 | A0A0H3P9V8 |
| A0A0H3PAL3 | Q7X518 | A0A0H3PIS5 | A0A0H3PBC8 | A0A0H3PHT8 |
| A0A0H3P9I4 | Q7X517 | A1W0G0 | Q0Q7K6 | A0A0H3PI11 |
| A1VZI4 | Q2M5Q2 | A0A0H3PAY0 | Q0Q7K2 | A0A0H3PDT4 |
| A0A0H3PEJ9 | Q939J8 | A0A0H3PDM3 | Q0Q7K5 | A0A0H3PE85 |
| Q29VV6 | A0A0H3P9U7 | A0A0H3PA17 | Q0Q7K1 | A0A0H3PAW5 |
| A0A0H3P9R1 | A1W0U6 | A0A0H3PAU0 | Q2M5Q3 | A0A0H3PA01 |
| A0A0H3PAP2 | Q5QKR7 | A0A0H3P9N4 | Q0Q7K4 | A0A0H3PIQ2 |
| A0A0H3PA70 | Q5QKR8 | A0A0H3PDF2 | A0A0H3PJF3 | A0A0H3PB39 |
| A1VZJ6 | A0A0H3PAW3 | A0A0H3P9R0 | A0A0H3PAY9 | A0A0H3PGL0 |
| A1VYQ1 | A0A0H3PE81 | A0A0H3PED0 | A0A0H3PA59 | Q8GJE8 |
| A1W0I0 | A0A0H3PE69 | A0A0H3PBQ0 | A0A0H3PEC2 | Q8GJE6 |
| A0A0H3P9U0 | A1VZG5 | A0A0H3PBI5 | A0A0H3PEW9 | Q8GJC6 |
| A0A0H3PI47 | A1VZE6 | Q2M5Q4 | A0A0H3PDG2 | Q8GJC5 |
| A0A0H3PE58 | A1W1J5 | A0A0H3P9P2 | A0A0H3PA31 | Q8GJA8 |
| A0A0H3P9U4 | A1W1V6 | A0A0H3PDG0 | A0A0H3PA30 | A1VZY1 |
| A0A0H3PAJ4 | A1VXH7 | A0A0H3PHZ5 | A0A0H3P9J3 | |
| A1VY36 | A1W1V8 | A0A0H3P9T3 | A0A0H3PBF1 | |
| A0A0H3PB29 | A0A0H3PB47 | A0A0H3P9H3 | A0A0H3PD80 | |

**Table 11**: Proteins identified by SILAC and not label-free analysis with SWATH

| T: Protein IDs | Gene name | Protein name | N: Razor + unique peptides | N: Q-value |
|---|---|---|---|---|
| A1VYG6 | *RpmB* | 50S ribosomal protein L28 | 6 | 0 |
| A1VZV2 | *RpmH* | 50S ribosomal protein L34 | 1 | 0 |
| A0A0H3PD07 | *DctA* | C4-dicarboxylate transport protein | 1 | 0 |
| A0A0H3P972 | CJJ81176_pTet0015 | CCP20 | 1 | 0 |
| A1VZQ6 | *CheR* | Chemotaxis protein methyltransferase | 2 | 0 |
| A0A0H3PJJ8 | *PheA* | Chorismate mutase/prephenate dehydratase | 6 | 0 |
| A0A0H3P9Y5 | CJJ81176_pTet0016 | Cpp21 | 3 | 0 |
| A0A0H3PA83 | CJJ81176_0885 | Cytochrome C | 1 | 0.0037267 |
| A0A0H3PHE9 | CJJ81176_0894 | Flagellin | 3 | 0 |
| A0A0H3PDZ8 | *FdhB* | Formate dehydrogenase, iron-sulfur | 3 | 0 |

| | | | | |
|---|---|---|---|---|
| | | subunit | | |
| A0A0H3P9F9 | CJJ81176_1025 | Mechanosensitive ion channel family protein | 1 | 0.0025773 |
| A0A0H3PAT6 | CJJ81176_1251 | Phosphatase, Ppx/GppA family | 3 | 0 |
| A0A0H3PGN8 | CJJ81176_0003 | Pseudouridine synthase | 4 | 0 |
| A0A0H3P9G2 | CJJ81176_1023 | Putative, Cell division protein FtsH | 3 | 0 |
| A0A0H3PJC4 | CJJ81176_0627 | Putative, Chemotaxis protein MotB | 1 | 0 |
| A0A0H3PAE0 | CJJ81176_1535 | RloH | 4 | 0 |
| A0A0H3PD83 | *LepB* | Signal peptidase I | 7 | 0 |
| A0A0H3PGQ9 | *SppA* | Signal peptide peptidase SppA, 36K type | 2 | 0 |
| A0A0H3P9Y3 | *FtsY* | Signal recognition particle receptor FtsY | 3 | 0 |
| A0A0H3PJ87 | CJJ81176_0413 | TPR domain protein | 4 | 0 |
| A0A0H3PEZ9 | CJJ81176_0207 | Uncharacterized protein | 3 | 0 |
| A0A0H3PHD0 | CJJ81176_1050 | Uncharacterized protein | 2 | 0 |
| A0A0H3PI03 | CJJ81176_1405 | Uncharacterized protein | 1 | 0 |

**Table 12**: Inconsistent quantification results in SILAC and SWATH

| T: Protein IDs | Gene name | C: T-test Significant | C: T-test Significant _T-test Significant | Expression in SWATH |
|---|---|---|---|---|
| A0A0H3PAI3 | *CJJ81176_0586* | | | Significantly downregulated |
| A0A0H3PAL0 | *cadF* | + | +_+ | Significantly downregulated |
| A0A0H3PB02 | *CJJ81176_0220* | | | Downregulated |
| A0A0H3PB43 | *CJJ81176_0637* | | | Significantly downregulated |
| A0A0H3PCI2 | *CJJ81176_0072* | + | +_+ | Significantly downregulated |
| A0A0H3PCP8 | *CJJ81176_1045* | | | Significantly downregulated |
| A0A0H3PCS4 | *ribE* | | | Significantly downregulated |
| A0A0H3PD29 | *cobE* | + | +_+ | Downregulated |
| A0A0H3PD33 | *sixA* | + | +_+ | Downregulated |
| A0A0H3PEX7 | *CJJ81176_0438* | + | +_+ | Downregulated |
| A0A0H3PH47 | *CJJ81176_1185* | + | +_+ | Significantly downregulated |
| A0A0H3PH83 | *ssb* | + | +_+ | Significantly downregulated |
| A0A0H3PJ30 | *nrdB* | + | +_+ | Downregulated |
| A1W0Z5 | *selA* | + | +_+ | Downregulated |
| A1W1H0 | *nuol* | + | +_+ | Downregulated |
| A1W1U3 | *rpsE* | + | +_+ | Downregulated |
| A1W1V5 | *rplV* | + | +_+ | Downregulated |
| Q29W27 | *kpsD* | | | Downregulated |
| Q3I354 | *luxS* | + | +_+ | Significantly downregulated |

## 4.7 Deletion of MazF (*cjp47*) affects growth of 81-176 in bile acids and decreased Caco-2 cell adherence and invasion in presence of bile acids.

The biological functions of all proteins that were differentially expressed in this study were matched with those in other Gram negative bacteria. This comparison led to the identification of a number of proteins which could be playing a role in the pathogenesis of 81-176 (Appendices 1 - 8). From this collection, gene *cjp47* was chosen for further characterization. A BLAST search showed that it was a toxin-toxin system gene and was closely related to *mazF* of *E. coli* (fig. 16). This type of toxin-toxin has been found to play a role in the survival and pathogenesis of other bacterial pathogens (Kędzierska and Hayes, 2016). It was hypothesized that *cjp47* could be playing a similar role in 81-176. Consequently,

a mutant and complement of *cjp47* was constructed (fig 17). The mutant, its complement and the wild type (WT) showed similar growth pattern in MHB (fig. 18). The growth of mutant in MHB that was supplement with CA 2.5mM, LCA 15mM, TCA 10mM, UDCA 15mM, DCA 2.5mM, CDCA 2.5mM, and GCA 2.5mM. The growth curves of mutant growing in MHB supplemented with these concentrations showed no significant difference between WT, mutant and complement between 0h and 20h ($p>0.05$). However, after 20h, the growth curves of mutant growing in MHB supplemented with CA 2.5mM, TCA 10mM, DCA 2.5mM, CDCA 2.5mM and GCA 2.5mM showed a significant difference between the mutant and WT and complement ($p<0.05$; fig. 19).

In the next experiment, GPA was used to test the effect of deleting gene *mazF* on the capability of 81-176 to adhere and invade Caco-2 cells. DMEM medium was supplemented with CA 2.5mM, LCA 15mM, TCA 10mM, UDCA 15mM, DCA 2.5mM, CDCA 2.5mM, and GCA 2.5mM. The mutant showed reduced adherence and invasion on Caco-2 cells in presence of these concentrations of bile acids (fig. 20). However, two-way ANOVA analysis revealed that the reduction in numbers related to adherences that were found in DCA, LCA, TCA, CDCA, UDCA and GCA were not significantly different from WT and complement. On the other hand, two-way ANOVA analysis of invasion results revealed that the reduced numbers that were found in DCA, LCA, CDCA, UDCA and GCA were not significantly different from the WT and complement ($p<0.05$). Therefore, deletion of *cjp47* (i) significantly reduced the adherence of 81-176 in the presence of low concentration of CA and (ii) significantly reduced the invasion of cj81-176 in the presence of low concentration of CA and TCA. In both experiments, there was no significant difference between mutant, WT and complement in GPA with DMEM that was not supplemented with all bile acids.

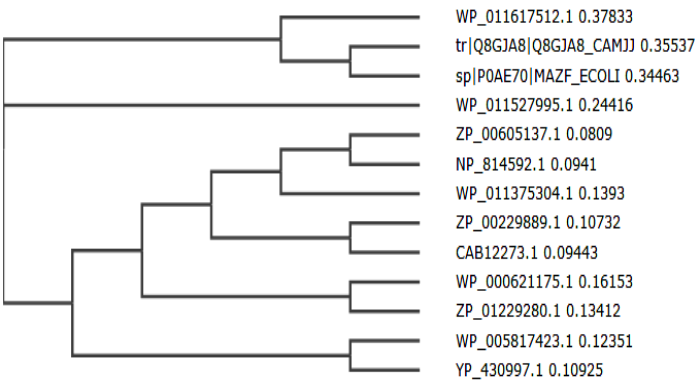


**Fig. 16**. **Phylogenetic analysis of the relationship of *cjp47* with *mazF* orthologs from other bacteria**. The analysis was done through CLUSTRAL multiple sequence alignment by MUSCLE. The accession umbers for each MazF of bacteria species are: tr|Q8GJA8|Q8GJA8_CAMJJ Uncharacterized protein OS=*Campylobacter jejuni* subsp. *jejuni* serotype O:23/36 (strain 81-176), sp|P0AE70|MAZF_*E. coli* Endoribonuclease MazF OS=*Escherichia coli* (strain K12), ABA71736.1 pemK-like protein (*Enterococcus faecalis*), WP_011617512.1 antitoxin (*Cupriavidus necator*), WP_005817423.1 MULTISPECIES: mRNA interferase PemK (*Desulfitobacterium*), WP_011375304.1 PemK family transcriptional regulator (*Lactobacillus sakei*), YP_430997.1 transcriptional modulator of MazE/toxin MazF (*Moorella thermoacetica* ATCC 39073), NP_814592.1 PemK family transcriptional regulator (*Enterococcus faecalis* V583), ZP_00229889.1 transcriptional regulator, PemK family (*Listeria monocytogenes* serotype 4b str. H7858), WP_011527995.1 toxin MazF (*Streptococcus pyogenes*), ZP_01229280.1 hypothetical protein CdifQ_02003809 (*Clostridium difficile* QCD-32g58), CAB12273.1 endoribonuclease toxin (*Bacillus subtilis* subsp. subtilis str. 168), WP_000621175.1 MULTISPECIES: mRNA interferase MazF (*Staphylococcus aureus*)

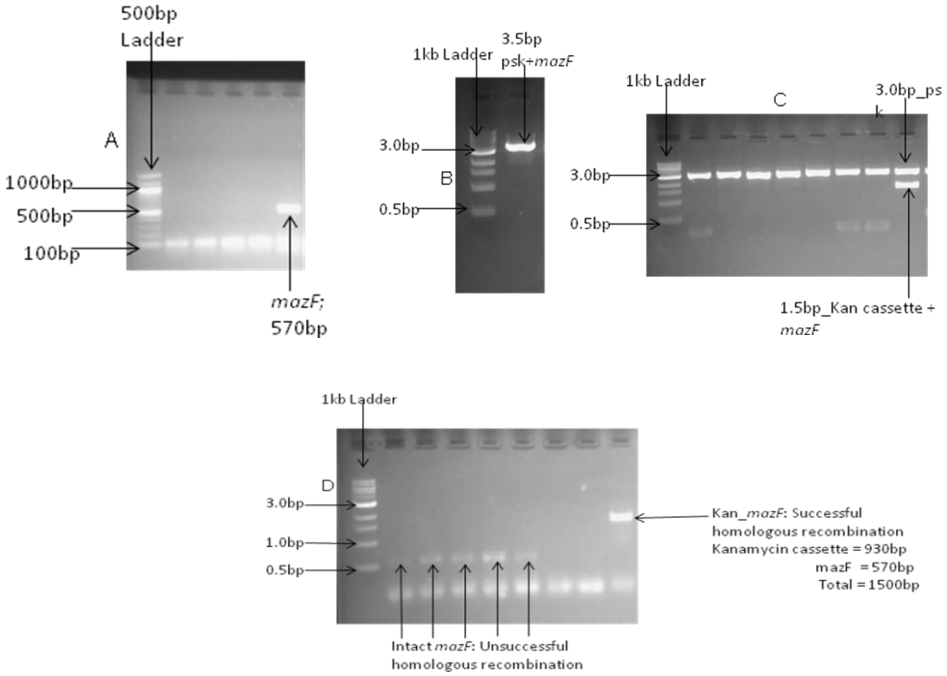



**Fig. 17**. **Gel pictures showing the mutant construction scheme**. A shows the size of *mazF* (570bp). B shows successful ligation of *mazF* and pbluescript vector (3.5bp). C shows successful construction of a sucide vector comprising ligation of pbsK (3bp), *mazF* and kanamysin cassette (1.5bp). D shows successful homologous in 81-176 upon transformation of suicide vector into 81-176.

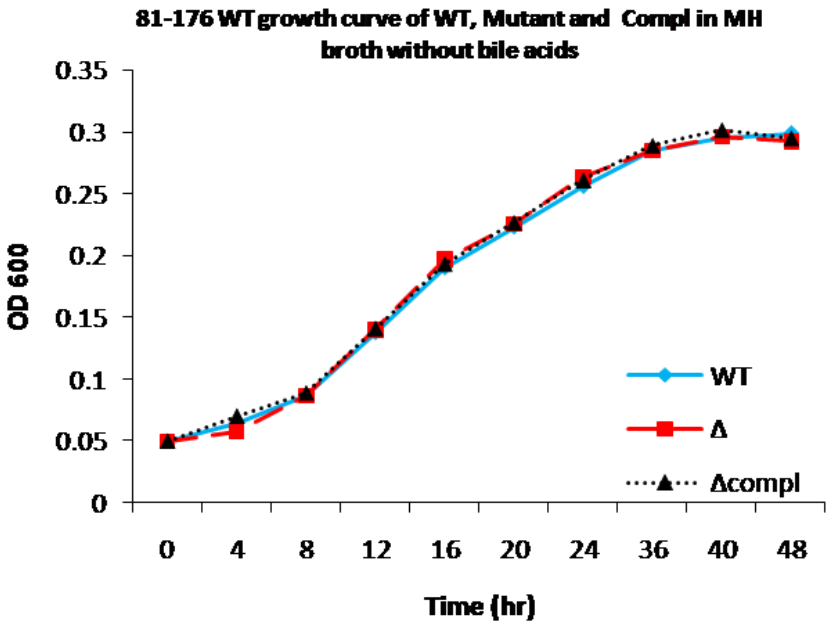



**Fig. 18**. Growth curves showing the comparison of 81-176 WT, *mazF* mutant and its complement in MHB at 37°C. The graph shows the average results of three independent experiments. However, due to a small standard deviation between the independent experiments, no error bars are visible.

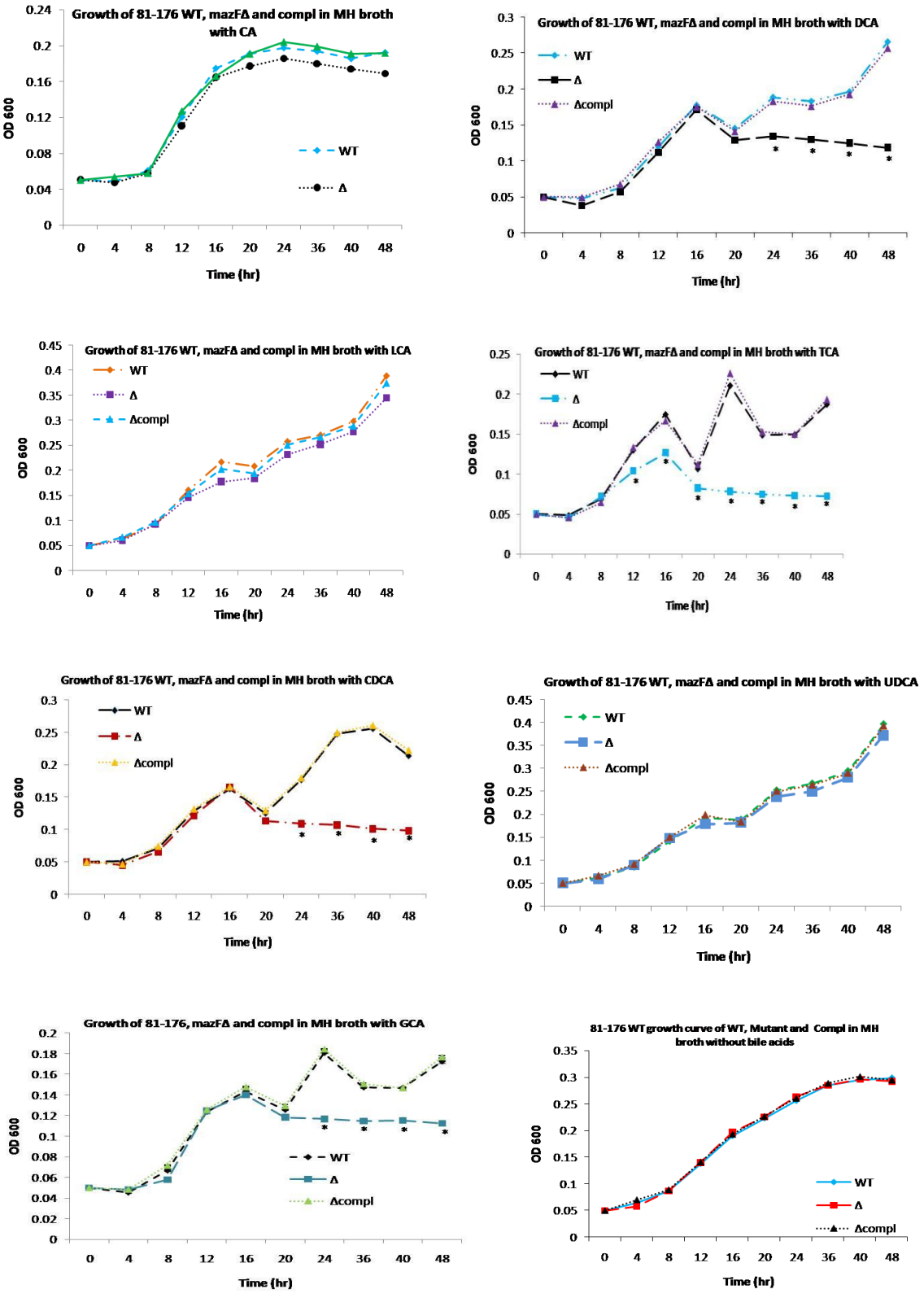



**Fig 19**: Growth curves of *mazF* mutant in bile acids used in this study. * on the graphs showed time points where significant differences between mutant and both wild type and complement were found ($p<0.05$). The graph shows the average results of three independent experiments. However, due to a small standard deviation between the independent experiments, no error bars are visible.

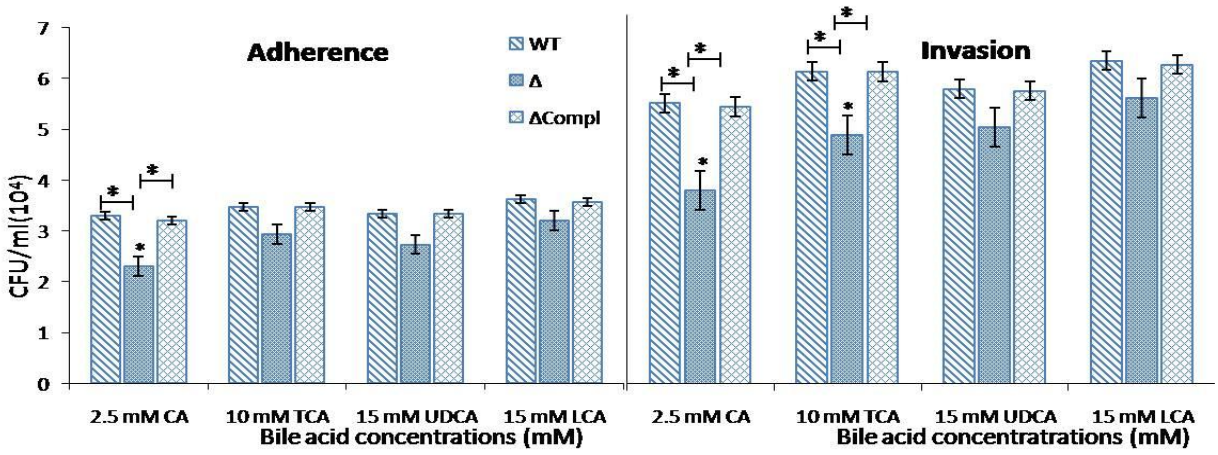



**Fig.20a. Comparison of adherence and invasion of Caco-2 cell by WT, mutant and complement in DMEM medium supplemented with 2.5mM CA, 15mM LCA, 10mM TCA and 15mM UDCA**. Two-way ANOVA analysis showed that the deletion of *mazF* significantly impaired the adherence of 81-176 to Caco-2 cells in 2.5mM CA ($p<0.05$). However, its deletion did not significantly impair the adherence of cj81-176 to Caco-2 cells in the presence of TCA, LCA and UDCA. On the other hand, Two-way ANOVA analysis showed that the deletion of *mazF* significantly impaired the invasion of Caco-2 cells by cj81-176 in the presence of 2.5mM CA and 10mM TCA ($p<0.05$). Howerver, its deletion did not significantly impair the invasion of Caco2-cells by cj81-176 in the presence of UDCA and LCA. The deletion reduced the number of 81-176 cells that were recovered from Caco-2 cells in all situations. The experiment was repeated three independent times.

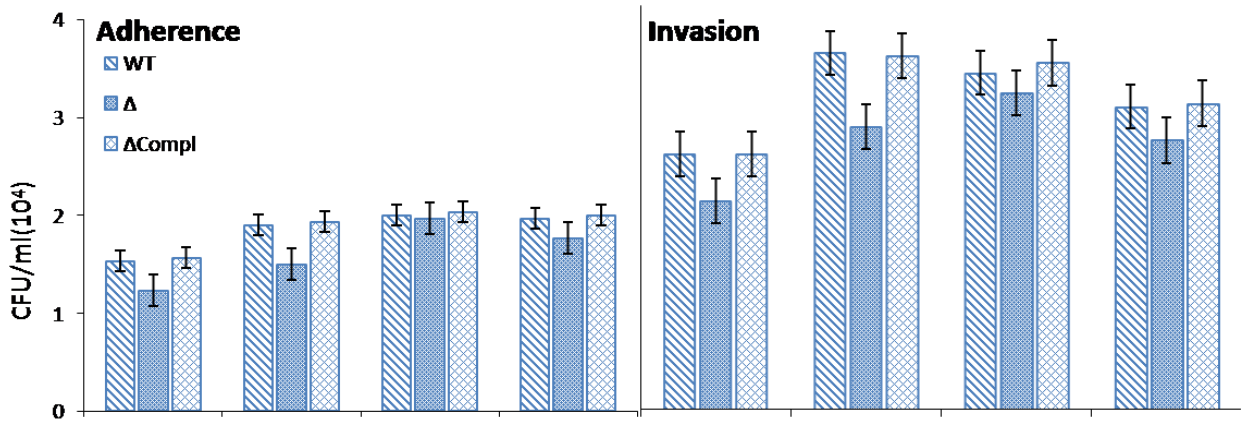



**Fig.20b. Comparison of adherence and invasion of Caco-2 cell by WT, mutant and complement in DMEM medium without bile acids**. Two-way ANOVA analysis showed that the deletion of *mazF* did not significantly impaired the adherence and invasion of 81-176 to Caco-2 cells ($p>0.05$). However, the delation of *mazF* reduced the number of cells that were recovered from Caco-2 cells. The experiment was repeated three independent times.

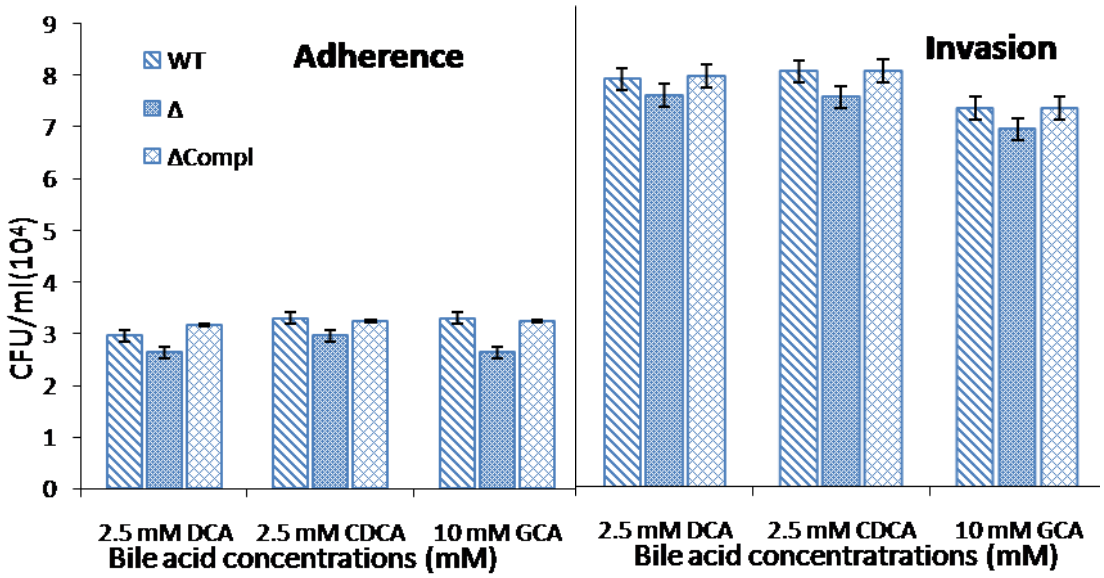



**Fig.20c. Comparison of adherence and invasion of Caco-2 cell by WT, mutant and complement in DMEM medium supplemented with 2.5mM DCA, 2.5mM CDCA and 10mM GCA**. Two-way ANOVA analysis showed that the deletion of mazF did not significantly impaired the adherence of 81-176 to Caco-2 cells ($p>0.05$). However, its deletion reduced the number of C81-176 cells that were recovered from Caco-2 cells. The experiment was repeated three independent times.

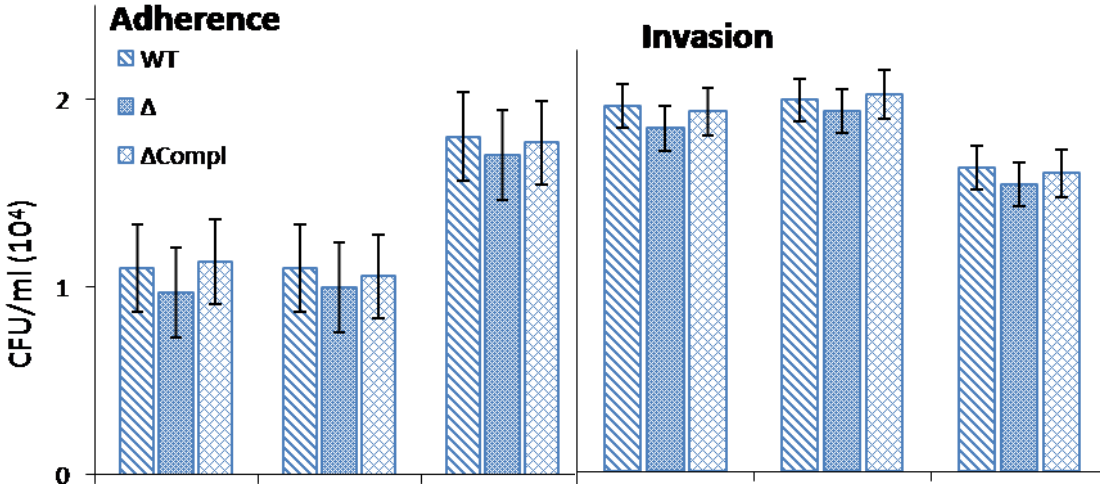



**Fig.20d. Comparison of adherence and invasion of Caco-2 cell by WT, mutant and complement in DMEM medium without bile acids**. Two-way ANOVA analysis showed that the deletion of *mazF* did not significantly impaired the adherence and invasion of 81-176 to Caco-2 cells ($p>0.05$). However, the delation of *mazF* reduced the number of cells that were recovered from Caco-2 cells. The experiment was repeated three independent times.

## 4.8 Metabolism proteins dominate number of significantly upregulated proteins under regular laboratory growth temperatures (37°C and 42°C)

The last objective of this study was to compare proteomic shifts in 81-176 cultured at 37°C and 42°C over a period of 24h. This objective was driven by the outcomes that are observed when *C.jejuni* colonizes two different hosts, namely, chicken and human beings. In chicken, gut colonization by *C .jejuni* does not result in campylobacteriosis (Beery et al., 1988b). But in human beings, colonization of small intestines with *C. jejuni* results in campylobacteriosis (Black et al., 1988). Temperature is one of the contrasting physiological factors between these two hosts. The natural temperature of chicken is 42°C while the natural temperature of human beings is 37°C (Richards, 1970; Sund-Levander et al., 2002). This difference in temperature prompted a need for further investigation into the role of temperature in pathogenesis of *C. jejuni*.

The first investigation focused on the proteomic shift in 81-176 growing at 37°C during 12h and 24h. The biological functional categorization criteria that was established in section 4.5.4 was used to group the expressed proteins into various biological processes. As a result, significantly differentiated proteins belonged to the following biological processes: cell cycle and cell division, outer membrane, chemotaxis, DNA replication and transcription, metabolism, protein synthesis, stress response, transport and two-component regulatory systems (Appendix 4, 5 and 6). Using 0h as the reference point, 242 proteins were differentially expressed at 12h; 43 significantly downregulated and 199 significantly upregulated (fig 21a and fig 21b) were detected. At 24h, 401 proteins were differentially expressed; 83 significantly downregulated proteins and 318 significantly upregulated proteins were detected. As shown in fig. 21a, the highest number of proteins that were significantly upregulated at 24h belonged to metabolism, stress response and protein synthesis. In addition, uncharacterized proteins. The additional stress response proteins that were identified at 24h include: CsrA, HslU, Cjj81176_1536, HypC, Cjj81176_1158, LuxS, SodB, Cjj81176_1101, NapD and PpiB. Interestingly, a pairwise comparison between differentially expressed at 12h and 24h revealed the following to be significantly expressed at 24h and not 12h (fig. 21c): (i) cell cycle: FtsZ, FtsA (ii) outer membrane: Ffh (iii) DNA replication and transcription: PolA, TopA, DnaX, DnaB, NusG, GreA, NusA (iv) metabolism: TrpE, HisD, GltD, PrsA, QueF, Fbp, AroQ (v) motility and chemotaxis: FliS, FliD, FliW, and CheW (vi) stress response: ClpX, CsrA, DnaJ, LuxS, SodB (vii) transportation: Fur and SecA.

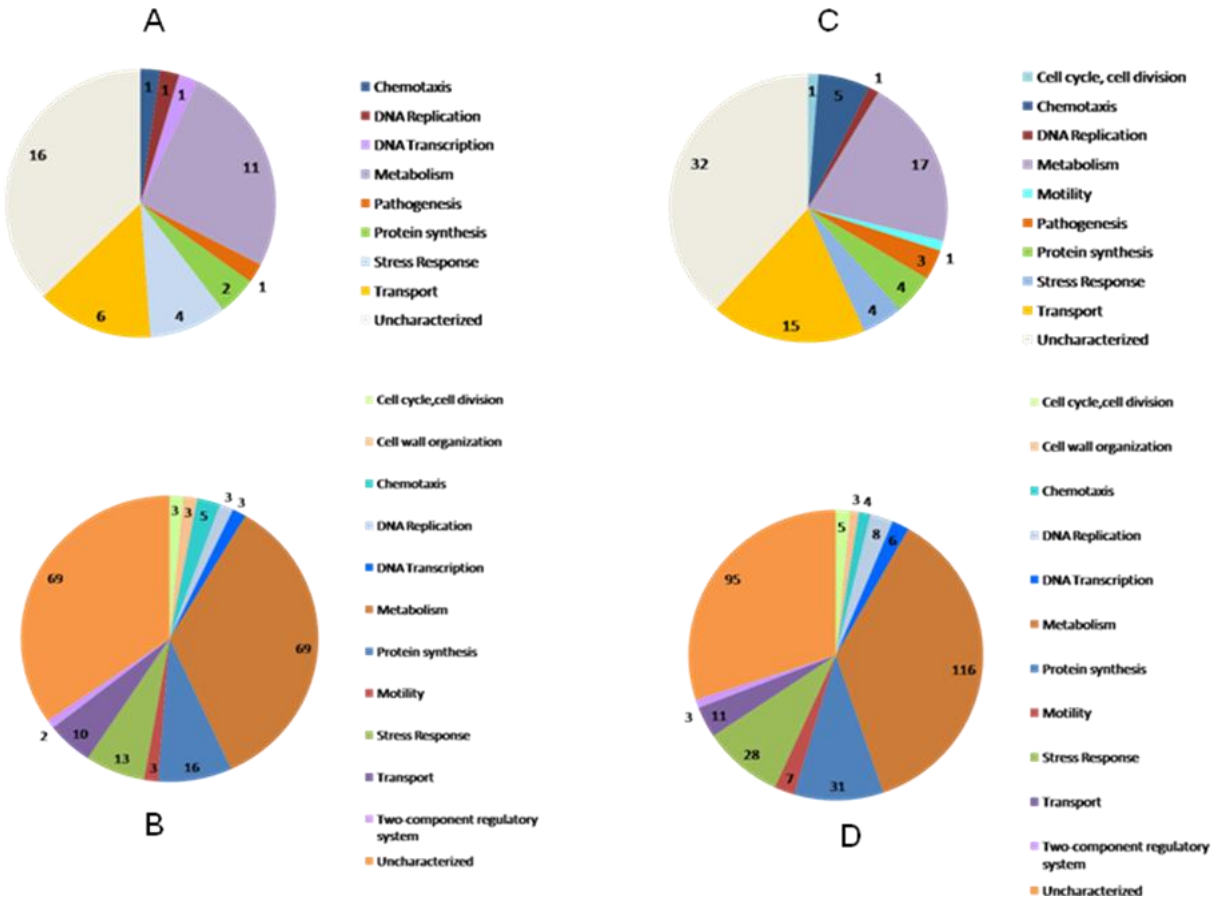



**Fig 21a. Functional categorization of significantly upregulated and downregulated proteins in 81-176 cultured for 12h at 37°C ( A and B) and cultured for 24h at 37°C (C and D)**. A and B shows number of significantly downregulated and upregulated proteins at 12h respectively and the biological processes they are involved. Similarly, C and D shows number of significantly downregulated and upregulated proteins at 24h respectively and the biological processes they are involved and B shows significantly upregulated proteins. Log2FC≤1 was interpreted as significantly downregulated and log2FC≥1 significantly upregulated.

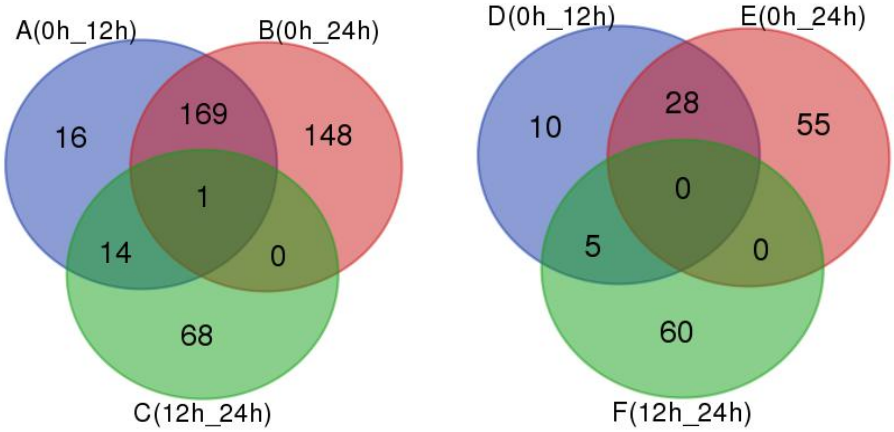



**Fig 21b. Venn diagram showing significantly differentiated proteins in an analysis comparing protein expression in 81-176 at 0h, 12h and 24h at 37°C**. In summary, A: 31 significantly distinct proteins were significantly upregulated at 12h; B: 149 proteins were significantly upregulated at 24h; , 169 significantly upregulated proteins were shared at 12h and 24h. C: 82 proteins were significantly upregulated between 12h and 24h (log2FC≥1). D: 15 proteins were significantly downregulated at 12h; E: 55 proteins were significantly downregulated at 24h; 28 proteins similar proteins were significantly downregulated at 12h and 24h; F: 65 proteins were significantly downregulated between 12h and 24h (Log2FC≤1).

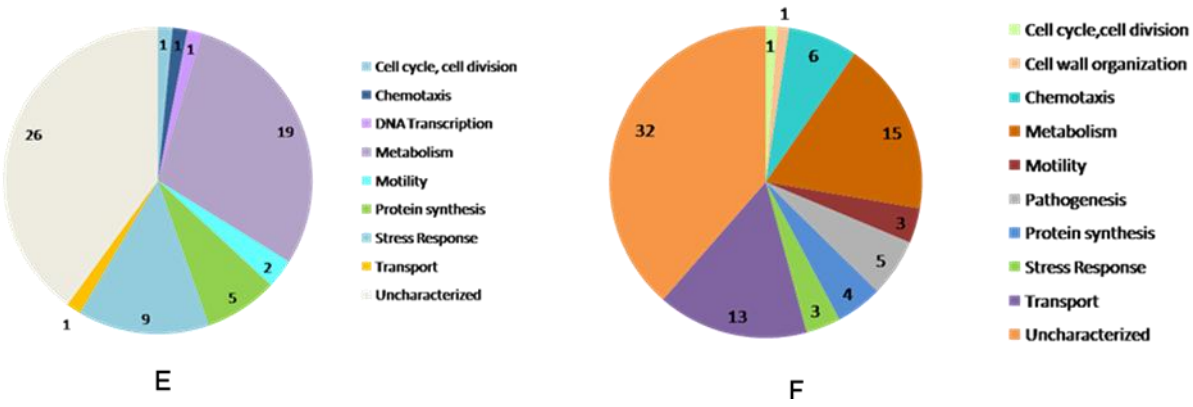



**Fig.21c. Functional categorization of significantly upregulated and downregulated distinct proteins at 12h and 24h at 37°C**. E shows number of significantly downregulated proteins (log2FC≤1) and biological processes where they are involded. F shows the number of significantly upregulated proteins (log2FC≥1) and biological processes where they are involved.

The second investigation entailed comparing proteins that were differentially expressed when 81-176 was cultured at 37°C and 42°C for 24h (Appendix7). The initial comparison of the proteins that are significantly differentiated at 42°C and 37°C identified proteins that were significantly differentiated in 81-176 cultured 42°C for 24h (fig. 22). These included: (i) metabolism: significantly upregulated PurE, SdaA, SdaC, NrfA, NrfH, PetA, PetC, NuoC, NuoG, CydA, Peb1C, Ppk,and FrdC; (ii) stress response: ClpB, DnaJ-1, GroL, DnaJ, DnaK, DsbA, GrpE and TatA (iii) transportation: TatB (iv) cell division: PbpA. Similarly, a comparative analysis of differentially expressed proteins in 81-176 cultured at 42°C in CDB for 24h and 81-176 cultured at 37°C in CDB supplemented with bile acids for 12h (section 4.5.4) was carried out. This analysis revealed additional essential proteins for adaptation to 42°C (table 13).

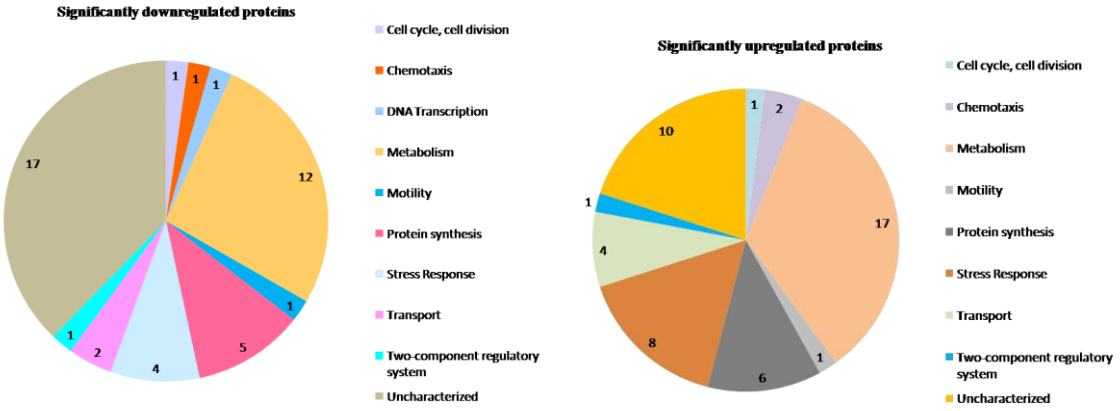



**Fig.22. Functional categorization of significantly upregulated and downregulated distinct proteins in 81-176 cultured for 24h at 42°C**.

**Table 13:** Key proteins likely to promote adaptation of 81-176 from CDB suppl with bile acids at 37°C to 42°C

| Biological category | Gene name | Expression level |
|---|---|---|
| Chaperone | *groL, groS, hypD, hypE, clpB, clpX* | Significantly upregulated |
| Chemotaxis | *cheB, cheY, cheA* | |
| Cell cycle and division | *ftsA, ftsZ, murC* | |
| Transport | *CJJ81176_0137, CJJ81176_0446, CJJ81176_0897* | |
| Two-component regulatory system | *trpS* | |
| Stress Response | *dnaK, grpE, csrA, napD, dnaJ, dnaJ-1, clpB, sodB* | |
| Protein transportation pathways | *tat and sec* | |
| Protein synthesis | *Rnc, rpsJ, rpsH, rpsT, rpsE, rpsU, rpsK, rpsB, hypE, hypB, trpS, hypB* | |
| Protein modification | *sixA, map* | |
| Metabolism | *CJJ81176_0111, sdaA, pur(C,E,F,M,N,S), hom, gltA, ggt, tyrA,hisA, hisl* | |
| Uncharacterized | 38 proteins | |
| Chemotaxis | *cjj81176_1204* | Significantly downregulated |
| Protein synthesis | *pepA, rpmJ* | |
| Metabolism | *aspA, proB, thiE* | |

# 5.0 DISCUSSION

*C. jejuni* remains to be one of the most important gastrointestinal bacterial pathogens (Kaakoush et al., 2015). As a result, efforts are being put into understanding how it interacts with the human host at the molecular level so that appropriate preventive and treatment measures can be developed (Masanta et al., 2013). Bile acids and temperature are some of the environmental components which *C. jejuni* constantly encounters. For example, bile acids are lethal to bacteria (Begley et al., 2005b). Therefore, *C. jejuni* must overcome the effects of bile acids to survive in the small intestines. The physiological and total proteomic response in *C. jejuni* to different bile acids which are present in the human small intestines remains poorly understood. As a result, this study has mainly utilized a quantitative proteomic approach known as label-free with SWATH analysis to investigate the global proteomic response in 81-176 to non-lethal concentrations of CA, DCA, LCA, TCA, CDCA, UDCA and GCA. In addition, there is necessity to look into how C. jejuni adapts into common temperature ranges. Therefore, this study: (i) has identified and compared adaption strategies that 81-176 utilizes to counter the antimicrobial activities of these bile acids (ii) has identified a broader role of bile acids in pathogenesis (iii) has detected previously unknown and uncharacterized proteins expressed in response to bile acids and (iv) has identified a toxin and characterized its influence of on the ability of *C. jejuni* to grow in bile acids and adhere and invade Caco-2 cells (v) identified key proteins which aid *C. jejuni* to adapt to live at 37$^{o}$C and 42$^{o}$C.

## 5.1 The choice of label-free analysis with SWATH for this study over SILAC

Both SILAC and label-free analysis with SWATH are known to be reliable quantitative proteomic methods (Gillet et al., 2012; Ong et al., 2002). However, the success of SILAC depends on the ability of a cell to effectively incorporate external heavy labeled arginine and lysine amino acids into its proteome (Ong and Mann, 2007); incorporation efficiency of 95% and above is recommended for good results (Kim et al., 2016). One the other hand, the success of label-free analysis with SWATH depends on good sample preparation (Huang et al., 2015). In the present study, statistical analysis of the 81-176 proteome exposed to DCA 0.05% at 37$^{o}$C for 12h emerging from label-free analysis with SWATH found 957 quantifiable proteins. Further, PCA analysis revealed good sample preparation and

reproducible results making it a choice for this study. However, statistical analysis of similar proteome resulting from SILAC found 500 quantifiable proteins.

The difference of number of quantifiable proteins between these two methods can be attributed to the following: first, poor incorporation efficiency of external heavy labeled amino acids. This is demonstrated by the observation that arginine achieved an incorporation efficiency of above 95% at 42$^{o}$C while lysine achieved a maximum of 80%. The inability of lysine to achieve the required incorporation efficiency of 95% hints at poor incorporation of external labeled amino acids in *C. jejuni*. This was also demonstrated by the auxotyping results which did not find a lysine auxotroph. These observations indicate that *C. jejuni* has robust internal lysine and arginine biosynthesis mechanism which prevented maximum incorporation of labeled heavy amino acids from an external source. These biosynthesis mechanisms seem to be present in other members of Epsilonproteobacteria. For example, a recent study on the SILAC of *Helicobacter pylori* evaluated the incorporation efficiency of heavy labeled 4, 5, 5 – $^{2}$H–lysine and obtained 78% lysine incorporation percentage (Müller et al., 2015). Second, heavy labeled arginine may have failed to achieve incorporation efficiency of 95% and above when 81-176 was cultured at 37$^{o}$C. Similar conclusion was made in a recent study that applied SILAC in *C. jejuni* at 37$^{o}$C and the expect proteomic quantification results were not achieved (Scanlan et al., 2017). *C. jejuni* 81-176 is known to have diverse metabolic pathways for survival in various environments (Hofreuter et al., 2006). Similarly, *C. jejuni* has been shown to synthesize arginine from glutamine through acetylation (Xu et al., 2007). Therefore, 81-176 could be having different arginine biosynthesis strategies at 37$^{o}$C and 42$^{o}$C. This phenomenon should be studied further and how it affects incorporation of external heavy labeled arginine determined.

In spite of the incorporation challenge, quantification results of SILAC revealed that it is more precise that SWATH. This argument is supported by the finding that SILAC quantified 23 proteins with SWATH didn't. This strength of SILAC can be attributed to its high accuracy (Lau et al., 2014). This accuracy is achieved through: first, mixing both the labeled and non-labeled samples hence reducing differences in samples (Ong and Mann, 2007); and second, measuring ratios of heavy and light amino acids in peptides which eliminates poor quantification (Schmidt et al., 2014). These statements should not be taken to mean that SWATH quantification is not comparable to SILAC quantification. In fact, SWATH quantification is as accurate and precise as SILAC quantification in simple proteomic experiments (Collins et al., 2013; Gillet et al., 2012; Lambert et al., 2008; Liu et al., 2013). However, its accuracy is inconsistent in complex proteomic experiments in which proteins

have different abundance ratios (Huang et al., 2015). A good example is demonstrated in table 13 of this thesis where SILAC and SWATH generated inconsistent quantification results of 19 proteins. Therefore, in complex *C. jejuni* proteomic experiments both SILAC and SWATH should be used to complement each other.

## 5.2 CDB and Auxotyping

CDB was developed and utilized in the proteomic section of this study. This broth was selected because its components, namely, trace elements, metals ions, amino acids, vitamins and pH 6.8 resembles the fluid environment that is present in the duodenum of human small intestines (Kararli, 1995). It was reasoned that this broth would provide an environment for 81-176 to synthesis proteins similar to those it does in when in the small intestines of human beings. In general, the growth of 81-176 in CDB was comparable to that in LB, MH and BHI. This finding agrees with the results of a previous study that was carried out by Birk and colleagues (Birk et al., 2012). Due to the composition of this broth, 81-176 expressed a good number of proteins. The DDA library was made up of a 1079 proteins which represents approximately 70% of the total number of proteins in *C. jejuni* were quantified and used to build a DDA library. Consequently, 957 differentially expressed proteins quantified representing 59.9% of the total number of proteins in 81-176 (Johnson et al., 2014).

CDB was used to performed auxotyping analysis. Interestingly, 285 *C. jejuni* strains were prototrophic, one strain was both arginine and serine auxotrophic and 17 strains were methionine auxotrophs. These findings bring forth three biological factors about *C. jejuni*. First, the finding that 285 *C. jejuni* strains are prototrophic show that majority of strains do not have a strict nutritional requirement. This could be one of the traits which enables *C. jejuni* to colonize the guts of various hosts which have different nutrient compositions. This observation is supported by findings of a previous study which found that *C. jejuni* expresses a particular set of genes in response to nutrients which it meets in a host (Gripp et al., 2011). The easy at which *C. jejuni* alters its genes to suit environmental nutrition helps it to adapt and thrive in various hosts (Dearlove et al., 2016; Sheppard et al., 2014). Second, identification of one arginine auxotroph and zero lysine auxotroph shows the challenge of successful application of metabolomic labeling proteomic techniques e.g. SILAC in *C. jejuni* research. This challenge has been demonstrated in this study where the number of SILAC quantifiable proteins did not march that of SWATH analysis. Similarly, Scanlan and co-workers arrived at a similar conclusion in their recent study where they used SILAC to study flagella associated proteins (Scanlan et al., 2017). Lastly, CDB lead to the identification of 17 methionine *C. jejuni* auxotrophs. A recent study that was carried out to investigate essential

genes in NCTC 11168 did not find known methionine synthesis genes (Metris et al., 2011). Tenover and co-workers also identified a reasonable number of methionine auxotrophs (Tenover et al., 1985). Therefore, efforts are required to understand methionine synthesis in *C. jejuni* and how its auxotrophic behaviour can be exploited to advance *C. jejuni* research.

## 5.3 Factors driving differential expression of proteins by CA, CDCA and biotransformants.

The proteomic results of this study show that combinations of similar and dissimilar groups of proteins were differentially expressed by each bile acid. TCA, GCA and DCA are almost synthesized from CA (Lefebvre et al., 2009). Therefore, it would be expected that they differentially express similar proteins. However, fig. 4, fig. 10 and table 13 show that this is not the case. Similarly, LCA and UDCA are synthesized from CDCA (Smet et al., 1995). Hence, it would be expected to differentially express similar proteins. But fig. 4, fig 10 and table reveal the contrary.

This diversity can be mainly attributed to the following: (i) chemical structure, (ii) hydrophobicity status, (iii) solubility, critical micelle concentration and (iv) critical micelle temperature of each bile acid (Armstrong and Carey, 1982; Heuman, 1989). However, hydrophobicity is the key factor. Primary bile acids are hydrophilic (Heuman, 1989). Taurine conjugation, glycine conjugation and biotransformation reduces the hydrophobicity status modified bile acids. As a result, taurine conjugates are more hydrophilic than glycine conjugates (Table 14). Therefore, the hydrophobic differences between these bile acids are responsible for the differences in differential expressed proteins that were observed in this study. For example:

a) Bile acids with almost similar hydrophobicity values expressed similar and almost equal number of proteins. This is demonstrated by DCA and CDCA. These bile acids are not biologically identical; DCA is a secondary bile acid which is synthesized from CA through biotransformation while CDCA is a primary bile acid. However, their hydrophobicity values are almost equal. Hence, this study found that they differentially expressed mostly similar proteins and numbers; (i) DCA significantly upregulated 119 proteins and CDCA 98 proteins (ii) DCA significantly downregulated 90 proteins and CDCA 88 proteins.
b) Extreme hydrophilicity and hydrophobicity influenced the type of differentially expressed proteins. As shown in Table 14, UDCA is slightly more hydrophilic than CA. Therefore, it can be naturally assumed that CA and UDCA should express almost

similar and equal number of proteins. Contrary to this school of thought, this study has shown that UDCA significantly differentiated the lowest number of proteins. In this study, UDCA significantly upregulated 4 proteins and CA 30 proteins; UDCA significantly downregulated 16 proteins and CA 37 proteins. Similarly, LCA is slightly more hydrophobic than DCA and CDCA. Naturally it can be assumed that LCA, DCA and CDCA significantly differentiate similar and equal number of proteins. However, this study found that LCA significantly upregulated 12 proteins and downregulated 28 proteins. This number of significantly differentiated proteins is way below those of both DCA and CDCA and lack similarity.

c) Conjugated bile acids (TCA and GCA) differentially expressed different number and type of proteins. TCA differentially upregulated 57 proteins and GCA 160 proteins. But TCA downregulated 69 proteins and GCA 25 proteins. This similar pattern was found to exist between primary bile acids and their corresponding secondary bile acids. Findings of this study show that CA, GCA, TCA and DCA did not differentially express similar and equal number of proteins. Similarly, CDCA, LCA and UDCA did not differentially express similar and equal number of proteins.

**Table 14**: Hydrophobicity levels of the bile acids used in this study

| **Bile acid** | **Hydrophobicity indices (Hlx)** | **Status** |
|---|---|---|
| CA | 0 | Hydrophilic |
| DCA | +0.69 | Hydrophobilc |
| LCA | +1.23 | Most hydrophobic |
| TCA | 0.90 | Hydrophilic |
| CDCA | +0.53 | Hydrophobic |
| UDCA | -0.47 | Most hydrophilic |
| GCA | 0.07 | Hydrophilic |

(Armstrong and Carey, 1982; Heuman, 1989; Roda et al., 1989)

## 5.4 Adaption strategies of 81-176 to antimicrobial activities of sub lethal concentrations of CA, DCA, LCA, TCA, CDCA, UDCA and GCA

As stated in the introduction, bile acids are very toxic to bacteria. MIC and $IC_{50}$ are used to measure the antimicrobial effects of a chemical. Consequently, in order to get a general overview of the antimicrobial effect of bile acids used in this study to *C. jejuni* 81-176, relevant $IC_{50}$ were investigated. The results were as follows: DCA (0.2%), CDCA (0.2%), CA (0.4%), GCA (1.4%), LCA (2%), TCA (0.2%) and UDCA (2%). These results implied that DCA, TCA and CDCA an almost similar toxicity effect in 81-176. Similarly, LCA and

UDCA have almost equal level of toxicity. These findings agree with findings of a study that was carried out by (Kurdi et al., 2006) which obtained similar CA and DCA MIC values for lactobacillus and bifidobacteria. In terms of toxicity strength, DCA, CDCA and TCA are very toxic to 81-176; followed CA and GCA; LCA and UDCA are less toxic. In a broader perspective, the proteomic and physiological response of a cell to the antimicrobial effects of a bile acid correlates to (i) the chemical structure and hydrophobicity status of a bile acid (ii) the ability of a bile acid to migrate across the cell wall and (iii) the proteins of a cell which interacts with the active sites of the bile acid (Perez, 2009).

This study has used sub-lethal concentrations of bile acids to gain an insight into the proteomic response in *C. jejuni* against antimicrobial effects of CA, DCA, LCA, TCA, CDCA, UDCA and GCA. These responses are discussed below:

### *5.4.1 Elevated synthesis of outer, inner membrane and periplasmic membrane proteins and general protein transport machinery*

Interestingly, in response to the antimicrobial activities of DCA and GCA, 81-176 synthesized more proteins for export to the outer membrane. This observation is supported by two findings: first, the significant upregulation of known outer membrane proteins, putative membrane proteins and putative periplasmic proteins (table 15). Second, the significant upregulation of the corresponding protein transport machineries, namely, (i) SecF and Cjj81176_0967 (outer membrane protein chaperone) by DCA and (ii) TatB, SecF, YajC, YidC and Cjj81176_0967 by GCA. In addition, TCA significantly upregulated two protein transport machineries: TatA and Cjj1584c. Hence, the results of this study suggest a raise in the synthesis of some proteins for export to the outer membrane in response to the antimicrobial activities of DCA, CDCA, TCA and GCA. From Table 15, it is evident that LCA and UDCA did not significantly upregulate as many outer, inner membrane and periplasmic membrane proteins as compared to CA, DCA, TCA, CDCA and GCA. The phenomenon of rise in outer, inner membrane and periplasmic membrane proteins under harsh environment is not unique to *C. jejuni* only. It has been established that most Gram-negative bacteria synthesize proteins targeted for the outer membrane in response to harsh environments (Rollauer et al., 2015). These proteins protect the synthesizing bacteria against effects of the harsh environment (Manning and Kuehn, 2011). However, this area requires further investigation to establish the proteins that are transported through the Sec and Tat protein transport systems and the role that they play in protecting *C. jejuni* against harsh environments.

**Table 15**: Significantly of upregulated proteins related to outer, inner and periplasmic membrane proteins and general transport machinery

| Protein | Gene Name | Location/Function | Bile acid |
|---|---|---|---|
| A0A0H3PAU3 | *cjj81176_0159* | Putative membrane protein | TCA, GCA |
| A0A0H3PC19 | *cjj81176_0428* | Putative membrane protein | GCA |
| A0A0H3PIU3 | *cjj81176_0188* | Putative membrane protein | GCA |
| A0A0H3P9L7 | *cjj81176_0128* | Putative periplasmic protein | TCA |
| A0A0H3PCN0 | *cjj81176_0127* | Putative periplasmic protein | GCA |
| A0A0H3PD99 | *cjj81176_0797* | Putative periplasmic protein | CA, LCA, TCA, GCA |
| A0A0H3PDT4 | *cjj81176_1617* | Putative periplasmic protein | CA, DCA, TCA, CDCA |
| A0A0H3PB39 | *cjj81176_1673* | Putative periplasmic protein | GCA |
| A0A0H3PF03 | *fabF* | Fatty acid biosynthesis | DCA |
| A1VYF9 | *acpP* | Fatty acid biosynthesis | DCA |
| A1VZI4 | *fbp* | Carbohydrate biosynthesis | DCA, CDCA |
| A1W0I0 | *gpsA* | Membrane lipid metabolism | DCA, CDCA, GCA |
| A0A0H3PEG0 | *lpxB* | lipid A biosynthetic process | CA, CDCA |
| A0A0H3PAD5 | *lpxD* | LPS lipid A biosynthesis | DCA, TCA, CDCA, GCA |
| Q29VW1 | *gmhA-2* | Carbohydrate biosynthesis | CDCA |
| A0A0H3P9T0 | *gmhA-1* | Carbohydrate biosynthesis | CDCA |
| A0A0H3P9C5 | *mapA* | Lipoprotein | CA |
| A0A0H3PCP8 | *cjj81176_1045* | Putative, Lipoprotein | CA, DCA, TCA |
| A0A0H3PH37 | *cjj81176_1222* | Putative, Lipoprotein | TCA, GCA |
| A0A0H3PA50 | *cjj81176_0126* | Putative, Lipoprotein | CA, DCA, TCA, GCA |
| A0A0H3PBE5 | *cjj81176_0430* | Putative, Lipoprotein | CDCA |
| A0A0H3PI86 | *cjj81176_1476* | Putative, Lipoprotein | GCA |
| A0A0H3PJC9 | *cjj81176_0518* | Putative, Lipoprotein | GCA |
| A1W0G0 | *tatA* | Protein secretion | TCA |
| A0A0H3PAY0 | *tatB* | Protein secretion | GCA |
| A0A0H3PAN7 | *secF* | Protein secretion | DCA, GCA |
| A0A0H3PEE2 | *secG* | Protein secretion | LCA |
| A0A0H3P9B1 | *yajC* | Protein secretion | GCA |
| A0A0H3PAC3 | *cjj81176_1161* | LOS sialylation | DCA, TCA, CDCA, GCA |

### *5.4.2 Chemotaxis and motility*

The results of this study showed that essential proteins of the chemotaxis and motility systems of *C. jejuni* were significantly upregulated in the presence of CA, DCA, CDCA and GCA (Table 16). A brief look into the link between chemotaxis and motility systems of *C. jejuni* will be helpful in understanding the role displayed by the findings of this study. The chemotaxis system of *C. jejuni* is made up of Che proteins, Transducer-like proteins (Tlps) and aerotaxis proteins (Day et al., 2012; Marchant et al., 2002). Che proteins are a two-component regulator-based backbone and comprises of CheW, CheY, CheB, CheR, CheA, CheV and CheZ. Transducer-like proteins (Tlps) are grouped into A, B and C. Group A is composed of Tlps 1, 2, 3, 4. 7 ($7_{mc}$ and $7_{m}$), 10, 11, 12 and 13 (Mund et al., 2016). This group

of Tlps is positioned in the membrane hence role is to sense and transmit the external stimuli (Blair, 1995; Krell et al., 2011). Group B comprise of Tlp9, Aer1 and Aer2 (Marchant et al., 2002). They are positioned both in the membrane and the cytoplasm and are responsible for energy taxis. Tlp9, Aer2 and Aer1 are also referred to as *Campylobacter* energy taxis A (CetA), *Campylobacter* energy taxis C (CetC), and *Campylobacter* energy taxis B (CetB) respectively (Hendrixson et al., 2001; Reuter and van Vliet, 2013). Group C proteins are distinctly found in the cytoplasm and include: Tlp 5, 6, $7_c$ and 8 (Marchant et al., 2002; Tareen et al., 2010). They are responsible for cytoplasmic chemotaxis signaling (Marchant et al., 2002; Zautner et al., 2012). Functionally, Tlps, Che proteins and flagella are interlinked. At the initial stage of response, Tlp become automatically methylated by CheR when chemosensors sense chemoeffectors (Aravind and Ponting, 1999; Kanungpean et al., 2011). Methylated Tlps interact with CheA making it phosphorylated (Blat et al., 1998). Phosphorylated CheA interacts with CheW which transduces the signal to CheY (Parrish et al., 2007). Subsequently, CheY communicates with flagella's FliM proteins directing the flagella to move towards a chemoattractant or away from a chemorepellent (Barak and Eisenbach, 2001).

Based on the foregoing, expression of Che proteins, Tlps, 1, 2 and 4, Cet proteins and FliM, FilY, FilG and FliL by CA, DCA, TCA, and GCA shows that *C. jejuni* responds to the antimicrobial activities of these bile acids by movement. This finding concurs with a previous study by Hugdahl and colleagues which found that CA, DCA, TCA and GCA were chemorepellents for *C. jejuni* (Hugdahl et al., 1988). Similar findings were observed in bile acid resistance studies on *Salmonella enterica* spp. *enterica* ser. Typhimurium (Hernández et al., 2012). Although, LCA, UDCA and CDCA significantly upregulated some chemotaxis proteins, this study did not clearly established there in the response of 81-176 to the antimicrobial activities of these bile acids. DCA, CDCA and GCA significantly upregulated Tlp5. These bile acids are readily transported into the cytoplasm (Armstrong and Carey, 1982; Heuman, 1989). Therefore, it is right to speculate that this protein senses the presences of DCA, CDCA and GCA in the cytoplasm and signals the *C. jejuni* to respond appropriately. This assumption resembles the actions of Tlp and Tar which mediate the responses of *E. coli* to changing levels of cytoplasmic pH (Pham and Parkinson, 2011).

**Table 16**: Significantly upregulated chemotaxis and motility proteins

| Protein | Gene name | Protein function | Bile acid |
|---|---|---|---|
| A0A0H3P9J9 | *aer1/CetB* | Energy taxis | TCA, GCA |
| A0A0H3P9P7 | *aer2/CetC* | Energy taxis | GCA |
| A0A0H3P9T7 | *tlp4* | Sensing external stimuli | GCA |
| A0A0H3PAG7 | *cheW* | Signal transducer | TCA, GCA |
| A0A0H3PAM0 | *cheA* | Transferase | GCA |
| A0A0H3PAN9 | *tlp9/CetA* | Signal transducer | CA, TCA, GCA |
| A0A0H3PB06 | *tlp5* | Sensing internal stimuli | DCA, CDCA, GCA |
| A0A0H3PB49 | *tlp10* | Sensing external stimuli | LCA, UDCA |
| A0A0H3PBN1 | *cheY* | Chemotaxis protein CheY | DCA, CDCA |
| A0A0H3PEF7 | *tlp 1* | Sensing external stimuli | CA, LCA, TCA, GCA |
| A0A0H3PEL1 | *tlp2* | Sensing external stimuli | CA, TCA, GCA |
| A0A0H3PAE1 | *cheR* | Methyltransferase | DCA, CDCA |
| A0A0H3P9L2 | *fliM* | C-ring protein | GCA |
| A0A0H3PA78 | *fliY* | Controls flagellar motor direction | DCA, GCA |
| A0A0H3PAL4 | *fliG* | C-ring protein | CA, DCA |
| A0A0H3PIF6 | *fliL* | Increases torque movement | CA, TCA, GCA |

### *5.4.3 General stresses response*

This study identified a number of reactive oxygen stress (ROS) defense proteins that were significantly upregulated (table 17). This implies that ROS defense mechanism is another important bile acid adaptation strategy that *C. jejuni* employs. Bile acids have been shown to damage cells by generating reactive ROS (Perez, 2009). Studies on microbial physiology have established that ROS in bacteria is generated through tricarboxylic acid cycle (TCA) (Fernie et al., 2004; Kelly, 2001). But ROS species which are generated during normal bacterial metabolic activities are neutralized by the oxidative defense mechanism present in bacteria (Kohanski et al., 2007; Mailloux et al., 2007).

An earlier study, (Kohanski et al., 2007) used microarray technique and showed that norfloxacin, ampicillin and kanamycin achieve their bactericidal activities in bacteria by stimulating TCA to generate uncontrollable quantities of hydroxyl radicals. Interestingly, results of this thesis strongly suggest that CA, DCA, TCA, CDCA and GCA use ROS to achieve their antimicrobial effect in *C. jejuni*. This is suggestion is supported by the significant upregulation of the following proteins in 81-176: arginine biosynthesis (ArgG), Acetyl-CoA biosynthesis (AckA, AcsA), glycolysis/gluconeogenesis (Pgi, Fbp), malate biosynthesis (Cjj81176_1304), C4-dicarboxylate transporters (DcuA and DucB), NAD (NadK), fumarate reductase C (FrdC) and succinate dehydrogenase (SdhB and SdhC). These proteins which were significantly upregulated play a vital role in the TCA cycle of

*C. jejuni* (Hofreuter, 2014; Stahl et al., 2012b). Therefore, it follows that this enhanced activities of TCA led to generation of ROS.

In line to the findings of Kohanski and colleagues, it is correct to assume that 81-176 significantly expressed three categories of defense proteins (table 17) against the detrimental effects of ROS. (i) Proteins for regulation of oxidative stress: Proteins that were grouped into in this category include: (a) SodB and AhpC which are known to convert $O_2^-$ to less harmful $H_2O_2$ and $O_2$ (Kim et al., 2015). (ii) Proteins for DNA repair: CJJ81176_1101, HtrA, RadA and RecN were significantly upregulated. Further they indicate presence of extensive DNA damage especially by TCA and GCA. A similar heavy response to DNA damages has been found in transcriptomics studies on DNA extracted from *C. jejuni* invasion experiments done under human conditions (Gaasbeek et al., 2009; Mills et al., 2012). (iii) Proteins for guarding protein misfolding: DnaJ-1, Nth, HtpG, LigA and DsbD. These results show that GCA and TCA are a leading cause of protein misfolding. Previous studies have found that *C. jejuni* mutants of these proteins show minimal survival in oxidative stress (Flint et al., 2014; Kim et al., 2015; Konkel et al., 1998).

**Table 17**: Significantly upregulated ROS defense proteins

| Protein | Gene name | Protein function | Bile acid |
|---|---|---|---|
| A0A0H3P9V7 | *cjj81176_1101* | DNA repair | DCA, GCA |
| A0A0H3PA52 | *htrA* | DNA repair | TCA |
| A0A0H3PAG5 | *radA* | DNA repair | TCA, GCA |
| A0A0H3PJI4 | *recN* | DNA repair | GCA |
| A0A0H3PB76 | *dnaJ-1* | Protein folding | CA, TCA |
| A0A0H3PEB4 | *nth* | Protein folding | GCA |
| A1VYN0 | *htpG* | Protein folding | GCA |
| A1VYU6 | *ligA* | Protein folding | GCA |
| A0A0H3PBJ5 | *dsbD* | Protein folding | TCA |
| A0A0H3PBY8 | *ahpC* | Regulation of oxidative stress | GCA |
| A1VXQ2 | *sodB* | Regulation of oxidative stress | DCA, CDCA |

### *5.4.4 General adaption responses*

Due to their ability to easily pass across the cell membrane, DCA, CDCA and GCA significantly upregulated: membrane and nucleotide biosynthesis systems and carbon utilization. Other bacteria have also been found to upregulate these biological processes during adaption to harsh environments (Brooks et al., 2011). Below is a brief discussion on the importance of each process in the adaptation of *C. jejuni* to bile acids.

(i) Nucleotide biosynthesis

Like other bacteria, DCA, GCA and CDCA significantly upregulated the following proteins which are essential in nucleotide biosynthesis: DCA upregulated Dcd, PurS, PyrG, PyrE, Apt, and PurM. GCA upregulated: PyrD and Apt. CDCA upregulated: PurS and Apt. This is not unexpected because studies that have looked into the adaptation of lactobacillus and bifidobacteria in bile acid have found a similar trend (Ruiz et al., 2013b). Upregulation of nucleotide biosynthesis assists the bacteria to replace the DNA sections that are destroyed by bile acids. Similarly, upregulation of nucleotide biosynthesis has been found to aid the survival and growth of *E. coli*, *Salmonella enterica* and *Bacillus anthracis* in human blood (Samant et al., 2008). Taken together, enhanced nucleotide biosynthesis is essential for the survival of *C. jejuni* in bile acids.

(ii) Carbon utilization

*C. jejuni* utilizes amino acids as carbon sources in a sequential pattern (Stahl et al., 2012a). However, this study has found that during growth in DCA, GCA and CDCA, 81-176 simultaneously upregulated proteins for the biosynthesis of arginine, serine, histidine, methionine, glutamine, cystine, lysine and leucine. According to (Ruiz et al., 2013b), this significant upregulation in requirements of energy assists bacteria growing in the presence of bile acids to actively synthesize various response mechanisms against effects of bile acids.

(iii) Lipid and carbohydrate biosynthesis

In this study, DCA, CDCA and GCA have significantly upregulated lipid biosynthesis proteins (IpxD, IpxB, cj88176, AcpP, GpsA), fatty acids biosynthesis proteins (FabF, AcpP) and carbohydrate biosynthesis proteins (RpIB, GmhA-1, Pgi, Fbp, GmhA-2). In addition, DCA, CDCA and GCA significantly upregulated protein CysQ. This protein guides the arrangement of proteins into the cell membrane, periplasmic membrane and cell wall (Di Paolo and De Camilli, 2006). Bile acids damage the cell wall of enteric bacteria (Begley et al., 2005b). Consequently, enteric bacteria have been found to maintain the integrity of their cell wall by synthesizing more lipids and carbohydrates (Merritt and Donaldson, 2009). This information together, reveals that *C. jejuni* upregulates lipid biosynthesis proteins, fatty acids biosynthesis proteins and carbohydrate biosynthesis proteins to maintain the integrity of the cell membrane, periplasmic membrane and cell wall.

### *5.4.5 Two-component and other regulatory systems*

This study identified three regulatory systems which sensed the environment and stimulated 81-176 to respond accordingly. They include: First, outer membrane protein R (OmpR) two-component regulatory system which is known to regulate the expression of OmpF and OmpC in response to osmotic stress, temperature and pH (Itou and Tanaka, 2001). However, this system has not been identified in *C. jejuni* before hence its clear function remains unknown. Second, CmeR two-component regulatory system which is known to regulate the multidrug efflux pump CmeABC, membrane transporters, capsular polysaccharide biosynthesis and $C_4$-dicarboxylate transport proteins (Guo et al., 2008). Lastly, *Campylobacter* bile resistance regulator (CbrR) which drives bile acid resistance in *C. jejuni* (Raphael et al., 2005). The role of CmeR and CbrR in relation to this study is to promote resistance against bile acids. Further, they have been found to play an important role in the colonization of *C. jejuni* in chicken (Guo et al., 2008; Raphael et al., 2005).

### *5.4.6 Adaptation to bile acid environment is a well managed process: A lesson from 81-176 response to DCA at 12h and 24h*

As shown in fig. 15 a total number of 111 proteins were significantly upregulated between 81-176 that was cultured in CDB supplemented with DCA at 24h and both 12h and CDB without DCA. Similarly, 116 proteins were significantly upregulated between 81-176 that was cultured in CDB supplemented with DCA at 12h and both 24h and CDB without DCA. Interestingly, the proteins that were significantly up- and downregulated at 24h were different from those that were up- and downregulated at 12h. But functional analysis showed that the proteins at 24h were enhancing the functions of those at 12h. This picture shows that 81-176 expresses a set of genes in response to the need hence adapting comfortably into each situation. This observation is supported by the following two examples.

First, at 12h only SodB stress response factor was significantly upregulated. This shows that at this stage DCA presents a superoxide stress. Hence, SodB is significantly expressed to neutralize its effect (Flint et al., 2014). But at 24h, diverse stress response factors were significantly upregulated. These include: (i) CmeABC and which shows that the level DCA in the cytoplasm was high. Hence the pump was activated to actively pump it from the cytoplasm to the external environment (Lin et al., 2003). (ii) KatA for the detoxification of raised levels of $H_2O_2$ (Day et al., 2000). Similarly, significant upregulation of MacA which has been proven to detoxify $H_2O_2$ in *S. enterica* serova Typhimurium (Bogomolnaya et al., 2013). Though its role in *C. jejuni* has not been investigated. (iii) chaperones DnaJ, DnaJ-1 and GroL were significantly upregulated. Studies in *E. coli* have shown that these chaperones

work jointly to protect proteins from being damaged through misfolding (Gragerov et al., 1992). It therefore implies that at 24h, DCA instituted misfolding of proteins in 81-176. Hence the upregulation of these chaperone to remedy the phenomenon. (iv) ClpX and FtsH were significantly upregulated to maintain appropriate level of essential protein in the cytosol. This is observation is supported by significant upregulation of genes for protein synthesis, namely, RplR,V,T,A,C, RpmC,G,T and RpsL,E,T. A previous study showed that DCA induced *Shigella* spp. to synthesis increased amounts of a group of proteins (Pope et al., 1995). Therefore, the significant upregulation of these genes indicates that DCA induced 81-176 protein synthesis machinery to synthesize large amount of necessary and unnecessary proteins. In response, 81-176 significantly upregulated ClpX and FtsH to eliminate those proteins that it did not need or harmful. This strategy is employed by *E. coli* during stressful situations to degrade proteins which are aimed at destroying its DNA (Ogura et al., 1999; Pruteanu and Baker, 2009).

Second, strengthening the integrity of the outer membrane. At 12h, fatty acid biosynthesis gene, Fab, and lipid biosynthesis gene, IpxD, were significantly expressed. This showed that fatty acids and lipids were synthesized to enhance the integrity of the outer membrane. This observation is supported by the findings in previous studies which showed that bacteria defend themselves against effects of DCA by enriching their outer membrane fatty acids and lipids (Merritt and Donaldson, 2009). At 24h, BamA, LptD, PorA and YidC were significantly upregulated. These genes have been shown to play an important role in maintaining the integrity of outer membrane of bacteria. PorA a is a major component of the cell wall which ordinarily facilitates the transportation of solutes into the cytoplasm and enhances the integrity of the outer membrane (Bolla et al., 1995). A recent study showed that it is aids *C. jejuni* to colonize mice gut (Islam et al., 2010). Also it has been shown that a mutation in porA produces an hypervirulent strain (Wu et al., 2016). BamA has been shown to play a central role in the continuous biogenesis of OMP in *E. coli, Neisseria gonorrhoeae* and *Borrelia burgdorferi* (Albrecht et al., 2014; Lenhart and Akins, 2010; Volokhina et al., 2013). It could be playing a similar role in *C. jejuni*. Equally, LptD has been proven to strengthen outer membrane integrity in *E. coli, S. enterica* serova Typhimurium and *S. flexneri* by inserting lipopolysaccarides into their outer membranes (Gu et al., 2015; Li et al., 2015). A similar role in *C. jejuni* is speculated. This is supported by the significant upregulation of AccB. Finally, YidC has been shown to mediate insertion of sec-independent proteins into the membrane in *E. coli* (Samuelson et al., 2000). This thesis speculates a similar role in *C. jejuni*. This speculation is firmly supported by the significant upregulation of SecD,

SecF and SecG. In addition, the significant upregulation of FtsH which has been shown to support the role of YidC (van Bloois et al., 2008). At 24h, this study identified numerous uncharacterized membrane, periplasmic and outer membrane proteins. Therefore, further investigations are required to link them to BamA, LptD and YidC and understand their role.

## 5.5 Sublethal concentration of bile acids and *Campylobacter* associated virulence factors

The link between bile acids and pathogenesis of enteric pathogens has been established. Two aspects have been of interest. First, the type of bile acid which induces expression of virulence genes. Second, the concentration of a particular bile acid which induces the expression of virulence genes. Both of these aspects were tested in this study. Initially, a survey was done to determine the influence of CA, DCA, LCA, TCA, CDCA, UDCA and GCA on 81-176 to adhere and invade Caco-2 cells. The results shown on fig. 3 have presented a loose picture about the influence of these bile acids on 81-176 to adhere and invade Caco-2 cells. These results show that CA, DCA, TCA, CDCA and GCA influenced 81-176 to adhere and invade Caco-2 cells. In addition, trend of the results on CDCA and DCA was similar to those that were found in a study on *Shigella* spp (Pope et al., 1995). However, the influence of LCA and UDCA on the invasion of Caco-2 cells was not clearly established. These findings are consistent to those that were obtained in the proteomic section of this study.

LCA and UDCA are synthesized from CDCA. Both have an MIC value of 1% indicating that at lower concentrations they are not toxic to 81-176. This observation is further supported by the proteomics results. From the proteomic results, LCA significantly upregulated 4 proteins and significantly downregulated 13 proteins. Similarly, UDCA significantly upregulated 2 proteins and significantly downregulated 4 proteins. Bile acids promote the capability of bacteria to adhere and invade cells by inducing a bacteria to synthesize and release proteins that facilitate the process (Pope et al., 1995). However, the proteins that LCA and UDCA significantly upregulated do not promote invasion. Importantly LCA and UDCA significantly upregulated Sec and Tat proteins. Also, they did not hinder the synthesis of colonization factors such as CiaC and MapA (Barrero-Tobon and Hendrixson, 2014; Johnson et al., 2014). Further, a previous study on invasion of cells by *Helicobacter pylori* found out that LCA didn't hinder invasion (Oliveira et al., 2006). Additionally, UDCA is widely used to treat hepatitis infections with minimal side effects (LU et al., 1995). All these taken together, agree that LCA and UDCA do play a role in promoting the capability of *C. jejuni* to colonize the host.

Interestingly, DCA, TCA, CDCA and GCA induced 81-176 to significantly upregulate and downregulate a high number of proteins. This response assumes the principle associated with bile acids and bacterial pathogenesis. A previous study postulated that bile acids play a significant role in the process (Malik-Kale et al., 2008b). To prove this hypothesis, it was shown that 1% DCA enhanced the ability of *C. jejuni* to invade epithelia cells by secreting invasion proteins. These proteins were called *Campylobacter* invasion antigens (Cia). Further, CiaB was the first DCA induced protein to be identified and characterized. Since then, CiaC (Christensen et al., 2009; Neal-McKinney and Konkel, 2012), CiaD (Samuelson et al., 2013) and CiaI (Buelow et al., 2011) have been identified and their role in the pathogenesis process have been characterized and understood.

DCA has been used to study the role of bile acids in the process of pathogenesis. The proteomic results of this study have identified that TCA, CDCA and GCA also play a role in the pathogenesis of 81-176 (Table 18). An earlier study had concluded that since CiaB was readily synthesized and secreted in the presence of DCA, the other bile acids will too induce it (Rivera-Amill et al., 2001). However, in this study only low concentrations of DCA and CDCA induced the significant upreguration of CiaB. Both CiaB and CiaC are required for maximum internalization of *C. jejuni* into host epithelia cells (Christensen et al., 2009; Konkel et al., 1999).

DCA, TCA, CDCA and GCA concurrently induced the significant expression of glycosylation proteins PseC, PseF, PseG and PseI and flagella proteins FliG and FliY. Similarly, TCA and GCA induced the significant expression of glycosylation protein Pgi, PseB and flagella proteins FliM, FliY, and FliL. N-glycosylation of the flagella is important in the pathogenesis of *C. jejuni* (Larsen et al., 2004; Linton et al., 2005). Mutants of 81-176 lacking *pse* glycosylation genes have demonstrated weak colonization power in chicken, inability to evade host immune and weak adherence and invasion of epithelia cells (Alemka et al., 2012) (Karlyshev, 2004). The functions of motility and chemotaxis are closely related to that of N-glycosylation. DCA, CDCA, TCA and GCA significantly upregulated motility factor. On the other hand, TCA and GCA significantly upregulated chemotaxis factors. A number of studies have shown that motility and chemotaxis play a significant role in aiding *C. jejuni* during colonization (Aihara et al., 2014; Lertsethtakarn et al., 2011).

DCA, TCA, CDCA and GCA induced the expression of various subunits of cytolethal distending toxin (Cdt) system. CDCA induced the significant expression of CdtA; CA, DCA, TCA and CDCA induced the significant expression of CdtC; and finally, CA, DCA, TCA, CDCA and GCA induced the significant expression of CdtB. The complete CdtABC toxin

system is made up of three subunits CdtA, CdtB and CdtC. These subunits play an important role in the pathogenesis of *C. jejuni* (Lee et al., 2003). Functionally, CdtA and CdtC bind onto the cell membrane while CdtB is delivered into the cytoplasm resulting in cell death (Lara-Tejero and Galan, 2001). Hence, CA, DCA, CDCA and GCA promote the activities of CdtABC.

DCA, TCA, CDCA and GCA induced the significant expression of outer membrane proteins. These proteins play an important role in the adherence and invasion of cells by *C. jejuni* (Watson et al., 2014). This observation is supported by significant upregulation of Sec and Tat protein transportation pathways by DCA, LCA, TCA, and GCA. These pathways have been postulated to play a significant role in pathogenesis of *C. jejuni* (Young et al., 2007). Specific virulence associated proteins which are transported by these pathways remain unidentified. However, a previous study has unequivocally shown that *C. jejuni* requires Tat pathway for effective colonization in chicken (Rajashekara et al., 2009). In terms of specific proteins, first, DCA, TCA, CDCA and GCA significantly upregulated Cjj81176_1161. This gene plays a role in the sialylation of lipooligosaccharide (LOS). Sialylation of LOS helps *C. jejuni* to evade the host immune response and also enhances adhesion and invasion of epithelia cells (Louwen et al., 2008). In addition, LOS sialylation plays a major role in the establishment of GBS (Bax et al., 2011). Second, TCA significantly upregulated HtrA which is important during the binding of host cells and *C. jejuni* (Baek et al., 2011). Third, a group of metabolism genes which play a direct role in pathogenesis. DCA significantly upregulated FabF which plays a role in fatty acid biosynthesis. *C. jejuni* has been found to require FabD, FabF, FabG, FabH and FabZ for chicken colonization (Hu et al., 2014; Vries et al., 2017). This finding show that they play an important role in the pathogenesis process of *C. jejuni*. Also DCA and CDCA significantly upregulated Tig. Mutants of Tig have been shown to have impaired colonization in chicken (Hoang et al., 2012). DCA and CDCA significantly expressed Peb4 while TCA and GCA significantly upregulated Peb1. Peb1, 2, 3 and 4 are cytoplasmic lipoproteins. They are important adherence factor. Their mutants have reduced adherence power (Asakura et al., 2007; Pei et al., 1998). Lastly, DCA, CDCA, TCA and GCA significantly upregulated 28, 32, 21 and 58 uncharacterized proteins. These proteins maybe playing a role in the pathogenesis of *C. jejuni*.

**Table 18**: Significantly upregulated known *Campylobacter* associated virulence factors

| Bile acid | Genes of upregulated virulence factor |
|---|---|
| DCA and CDCA | *fabF*, *tig*, *ipxD*, *pseC*, *pseD*, *cbf2* (*peb4*), *cdtC*, *cdtA*, *CJJ81176_1161*, motility (*fliY*, *cj81176_08473*, *cj81176_0342*) and uncharacterized proteins (DCA = 28; CDCA = 32) |
| TCA | *tatA*, *htrA*, *pebC1*, *cdtC*, *cdtB*, *CJJ81176_1161*, chemotaxis (*cheW*, *cheA*, *cj81176_1498*, *cj81176_1128*, *cj81176_1205*, *cj81176_08473*, *cj81176_0180*, *cj81176_0046*, *cj81176_0289*), *pgi*, motility (*fliY*, *fliL*, *fliM*), *peb1*, *ciaC* and 21 uncharacterized proteins. |
| GCA | chemotaxis (*cheW*, *cheA*, *cj81176_1498*, *cj81176_1128*, *cj81176_1205*, *cj81176_08473*, *cj81176_0180*, *cj81176_0046*, *cj81176_0289*), *cdtB*, *pgi*, Motility (*fliY*, *fliM*, *fliL*), *CJJ81176_1161*, *peb1*, *tatB*, *ciaC*, *cj81176_1161*, and 58 uncharacterized proteins. |

## 5.6 Gene c*jp47* influences the ability of *C. jejuni* to survive in bile acids, adhere and invade caco-2 cells in presence of bile acids

Bioinformatics analysis revealed that the gene *cpj47* is a MazF toxin. This toxin promotes the survival of a bacterium in harsh environments by inhibiting global mRNA translation (Starosta et al., 2014). A previous bioinformatics study showed the presence of gene *mazF* in *C. jejuni* (Yan et al., 2012). However, the conditions which swing it into action remain unknown. This thesis reports for the first time that bile acids induce the expression of *mazF*. The growth curve results reveal that *mazF* enhances the survival of *C. jejuni* in these bile acids. $IC_{50}$ values of each of the bile acid used in this study show that each of them is toxic to *C. jejuni*. Therefore, these findings demonstrate an important role of *mazF* in the survival of *C. jejuni* in bile acids. Previous studies have shown the importance of *mazF* in the survival of bacteria in harsh conditions has been observed in other gastrointestinal pathogens. For example: *mazF* has been found to aid (i) *Listeria monocytogenes* to survive in ampicillin and gentamicin (Curtis et al., 2017), (ii) *Staphylococcus aureus* to survive in penicillin G and oxacillin as well as other harsh environments (Schuster et al., 2015) and (iii) *E. coli* to survive in multiple antibiotics, nutrient starvation, oxidative stress, DNA damage, high temperature (Aizenman et al., 1996; Hazan et al., 2004; Tripathi et al., 2014).

In addition, the growth curves showed that *mazF* in 81-176 is activated in response to a particular stress at a particular time. This is shown by the fact that its effects were seen after 20h in CA, DCA, TCA, CDCA and GCA. This observation is supported by the growth curve

of 81-176 cultured in CDB supplemented with DCA 0.05% differentially expressed different proteins at 12h and 24h. Indicating that the stress response after 20h necessitated the activation of *mazF*. A similar strategy is utilized by *mazF* in *E. coli* where it is activated by elevated levels of ROS (Engelberg-Kulka et al., 2009). However, this phenomenon contradicts the situation in *S. aureus* where *mazF* is always activated (Schuster et al., 2015).

In addition to aiding survival of *C. jejuni* in bile acids, *mazF* toxin was found to play a role in influencing the ability of *C. jejuni* to adhere and invade Caco-2 cells. This implies that *mazF* could be playing a role in the pathogenesis of campylobacteriosis. In comparison to Type-II toxins, the role of *mazF* in pathogenesis is poorly investigated. Nevertheless, the contribution of *mazF* to pathogenesis of *Leptospira interrogans* has been established (Komi and et al., 2015). The cellular damages of this pathogen on a host includes macrophage apoptosis. However, mutants lacking *mazF* failed to induce late stage as compared to wild-type. The role of type-II toxins has been found to play a role in the pathogenesis of the following bacteria: *Staphylococcus aureus* (Zhu et al., 2009), *Enterococcus faecalis* (Michaux et al., 2014), *Mycobacterium tuberculosis* (Tiwari et al., 2015), uropathogenic *E. coli* (Norton and Mulvey, 2012), *Salmonella enterica* ssp. *enterica* ser. Typhimurium (Helaine and Kugelberg, 2014; Lobato-Márquez et al., 2015), *Vibrio cholerae* (Wang et al., 2015), *Helicobacter pylori* (Cárdenas-Mondragón et al., 2016) and *Heamophilus influenzae* (Ren et al., 2012). This information taken together, strengthens the findings of this thesis that *mazF* plays a role in the pathogenesis of campylobacteriosis.

## 5.7 Adaptation of 81-176 to 37$^{o}$C and 42$^{o}$C

Regarding temperature adaptation, this study explored adaptation of 81-176 to three scenarios, namely, adaptation to growth at 37$^{o}$C for 24h, adaptation to growth at 42$^{o}$C for 24h and a computer simulated adaptation at 42$^{o}$C for 24h after exposure to bile acids. As shown in fig. 18, during 24h 81-176 is moving into stationary phase. Hence, a comparison between differential expression at 12h and 24h of the above scenarios shows that adaptation to the scenarios involved a shift of genes belonging to the following biological processes: metabolism, stress response, chemotaxis and motility. The key genes in all these categories are briefly described below.

The results show that at 24h in both 37$^{o}$C and 42$^{o}$C, 81-176 utilized diverse branches of its respiration system to generate energy for adaptation. First, the significant upregulation of NuoC, NuoG, Cyf, PetA, and PetC pointed towards usage of aerobic respiration system (Hoffman and Goodman, 1982; Smith et al., 2000; Weerakoon and Olson, 2008). Second, significant upregulation of NrfA, NrfH, FrdC, DcuA and DcuB reflects usage of anaerobic

respiration system (Pittman et al., 2007; Sellars et al., 2002). This respiration diversity has been shown to favour the survival of *C. jejuni* in host cells (Liu et al., 2012). Other sources of carbon were identified. First, the significant upregulation of SdaA and SdaC showed that 81-176 utilized serine as a carbon source. Serine has been shown to aid *C. jejuni* in colonizing chicken gut (Hofreuter et al., 2012; Velayudhan et al., 2004). Second, utilization of proline was shown by the significant upregulation of ProB. This is one of the enzyme which synthesizes proline from glutamine (Arentson et al., 2012). Proline is one of the important amino acids which *C. jejuni* uses as a carbon source when others have been extinguished (Wagley et al., 2014). Lastly, the significant upregulation of HisA and Hisl showed usage of histidine. *C. jejuni* utilizes histidine as a carbon source (Awad et al., 2015). Interesting, 81-176 cultured at 37$^{o}$C for 24h significantly upregulated Peb1C, CadF, MapA, CdtB and CiaC. These proteins play an important role in the pathogenesis of *C. jejuni* (Ó Cróinín and Backert, 2012). Equally, NuoC, NuoG, Cyf, PetA, PetC NrfA, NrfH, FrdC, DcuA, DcuB, SdaC, SdaA, and HisD are essential for *C. jejuni* to colonize the gut of chicken (Hofreuter, 2014). These taken together, show that most *C. jejuni* associated adherence, invasion and colonization factors are from its metabolic processes.

The next group of intertwined adaptation factors are motility, chemotaxis and stress response proteins. The number of these group of factors was higher than at 12h. This can be explained in two ways. First, the significantly upregulated stress response proteins paint a picture of the stresses which 81-176 faced at 24h. These include: (i) chaperones (ClpB, DnaJ, GroL, DnaK, GrpE) which prevent protein misfolding (Andersen, 2005; Holmes et al., 2010), (ii) carbon starvation protein A (CstA) which regulates oxidative stress response, biofilm formation and adherence in *C. jejuni* (Fields and Thompson, 2008; Rasmussen et al., 2013), (iii) SodB which neutralizes $O_2$ and $H_2O_2$ stresses (Flint et al., 2014) and (iv) cyanide-sensitive oxidase (CydA) which eliminates cyanide arising during respiration (Jackson et al., 2007). The second explanation is the reduced level of nutrients in the broth. This is supported by the significant upregulation of CstA and a previous study which showed reduced nutrients at 24h (Wright et al., 2009b). Taken together, 81-176 activated the flagella and chemotaxis to search for nutrients and less stressful environment.

# 6.0 CONCLUSION AND FUTURE WORK

## 6.1 Conclusions

This study has established the following: First, large scale *C. jejuni* proteomic research requires usage of complementary quantitative proteomic techniques. For example, the proteomic results of this study clearly showed that SILAC and SWATH have different advantages and disadvantages. But there complementary application could have generated improved data. Second, all bile acids are toxic to *C. jejuni*. As results, they lead to shift in metabolism pathways in *C. jejuni*. However, the shift depends on the ability of the bile acid to pass across the membrane. For example, DCA, CDCA and GCA easily migrate across the membrane of *C. jejuni* hence because increased energy biosynthesis. On the other side, LCA and UDCA find it difficult to pass across the membrane hence cause little shift in normal metabolism. Third, CA, DCA, LCA, TCA, CDCA, UDCA and GCA stimulates *C. jejuni* synthesize more membrane proteins. This implies that these bile acids damage the membrane hence the need to replenish it. Fouth, though DCA, CDCA, GCA and TCA migrate across *C. jejuni* membrane, they generate different reactive oxidative defense mechanisms. Fifth, this study found *mazF* toxin and a putative *relBE* toxin. These genes are expressed in the presence of different concentrations of bile acids: *mazF* is significantly expressed in the presence of low concentrations of CA, LCA, TCA and UDCA while putative *relBE* is significantly expressed in the presence of low concentrations of DCA, CDCA and GCA. This confirms that *C. jejuni* has a robust toxin-antitoxin system which assists it to survive in adverse environments. Sixth, all bile acids have the potential to stimulate *C. jejuni* to synthesize factors which aid it to adhere and invade epithelia cells. Adherence and invasion is driven by different proteins including lipoproteins, outer membrane proteins, chemotaxis, toxins and motility proteins, toxin-antitoxin proteins, proteins resulting from metabolism in adverse environments and transportation channels. Finally, this study has shown the *mazF* is important in the survival of *C. jejuni* in all bile acids but at different periods. Also in the adherence and invasion of epithelia cells in the presence of these bile acids. In conclusion, the information that this study has generated information that will further the understanding of the biology of *C. jejuni*.

## 6.2 Future work

To the best of my knowledge, this is the first study which has comprehensively examined the proteomic response of 81-176 to sub lethal concentrations of dominant bile acids in the human small intestines. It has generated interesting results which have opened a door to a number of interesting questions which need further investigations. These include:

a) Numerous uncharacterized outer, periplasmic and innre membrane proteins were differentially expressed. Therefore there is need to characterize them and understand their role in protecting the membrane against damage by bile acids.
b) Bile acids uptake systems was unclear. There is a need for further investigation. There identity could be potential drug targets.
c) A number of uptake systems were differentially expressed. Therefore, there is need for further investigations to understand which uptake systems transport bile acids across the cell membrane.
d) Up-and-down-regulation of protein transport channels to the outer membrane, periplasmic membrane and inner membrane show the bile acids triggers rearrangement of proteins in these regions. Therefore, there is a need to investigate these changes.
e) Differential expression and identification of putative YaaA. Therefore, further investigation is required to characterize it and understand its role in oxidative defense response.
f) Differential expression of MazF and putative RelE toxins. Hence, further investigations are required to understand the molecular basis of their actions.
g) All know virulence factors were differentially expressed. Hence, there is need for further investigation to understand the role of bile acids driven metabolism in the pathogenesis of *C. jejuni*.
h) A number of lipoproteins were differentially expressed. Hence, further investigation is required to understand lipoprotein export channels to the outer membrane and their role in protecting *C. jejuni* against the antimicrobial activities of bile acids.
i) There is need to identify and compare proteins that are induced in 81-176 at 16h, 20h, 24h, 36h and 48h. This analysis will provide insight into how 81-176 responds to bile acid generated stresses that are presented over a period of time.

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# APPENDICES

## **Appendix 1**: SILAC - Quantified Proteins

| T: Protein IDs | C: T-test Significant | C: Filter | C: T-test Significant _T-test Significant | Protein name | N: Razor + unique peptides | N: Q-value |
|---|---|---|---|---|---|---|
| A0A0H3ADZ7 | | Discard | | DNA binding protein HU | 2 | 0 |
| A0A0H3P979 | | Discard | | Hydrolase, carbon-nitrogen family | 4 | 0 |
| A0A0H3P984 | | Discard | | Uncharacterize protein | 1 | 0 |
| A0A0H3P987 | | Discard | | Putative, oxaloacetate decarboxylase | 7 | 0 |
| A0A0H3P999 | | Discard | | Uncharacterized protein | 5 | 0 |
| A0A0H3P9B7 | + | Discard | +_+ | Cytochrome c553 | 1 | 0 |
| A0A0H3P9D3 | | Discard | | Putative, dihydroorotase | 2 | 0 |
| A0A0H3P9D4 | | Discard | | Oligoendopeptidase F | 7 | 0 |
| A0A0H3P9D5 | | Discard | | ATP-dependent zinc metallopeptidase | 9 | 0 |
| A0A0H3P9E6 | | Discard | | Uncharacterized protein | 4 | 0 |
| A0A0H3P9F6 | | Discard | | CCP47 | 4 | 0 |
| A0A0H3P9F9 | | Discard | | Mechanosensitive ion channel family protein | 1 | 0.0025773 |
| A0A0H3P9G7 | | Discard | | Coproporphyrinogen III oxidase | 8 | 0 |
| A0A0H3P9H5 | | Discard | | D-3-phosphoglycerate dehydrogenase | 6 | 0 |
| A0A0H3P9I8 | + | Discard | +_+ | Arginine decarboxylase | 3 | 0 |
| A0A0H3P9J4 | | Discard | | Arylsulfate sulfotransferase, degenerate | 15 | 0 |
| A0A0H3P9J6 | | Discard | | Phosphate acetyltransferase | 5 | 0 |
| A0A0H3P9K6 | | Discard | | Type II restriction-modification enzyme | 6 | 0 |
| A0A0H3P9K8 | | Discard | | Iron-sulfur cluster binding protein | 10 | 0 |
| A0A0H3P9L2 | | Discard | | Flagellar motor switch protein FliM | 9 | 0 |
| A0A0H3P9L6 | | Discard | | Cell division protein FtsA | 1 | 0 |
| A0A0H3P9L7 | + | Discard | +_+ | Uncharacterized protein | 7 | 0 |
| A0A0H3P9M5 | + | Discard | +_+ | Adenylosuccinate lyase | 10 | 0 |
| A0A0H3P9M7 | | Discard | | Aconitate hydratase B | 22 | 0 |
| A0A0H3P9N0 | | Discard | | Flagellar biosynthetic protein FlhF | 2 | 0 |
| A0A0H3P9N5 | | Discard | | Cytochrome c family protein | 3 | 0 |
| A0A0H3P9N6 | | Discard | | Conserved domain protein | 2 | 0 |
| A0A0H3P9P7 | | Discard | | Methyl-accepting chemotaxis protein | 11 | 0 |
| A0A0H3P9P8 | | Discard | | Transketolase | 6 | 0 |
| A0A0H3P9P9 | | Discard | | Inosine-5'-monophosphate dehydrogenase | 13 | 0 |
| A0A0H3P9Q2 | | Discard | | Cytochrome c-552 | 9 | 0 |
| A0A0H3P9Q4 | + | Discard | +_+ | Catalase | 6 | 0 |
| A0A0H3P9Q8 | | Discard | | Oxidoreductase, 2-nitropropane dioxygenase family | 11 | 0 |
| A0A0H3P9R4 | | Discard | | L-serine ammonia-lyase | 3 | 0 |
| A0A0H3P9R9 | | Discard | | Cytochrome c oxidase, cbb3-type, subunit II | 5 | 0 |
| A0A0H3P9S2 | + | Discard | +_+ | S-adenosylmethionine synthetase | 5 | 0 |
| A0A0H3P9T1 | + | Discard | +_+ | Glyceraldehyde-3-phosphate dehydrogenase | 6 | 0 |
| A0A0H3P9T4 | + | Discard | +_+ | Nitroreductase family protein | 7 | 0 |
| A0A0H3P9T7 | | Discard | | Methyl-accepting chemotaxis protein | 5 | 0 |
| A0A0H3P9W4 | | Discard | | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase | 3 | 0 |
| A0A0H3P9X6 | + | Discard | +_+ | Nitroreducatase family protein | 3 | 0 |
| A0A0H3P9Y0 | | Discard | | Peptidase, M23/M37 family | 6 | 0 |
| A0A0H3P9Y3 | | Discard | | Signal recognition particle receptor FtsY | 3 | 0 |

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|---|---|---|---|---|---|---|
| A0A0H3P9Y5 | | Discard | | Cpp21 | 3 | 0 |
| A0A0H3P9Y9 | | Discard | | L-lactate dehydrogenase | 2 | 0 |
| A0A0H3PA02 | | Discard | | Peptidase, M16 family | 3 | 0 |
| A0A0H3PA13 | | Discard | | Putative sugar transferase | 0 | |
| A0A0H3PA14 | | Discard | | Ribose-phosphate pyrophosphokinase | 7 | 0 |
| A0A0H3PA15 | + | Discard | +_+ | Oxidoreductase, dehydrogenase/reductase family | 6 | 0 |
| A0A0H3PA46 | | Discard | | DNA topoisomerase 1 | 10 | 0 |
| A0A0H3PA47 | | Discard | | Ribonuclease J | 4 | 0 |
| A0A0H3PA50 | | Discard | | Putative, Lipoprotein | 7 | 0 |
| A0A0H3PA52 | | Discard | | Protease DO | 5 | 0 |
| A0A0H3PA63 | | Discard | | Uncharacterized protein | 3 | 0 |
| A0A0H3PA64 | + | Discard | +_+ | Gamma-glutamyltransferase | 6 | 0 |
| A0A0H3PA74 | | Discard | | Periplasmic nitrate reductase, electron transfer subunit | 3 | 0 |
| A0A0H3PA81 | | Discard | | Flavin-dependent thymidylate synthase | 8 | 0 |
| A0A0H3PA82 | | Discard | | Phosphomannomutase/ phosphoglucomutase | 5 | 0 |
| A0A0H3PA83 | | Discard | | Cytochrome C | 1 | 0.0037267 |
| A0A0H3PA85 | | Discard | | DNA gyrase subunit B | 9 | 0 |
| A0A0H3PA88 | + | Discard | +_+ | Putative, Lipoprotein | 4 | 0 |
| A0A0H3PA93 | | Discard | | Putative, periplasmic solute binding protein | 5 | 0 |
| A0A0H3PA94 | + | Discard | +_+ | Putative, Peptidase | 4 | 0 |
| A0A0H3PA97 | | Discard | | Putative, TolB protein | 5 | 0 |
| A0A0H3PA98 | | Discard | | Uncharacterized protein | 3 | 0 |
| A0A0H3PAA8 | | Discard | | DegT/DnrJ/EryC1/StrS aminotransferase family | 2 | 0 |
| A0A0H3PAB0 | + | Discard | +_+ | Uncharacterized protein | 5 | 0 |
| A0A0H3PAB8 | | Discard | | Amidophosphoribosyltransferase | 11 | 0 |
| A0A0H3PAB9 | | Discard | | Putative, Endoribonuclease L-PSP | 1 | 0 |
| A0A0H3PAC1 | | Discard | | NADH-quinone oxidoreductase, G subunit | 5 | 0 |
| A0A0H3PAC6 | + | Discard | +_+ | Delta-aminolevulinic acid dehydratase | 4 | 0 |
| A0A0H3PAD0 | | Discard | | Pyruvate-flavodoxin oxidoreductase | 22 | 0 |
| A0A0H3PAD5 | | Discard | | UDP-3-O-acylglucosamine N-acyltransferase | 2 | 0 |
| A0A0H3PAE0 | | Discard | | RloH | 4 | 0 |
| A0A0H3PAE3 | | Discard | | Quinone-reactive Ni/Fe-hydrogenase | 4 | 0 |
| A0A0H3PAE4 | + | Discard | +_+ | RND efflux system | 7 | 0 |
| Q5QKR6;A0A0H3PAF7 | | Discard | | Acetyl-CoA carboxylase, biotin carboxylase | 5 | 0 |
| A0A0H3PAG1 | | Discard | | Pyridine nucleotide-disulphide oxidoreductase family protein | 1 | 0 |
| A0A0H3PAG7 | | Discard | | Purine-binding chemotaxis protein CheW | 7 | 0 |
| A0A0H3PAG9 | | Discard | | Hydrolase, TatD family | 3 | 0 |
| A0A0H3PAH7 | | Discard | | 2-oxoglutarate:acceptor oxidoreductase, alpha subunit | 11 | 0 |
| A0A0H3PAI4 | | Discard | | Isoleucine--tRNA ligase | 17 | 0 |
| A0A0H3PAI7 | | Discard | | Uncharacterized protein | 1 | 0 |
| A0A0H3PAI9 | | Discard | | UDP-glucose 4-epimerase | 8 | 0 |
| A0A0H3PAJ7 | | Discard | | Quinone-reactive Ni/Fe-hydrogenase, large subunit | 10 | 0 |
| A0A0H3PAJ8 | | Discard | | High affinity branched-chain amino acid ABC transporter, ATP-binding protein | 2 | 0 |
| A0A0H3PAK2 | | Discard | | Nitrogen fixation protein NifU | 4 | 0 |
| A0A0H3PAK5 | | Discard | | RNA polymerase sigma factor RpoD | 6 | 0 |
| A0A0H3PAK6 | + | Discard | +_+ | TonB-dependent heme receptor | 6 | 0 |
| A0A0H3PAL7 | | Discard | | Putative, Malate:quinone oxidoreductase | 2 | 0 |
| A0A0H3PAM0 | | Discard | | Chemotaxis protein CheA | 12 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| A0A0H3PAM2 | | Discard | | Cell shape-determining protein MreB | 9 | 0 |
| A0A0H3PAM8 | | Discard | | Hydrolase, carbon-nitrogen family | 3 | 0 |
| A0A0H3PAN9; A0A0H3P9S7 | | Discard | | Methyl-accepting chemotaxis protein | 0 | |
| A0A0H3PAP4; Q29W34 | | Discard | | Putative, Oxidoreductase | 13 | 0 |
| A0A0H3PAS0 | | Discard | | UvrABC system protein B | 6 | 0 |
| A0A0H3PAT0 | | Discard | | Cysteine synthase A | 3 | 0 |
| A0A0H3PAT6 | | Discard | | Phosphatase, Ppx/GppA family | 3 | 0 |
| A0A0H3PAT9 | | Discard | | UvrABC system protein A | 10 | 0 |
| A0A0H3PAU4 | | Discard | | Putative, Molybdopterin biosynthesis MoeA protein | 3 | 0 |
| A0A0H3PAU9 | | Discard | | Uncharacterized protein | 3 | 0 |
| A0A0H3PAV5 | | Discard | | Cystathionine beta-lyase | 8 | 0 |
| A0A0H3PAY8 | + | Discard | +_+ | 50S ribosomal protein L13 | 4 | 0 |
| A0A0H3PB06 | | Discard | | Methyl-accepting chemotaxis protein | 2 | 0 |
| A0A0H3PB11 | | Discard | | DNA helicase | 4 | 0 |
| A0A0H3PB14 | | Discard | | D-isomer specific 2-hydroxyacid dehydrogenase family protein | 1 | 0 |
| A0A0H3PB21 | | Discard | | Iron-sulfur cluster carrier protein | 3 | 0 |
| A0A0H3PB24 | | Discard | | Fibronectin type III domain protein | 11 | 0 |
| A0A0H3PB33 | | Discard | | Biotin sulfoxide reductase | 11 | 0 |
| A0A0H3PB37 | | Discard | | Transcriptional regulator, MerR family | 3 | 0 |
| A0A0H3PB56 | | Discard | | UTP--glucose-1-phosphate uridylyltransferase | 4 | 0 |
| A0A0H3PB58 | + | Discard | +_+ | Aspartokinase | 8 | 0 |
| A0A0H3PB61 | + | Discard | +_+ | Transcription termination/antitermination protein NusA | 5 | 0 |
| A0A0H3PB79 | + | Discard | +_+ | RND efflux system, inner membrane transporter CmeB | 7 | 0 |
| A0A0H3PB91 | + | Discard | +_+ | Ferredoxin, 4Fe-4S | 4 | 0 |
| A0A0H3PB93 | | Discard | | ModE repressor domain protein | 5 | 0 |
| A0A0H3PBA0 | | Discard | | Carbamoyl-phosphate synthase small chain | 4 | 0 |
| A0A0H3PBA4 | + | Discard | +_+ | Oxidoreductase, putative | 10 | 0 |
| A0A0H3PBB6 | | Discard | | Anthranilate synthase component I | 3 | 0 |
| A0A0H3PBF3 | + | Discard | +_+ | DNA-binding response regulator | 5 | 0 |
| A0A0H3PBG3 | | Discard | | Hydrogenase expression/formation protein | 4 | 0 |
| Q7X516;A0A0H3PBG5 | + | Discard | +_+ | Flagellin | 2 | 0 |
| A0A0H3PBH7 | | Discard | | Carboxynorspermidine/carboxyspermidine decarboxylase | 4 | 0 |
| A0A0H3PBI3 | | Discard | | Bifunctional protein PutA | 4 | 0 |
| A0A0H3PBK9 | + | Discard | +_+ | Flagellar protein FlaG | 4 | 0 |
| A0A0H3PBL4 | | Discard | | Hydrogenase expression/formation protein HypE | 3 | 0 |
| A0A0H3PBN1 | | Discard | | DNA-binding response regulator | 4 | 0 |
| A0A0H3PBP8 | | Discard | | Uncharacterized protein | 2 | 0 |
| A0A0H3PBQ2 | | Discard | | Succinate dehydrogenase, flavoprotein subunit | 18 | 0 |
| A0A0H3PBR9 | + | Discard | +_+ | Cytochrome c551 peroxidase | 1 | 0 |
| A0A0H3PBT1 | + | Discard | +_+ | Uncharacterized protein | 3 | 0 |
| A0A0H3PBT4 | | Discard | | SPFH domain / Band 7 family protein | 7 | 0 |
| A0A0H3PBV6 | | Discard | | Putative, Cysteine desulfurase | 6 | 0 |
| A0A0H3PBV9 | | Discard | | 2-oxoglutarate:acceptor oxidoreductase, delta subunit | 4 | 0 |
| A0A0H3PBW9 | | Discard | | ATP-dependent chaperone protein ClpB | 9 | 0 |
| A0A0H3PCA0 | | Discard | | Cpp12 | 4 | 0 |
| A0A0H3PCE2 | | Discard | | Carbon starvation protein A | 1 | 0.0026008 |
| A0A0H3PCF8 | | Discard | | Putative, Membrane protein | 2 | 0 |
| A0A0H3PCH2 | | Discard | | Rubrerythrin | 4 | 0 |

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|---|---|---|---|---|---|---|
| A0A0H3PCH6 | + | Discard | +_+ | Ribosomal protein L3 | 6 | 0 |
| A0A0H3PCJ0 | | Discard | | Site-determining protein | 3 | 0 |
| A0A0H3PCK6 | | Discard | | L-asparaginase | 1 | 0 |
| A0A0H3PCL3 | | Discard | | Cytolethal distending toxin, subunit B | 7 | 0 |
| A0A0H3PCL7 | | Discard | | Ribonucleoside-diphosphate reductase | 6 | 0 |
| A0A0H3PCM5 | | Discard | | Homoserine O-acetyltransferase | 5 | 0 |
| A0A0H3PCN0 | | Discard | | Uncharacterized protein | 5 | 0 |
| A0A0H3PCQ6 | | Discard | | High affinity branched-chain amino acid ABC transporter, periplasmic amino acid-binding protein | 2 | 0 |
| A0A0H3PCR0 | | Discard | | Cytochrome b | 1 | 0 |
| A0A0H3PCT8 | | Discard | | Cytochrome c biogenesis protein, CcmF/CycK/CcsA family | 9 | 0 |
| A0A0H3PCU9 | | Discard | | DNA gyrase subunit A | 17 | 0 |
| A0A0H3PD07 | | Discard | | C4-dicarboxylate transport protein | 1 | 0 |
| A0A0H3PD54 | + | Discard | +_+ | Acetyl-CoA carboxylase, biotin carboxylase | 15 | 0 |
| A0A0H3PD61 | | Discard | | Uncharacterized protein | 2 | 0 |
| A0A0H3PD65 | | Discard | | High affinity branched-chain amino acid ABC transporter, periplasmic amino acid-binding protein | 6 | 0 |
| A0A0H3PD77 | | Discard | | Uncharacterized protein | 2 | 0 |
| A0A0H3PD83 | | Discard | | Signal peptidase I | 7 | 0 |
| A0A0H3PD97 | | Discard | | Signal recognition particle protein | 9 | 0 |
| A0A0H3PD99 | | Discard | | Uncharacterized protein | 3 | 0 |
| A0A0H3PDC4 | | Discard | | DNA-directed DNA polymerase | 6 | 0 |
| A0A0H3PDD3 | + | Discard | +_+ | Molybdenum cofactor biosynthesis protein | 3 | 0 |
| A0A0H3PDJ1 | | Discard | | Oxidoreductase, zinc-binding dehydrogenase family | 3 | 0 |
| A0A0H3PDK8 | | Discard | | Uncharacterized protein | 5 | 0 |
| A0A0H3PDT1 | | Discard | | Uncharacterized protein | 3 | 0 |
| A0A0H3PDU5 | | Discard | | Tyrosine--tRNA ligase | 5 | 0 |
| A0A0H3PDV4 | + | Discard | +_+ | Putative methyltransferase | 3 | 0 |
| A0A0H3PE30 | | Discard | | Adenylosuccinate lyase | 4 | 0 |
| A0A0H3PE83 | | Discard | | PDZ domain protein | 5 | 0 |
| A0A0H3PE88 | | Discard | | Uncharacterized protein | 8 | 0 |
| A0A0H3PEA7 | | Discard | | 2-oxoglutarate:acceptor oxidoreductase, beta subunit | 9 | 0 |
| A0A0H3PED8 | + | Discard | +_+ | Major antigenic peptide PEB3 | 4 | 0 |
| A0A0H3PEE7 | | Discard | | 2-oxoglutarate:acceptor oxidoreductase, gamma subunit | 3 | 0 |
| A0A0H3PEF4 | | Discard | | Fumarate reductase, flavoprotein subunit | 20 | 0 |
| A0A0H3PEG8 | + | Discard | +_+ | Putative, Phosphate ABC transporter, periplasmic phosphate-binding protein | 2 | 0 |
| A0A0H3PEI3 | | Discard | | Rare lipoprotein A | 6 | 0 |
| A0A0H3PEJ1 | | Discard | | Acetolactate synthase | 4 | 0 |
| A0A0H3PEK3 | | Discard | | Branched-chain amino acid aminotransferase | 7 | 0 |
| A0A0H3PEL1; A0A0H3PEF7 | | Discard | | Methyl-accepting chemotaxis protein | 0 | |
| A0A0H3PEN5 | | Discard | | Uncharacterized protein | 1 | 0 |
| A0A0H3PEP2 | | Discard | | DNA polymerase | 4 | 0 |
| A0A0H3PET5 | + | Discard | +_+ | Putative, TonB-dependent receptor | 8 | 0 |
| A0A0H3PEU8 | + | Discard | +_+ | Peptidoglycan-associated lipoprotein Omp18 | 4 | 0 |
| A0A0H3PEV5 | + | Discard | +_+ | Uncharacterized protein | 1 | 0 |
| A0A0H3PEV8 | | Discard | | Penicillin-binding protein 1A | 5 | 0 |
| A0A0H3PEW2 | | Discard | | Iron ABC transporter, periplasmic iron-binding protein | 3 | 0 |
| A0A0H3PEW6 | + | Discard | +_+ | Uncharacterized protein | 3 | 0 |
| A0A0H3PEZ1 | | Discard | | Succinate dehydrogenase iron-sulfur | 8 | 0 |

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|---|---|---|---|---|---|---|
| | | | | subunit | | |
| A0A0H3PF03 | | Discard | | 3-oxoacyl-[acyl-carrier-protein] synthase 2 | 5 | 0 |
| A0A0H3PF06 | | Discard | | Aminotransferase, classes I and II | 11 | 0 |
| A0A0H3PF18 | | Discard | | ABC transporter, ATP-binding protein | 15 | 0 |
| A0A0H3PF34 | | Discard | | Flagellar M-ring protein | 9 | 0 |
| A0A0H3PGI9 | | Discard | | Uncharacterized protein | 4 | 0 |
| A0A0H3PGK7 | | Discard | | Probable cytosol aminopeptidase | 6 | 0 |
| A0A0H3PGM1 | + | Discard | +_+ | Aspartate ammonia-lyase | 12 | 0 |
| A0A0H3PGP7 | + | Discard | +_+ | Flagellar hook protein FlgE | 15 | 0 |
| A0A0H3PGQ5 | | Discard | | GTP-binding protein TypA | 16 | 0 |
| A0A0H3PGQ9 | | Discard | | Signal peptide peptidase SppA, 36K type | 2 | 0 |
| A0A0H3PGR5 | | Discard | | Dissimilatory sulfite reductase | 7 | 0 |
| A0A0H3PGS5 | | Discard | | 3-isopropylmalate dehydrogenase | 2 | 0 |
| A0A0H3PGV5 | + | Discard | +_+ | Molybdopterin oxidoreductase family protein | 6 | 0 |
| A0A0H3PGW7 | | Discard | | Lon protease | 11 | 0 |
| A0A0H3PGY0 | + | Discard | +_+ | Peptidyl-prolyl cis-trans isomerase | 2 | 0 |
| A0A0H3PHD0 | | Discard | | Uncharacterized protein | 2 | 0 |
| A0A0H3PHD6 | + | Discard | +_+ | Glutamine synthetase | 12 | 0 |
| A0A0H3PHD8 | | Discard | | Valine--tRNA ligase | 7 | 0 |
| A0A0H3PHE2 | + | Discard | +_+ | Lipoprotein, NLPA family | 4 | 0 |
| A0A0H3PHE9 | | Discard | | Flagellin | 3 | 0 |
| A0A0H3PHJ0 | | Discard | | 5-hydroxyisourate hydrolase | 1 | 0 |
| A0A0H3PHL6 | | Discard | | Major antigenic peptide PEB2 | 2 | 0 |
| A0A0H3PHR2 | | Discard | | Phenylalanine--tRNA ligase beta subunit | 8 | 0 |
| A0A0H3PHX6 | | Discard | | Uncharacterized protein | 3 | 0 |
| A0A0H3PHZ1 | + | Discard | +_+ | Fumarate hydratase class II | 4 | 0 |
| A0A0H3PI03 | | Discard | | Uncharacterized protein | 1 | 0 |
| A0A0H3PI37 | | Discard | | NADH-quinone oxidoreductase | 5 | 0 |
| A0A0H3PI41 | | Discard | | Uncharacterized protein | 3 | 0 |
| A0A0H3PI52 | | Discard | | 50S ribosomal protein L15 | 5 | 0 |
| A0A0H3PI76 | | Discard | | Hydrogenase, (NiFe)/(NiFeSe) small subunit family | 3 | 0 |
| A0A0H3PI81 | | Discard | | Citrate synthase | 15 | 0 |
| A0A0H3PI91 | + | Discard | +_+ | Major outer membrane protein | 6 | 0 |
| A0A0H3PI95 | + | Discard | +_+ | DNA-binding response regulator | 7 | 0 |
| A0A0H3PIA8 | + | Discard | +_+ | Enoyl-[acyl-carrier-protein] reductase [NADH] | 3 | 0 |
| A0A0H3PID6 | | Discard | | NADP-dependent malic enzyme, truncation | 5 | 0 |
| A0A0H3PIE6 | + | Discard | +_+ | Uncharacterized protein | 4 | 0 |
| A0A0H3PIR1 | | Discard | | Formate dehydrogenase, alpha subunit, selenocysteine-containing | 6 | 0 |
| A0A0H3PIS8 | | Discard | | ATP-dependent Clp protease ATP-binding subunit ClpX | 6 | 0 |
| A0A0H3PIV6 | | Discard | | 3,4-dihydroxy-2-butanone 4-phosphate synthase | 2 | 0 |
| A0A0H3PIW2 | + | Discard | +_+ | Antioxidant, AhpC/Tsa family | 7 | 0 |
| A0A0H3PIY7 | + | Discard | +_+ | Pyruvate kinase | 8 | 0 |
| A0A0H3PJ06 | | Discard | | Cyclic dehypoxanthine futalosine synthase | 10 | 0 |
| A0A0H3PJ11 | | Discard | | PP-loop family protein | 5 | 0 |
| A0A0H3PJ24 | | Discard | | Isocitrate dehydrogenase, NADP-dependent | 13 | 0 |
| A0A0H3PJ47 | | Discard | | Outer membrane protein assembly factor BamA | 12 | 0 |
| A0A0H3PJ87 | + | Discard | +_+ | TPR domain protein | 4 | 0 |
| A0A0H3PJB7 | | Discard | | Succinate dehydrogenase, iron-sulfur protein subunit | 8 | 0 |
| A0A0H3PJC4 | | Discard | | Putative, Chemotaxis protein MotB | 1 | 0 |
| A0A0H3PJH5 | | Discard | | Carbamoyl-phosphate synthase large chain | 15 | 0 |

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|---|---|---|---|---|---|---|
| A0A0H3PJH9 | | Discard | | Carboxyl-terminal protease | 6 | 0 |
| A0A0H3PJJ8 | + | Discard | +_+ | Chorismate mutase/prephenate dehydratase | 6 | 0 |
| A0A0H3PJM2 | | Discard | | Saccharopine dehydrogenase | 5 | 0 |
| A1VX91 | | Discard | | Dihydroxy-acid dehydratase (DAD) | 9 | 0 |
| A1VXA6 | | Discard | | CTP synthase | 11 | 0 |
| A1VXG4 | | Discard | | Cytolethal distending toxin subunit A | 3 | 0 |
| A1VXH8 | | Discard | | 50S ribosomal protein L27 | 6 | 0 |
| A1VXI7 | | Discard | | ATP synthase subunit delta | 5 | 0 |
| A1VXI8 | | Discard | | ATP synthase subunit alpha | 16 | 0 |
| A1VXJ0 | + | Discard | +_+ | ATP synthase subunit beta | 9 | 0 |
| A1VXL9 | | Discard | | Translation initiation factor IF-2 | 7 | 0 |
| A1VXS0 | + | Discard | +_+ | ATP-dependent Clp protease proteolytic subunit | 7 | 0 |
| A1VXS1 | | Discard | | Trigger factor | 5 | 0 |
| A1VXS2 | + | Discard | +_+ | GTP cyclohydrolase 1 | 4 | 0 |
| A1VXS5 | | Discard | | 4-hydroxy-tetrahydrodipicolinate reductase | 9 | 0 |
| A1VXT5 | | Discard | | Threonine--tRNA ligase | 10 | 0 |
| A1VXT6 | | Discard | | Translation initiation factor IF-3 | 5 | 0 |
| A1VXW7 | + | Discard | +_+ | 50S ribosomal protein L20 | 6 | 0 |
| A1VXZ7 | | Discard | | 3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ | 2 | 0 |
| A1VXZ8 | | Discard | | UDP-N-acetylglucosamine acyltransferase | 8 | 0 |
| A1VY04 | | Discard | | Transaldolase | 5 | 0 |
| A1VY10 | + | Discard | +_+ | Transcription elongation factor GreA | 3 | 0 |
| A1VY17 | | Discard | | Pantothenate synthetase | 2 | 0 |
| A1VY30 | + | Discard | +_+ | 50S ribosomal protein L25 | 5 | 0 |
| A1VY45 | | Discard | | Phosphoserine aminotransferase | 2 | 0 |
| A1VY47 | | Discard | | 3-oxoacyl-[acyl-carrier-protein] synthase 3 | 2 | 0 |
| A1VY51 | | Discard | | Nucleoside diphosphate kinase | 2 | 0 |
| A1VY69 | | Discard | | Tryptophan synthase beta chain | 3 | 0 |
| A1VY80 | | Discard | | Phosphoglucosamine mutase | 7 | 0 |
| A1VY90 | | Discard | | 30S ribosomal protein S21 | 5 | 0 |
| A1VY92 | | Discard | | Putative, Lipoprotein | 2 | 0 |
| A1VY95 | | Discard | | Uncharacterized protein | 2 | 0 |
| A1VYA4 | | Discard | | 2-dehydro-3-deoxyphosphooctonate aldolase | 4 | 0 |
| A1VYA9 | | Discard | | Serine--tRNA ligase | 5 | 0 |
| A1VYC1 | | Discard | | Lysine--tRNA ligase | 12 | 0 |
| A1VYC2 | | Discard | | Serine hydroxymethyltransferase | 11 | 0 |
| A1VYF1 | | Discard | | 2,3-bisphosphoglycerate-independent phosphoglycerate mutase | 3 | 0 |
| A1VYG6 | | Discard | | 50S ribosomal protein L28 | 6 | 0 |
| A1VYG9 | + | Discard | +_+ | Phosphomethylpyrimidine synthase | 4 | 0 |
| A1VYH4 | | Discard | | tRNA-2-methylthio-N(6)-dimethylallyladenosine synthase | 4 | 0 |
| A1VYJ0 | + | Discard | +_+ | 50S ribosomal protein L11 | 4 | 0 |
| A1VYJ1 | + | Discard | +_+ | 50S ribosomal protein L1 | 3 | 0 |
| A1VYJ2 | + | Discard | +_+ | 50S ribosomal protein L10 | 2 | 0 |
| A1VYJ4 | | Discard | | DNA-directed RNA polymerase subunit beta | 23 | 0 |
| A1VYJ5 | | Discard | | DNA-directed RNA polymerase subunit beta' | 26 | 0 |
| A1VYJ6 | | Discard | | 30S ribosomal protein S12 | 4 | 0 |
| A1VYJ7 | | Discard | | 30S ribosomal protein S7 | 6 | 0 |
| A1VYJ8 | | Discard | | Elongation factor G | 15 | 0 |
| A1VYL8 | | Discard | | Alanine--tRNA ligase | 8 | 0 |
| A1VYN0 | | Discard | | Chaperone protein HtpG | 5 | 0 |
| A1VYP2 | | Discard | | Succinate--CoA ligase [ADP-forming] subunit beta | 4 | 0 |
| A1VYQ2 | | Discard | | Proline--tRNA ligase | 4 | 0 |
| A1VYU6 | | Discard | | DNA ligase | 5 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| A1VYV7 | | Discard | | Fructose-bisphosphate aldolase | 5 | 0 |
| A1VYZ2 | + | Discard | +_+ | Ketol-acid reductoisomerase (NADP(+)) | 5 | 0 |
| A1VYZ9 | | Discard | | Adenylate kinase | 5 | 0 |
| A1VZ00 | | Discard | | Aspartate--tRNA(Asp/Asn) ligase | 23 | 0 |
| A1VZ24 | | Discard | | Argininosuccinate synthase | 11 | 0 |
| A1VZ41 | | Discard | | 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (flavodoxin) | 7 | 0 |
| A1VZ44 | | Discard | | Acetate kinase | 8 | 0 |
| A1VZ59 | | Discard | | Glycine--tRNA ligase alpha subunit | 3 | 0 |
| A1VZ65 | | Discard | | 30S ribosomal protein S16 | 6 | 0 |
| A1VZ69 | | Discard | | 50S ribosomal protein L19 | 14 | 0 |
| A1VZC8 | | Discard | | Periplasmic nitrate reductase | 21 | 0 |
| A1VZF4 | | Discard | | 4-hydroxy-tetrahydrodipicolinate synthase | 2 | 0 |
| A1VZF8 | | Discard | | NH(3)-dependent NAD(+) synthetase | 2 | 0 |
| A1VZI8 | | Discard | | Glutamate--tRNA ligase 1 | 7 | 0 |
| A1VZL9 | | Discard | | 30S ribosomal protein S15 | 5 | 0 |
| A1VZM0 | | Discard | | DNA translocase FtsK | 3 | 0 |
| A1VZN1 | | Discard | | Phenylalanine--tRNA ligase alpha subunit | 8 | 0 |
| A1VZQ4 | + | Discard | +_+ | Major cell-binding factor | 2 | 0 |
| A1VZQ6 | + | Discard | +_+ | Chemotaxis protein methyltransferase | 2 | 0 |
| A1VZR5 | | Discard | | Phosphoenolpyruvate carboxykinase (ATP) | 6 | 0 |
| A1VZT4 | | Discard | | Protein translocase subunit SecA | 18 | 0 |
| A1VZU4 | | Discard | | Bifunctional purine biosynthesis protein PurH | 3 | 0 |
| A1VZU6 | + | Discard | +_+ | Phosphoribosylformylglycinamidine synthase subunit PurL | 6 | 0 |
| A1VZZ6 | + | Discard | +_+ | 3-dehydroquinate synthase | 2 | 0 |
| A1VZZ8 | | Discard | | Queuine tRNA-ribosyltransferase | 4 | 0 |
| A1W057 | | Discard | | 30S ribosomal protein S6 | 3 | 0 |
| A1W059 | | Discard | | 30S ribosomal protein S18 | 4 | 0 |
| A1W078 | | Discard | | Leucine--tRNA ligase | 8 | 0 |
| A1W085 | | Discard | | Aspartate carbamoyltransferase | 8 | 0 |
| A1W0A5 | + | Discard | +_+ | Chemotaxis protein CheY homolog | 3 | 0 |
| A1W0F6 | | Discard | | Putative, transcriptional regulatory protein | 3 | 0 |
| A1W0F9 | | Discard | | Arginine--tRNA ligase | 3 | 0 |
| A1W0G5 | | Discard | | Elongation factor Ts | 6 | 0 |
| A1W0G6 | | Discard | | 30S ribosomal protein S2 | 9 | 0 |
| A1W0H2 | | Discard | | tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG | 9 | 0 |
| A1W0I1; | | Discard | | Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B | 12 | 0 |
| A1W0I5 | | Discard | | 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase | 10 | 0 |
| A1W0J3 | | Discard | | Ribonuclease Y | 9 | 0 |
| A1W0K3 | | Discard | | 10 kDa chaperonin | 2 | 0 |
| A1W0M7 | | Discard | | Uroporphyrinogen decarboxylase | 3 | 0 |
| A1W0N3 | | Discard | | GMP synthase [glutamine-hydrolyzing] | 11 | 0 |
| A1W0N8 | | Discard | | Polyribonucleotide nucleotidyltransferase | 10 | 0 |
| A1W0P5 | | Discard | | Chaperone protein DnaJ | 4 | 0 |
| A1W0Q9 | + | Discard | +_+ | Uridylate kinase | 2 | 0 |
| A1W0S2 | | Discard | | Glutamate--tRNA ligase 2 | 12 | 0 |
| A1W0X7 | | Discard | | Polyphosphate kinase | 3 | 0 |
| A1W116 | | Discard | | Phosphoglycerate kinase | 6 | 0 |
| A1W163 | | Discard | | Peptide chain release factor 2 | 4 | 0 |
| A1W187 | | Discard | | 30S ribosomal protein S9 | 4 | 0 |

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|---|---|---|---|---|---|---|
| A1W1A5 | | Discard | | Adenylosuccinate synthetase | 11 | 0 |
| A1W1E0 | + | Discard | +_+ | Putative, protein | 3 | 0 |
| A1W1H5 | | Discard | | NADH-quinone oxidoreductase subunit D | 4 | 0 |
| A1W1J4 | | Discard | | 30S ribosomal protein S13 | 5 | 0 |
| A1W1J6 | | Discard | | 30S ribosomal protein S4 | 30 | 0 |
| A1W1J7 | | Discard | | DNA-directed RNA polymerase subunit alpha | 2 | 0 |
| A1W1J8 | | Discard | | 50S ribosomal protein L17 | 6 | 0 |
| A1W1K1 | | Discard | | Histidine biosynthesis bifunctional protein HisB | 3 | 0 |
| A1W1K3 | | Discard | | 1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase | 3 | 0 |
| A1W1L3 | | Discard | | 30S ribosomal protein S20 | 4 | 0 |
| A1W1L4 | | Discard | | Peptide chain release factor 1 | 7 | 0 |
| A1W1S4 | + | Discard | +_+ | Enolase | 10 | 0 |
| A1W1S5 | | Discard | | Protein RecA | 5 | 0 |
| A1W1U5 | | Discard | | 50S ribosomal protein L6 | 3 | 0 |
| A1W1U6 | | Discard | | 30S ribosomal protein S8 | 5 | 0 |
| A1W1U8 | | Discard | | 50S ribosomal protein L5 | 3 | 0 |
| A1W1V0 | | Discard | | 50S ribosomal protein L14 | 10 | 0 |
| A1W1V1 | | Discard | | 30S ribosomal protein S17 | 6 | 0 |
| A1W1V2 | + | Discard | +_+ | 50S ribosomal protein L29 | 1 | 0 |
| A1W1V4 | | Discard | | 30S ribosomal protein S3 | 13 | 0 |
| A1W1V7 | | Discard | | 50S ribosomal protein L2 | 12 | 0 |
| A1W1V9 | + | Discard | +_+ | 50S ribosomal protein L4 | 6 | 0 |
| A1W1W1 | | Discard | | 30S ribosomal protein S10 | 8 | 0 |
| A1W1X5 | + | Discard | +_+ | NADPH-dependent 7-cyano-7-deazaguanine reductase | 3 | 0 |
| Q0Q7I9 | | Discard | | Glutamine--fructose-6-phosphate aminotransferase [isomerizing] | 7 | 0 |
| Q0Q7K7 | | Discard | | Chaperone protein DnaK | 7 | 0 |
| Q0Q7K8 | | Discard | | Protein GrpE | 2 | 0 |
| Q1HG72 | + | Discard | +_+ | Glutamate synthase, small subunit | 4 | 0 |
| Q1HG73 | | Discard | | Uncharacterized protein | 10 | 0 |
| Q1HG74 | | Discard | | Glutamate synthase, large subunit | 11 | 0 |
| Q29VH0 | | Discard | | Arabinose-5-phosphate isomerase | 2 | 0 |
| Q29VV5 | | Discard | | Uncharacterized protein | 0 | |
| Q29VV7 | | Discard | | GDP-mannose 4,6-dehydratase | 4 | 0 |
| Q2M5Q5 | | Discard | | Putative, 3-oxoacyl-(Acyl-carrier-protein) synthase | 2 | 0 |
| Q2M5Q6 | | Discard | | FkbH domain-containing protein | 6 | 0 |
| Q2M5R1 | | Discard | | Motility accessory factor | 2 | 0 |
| Q2M5R2 | + | Discard | +_+ | Flagellin | 8 | 0 |
| Q8GJA7 | | Discard | | Uncharacterized protein | 2 | 0 |
| Q8GJE2 | | Discard | | DNA topoisomerase I | 5 | 0 |
| Q8GJE5 | | Discard | | Uncharacterized protein | 6 | 0 |
| Q939J7 | | Discard | | Flagellin modification protein, PseA | 12 | 0 |
| Q9KIS1 | | Discard | | VirB9 | 1 | 0 |
| A0A0H3P972 | + | Keep | +_+ | CCP20 | 1 | 0 |
| A0A0H3P982 | | Keep | | Ribose 5-phosphate isomerase B | 4 | 0 |
| A0A0H3P9A5 | | Keep | | Cysteine-rich domain protein | 3 | 0 |
| A0A0H3P9B2 | | Keep | | ThiH protein | 5 | 0 |
| A0A0H3P9G2 | | Keep | | Putative, Cell division protein FtsH | 3 | 0 |
| A0A0H3P9G3 | + | Keep | +_+ | Transcription termination termination factor Rho | 12 | 0 |
| A0A0H3P9I1 | | Keep | | Uncharacterized protein | 2 | 0 |
| A0A0H3P9I9 | | Keep | | Ribosome-binding ATPase YchF | 2 | 0 |
| A0A0H3P9J5 | | Keep | | Invasion antigen B | 10 | 0 |
| A0A0H3P9K9 | | Keep | | Oxidoreductase | 4 | 0 |
| A0A0H3P9L9 | | Keep | | Ribosomal protein S1 | 5 | 0 |
| A0A0H3P9M4 | + | Keep | +_+ | Aspartate aminotransferase | 5 | 0 |
| A0A0H3P9Q6 | | Keep | | Cytochrome P450 family protein | 7 | 0 |

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|---|---|---|---|---|---|---|
| A0A0H3P9R8 | + | Keep | +_+ | Sigma-54 dependent DNA-binding response regulator | 7 | 0 |
| A0A0H3P9T6 | + | Keep | +_+ | GTP cyclohydrolase-2 | 2 | 0 |
| A0A0H3P9Z0 | + | Keep | +_+ | Cpp14 | 16 | 0 |
| A0A0H3P9Z2 | | Keep | | Putative, Soluble lytic murein transglycosylase | 5 | 0 |
| A0A0H3PA08 | + | Keep | +_+ | Uncharacterized protein | 2 | 0 |
| Q5QKR4;A0A0H3PA20 | + | Keep | +_+ | dCTP deaminase | 2 | 0 |
| A0A0H3PA65 | + | Keep | +_+ | Methionine aminopeptidase | 4 | 0 |
| A0A0H3PA86 | | Keep | | Peptidase, U32 family | 5 | 0 |
| A0A0H3PA90 | | Keep | | Putative, 3-octaprenyl-4-hydroxybenzoate carboxy-lyase | 5 | 0 |
| A0A0H3PAD1 | + | Keep | +_+ | Aminopyrimidine aminohydrolase | 2 | 0 |
| A0A0H3PAD3 | | Keep | | Sulfurtransferase FdhD | 2 | 0 |
| A0A0H3PAF2 | | Keep | | Protein translocase subunit SecD | 5 | 0 |
| A0A0H3PAG3 | + | Keep | +_+ | Succinate dehydrogenase, C subunit | 1 | 0 |
| A0A0H3PAG6 | | Keep | | Triosephosphate isomerase | 1 | 0 |
| A0A0H3PAI3 | | Keep | | Uncharacterized protein | 5 | 0 |
| A0A0H3PAK7 | | Keep | | Peptidase, M24 family | 3 | 0 |
| A0A0H3PAL0 | + | Keep | +_+ | Fibronectin-binding protein | 11 | 0 |
| A0A0H3PAL4 | | Keep | | Flagellar motor switch protein FliG | 6 | 0 |
| A0A0H3PAQ3 | | Keep | | Uncharacterized protein | 3 | 0 |
| A0A0H3PAQ5 | | Keep | | Threonine synthase | 5 | 0 |
| A0A0H3PAQ8 | | Keep | | HAD-superfamily hydrolase, subfamily IIA | 2 | 0 |
| A0A0H3PAU2 | | Keep | | MloB | 8 | 0 |
| A0A0H3PAU5 | | Keep | | Paralyzed flagella protein PflA | 2 | 0 |
| A0A0H3PAV9 | | Keep | | Putative, Class I glutamine amidotransferase | 4 | 0 |
| A0A0H3PB02 | | Keep | | Uncharacterized protein | 2 | 0 |
| A0A0H3PB43 | | Keep | | Outer membrane efflux protein | 2 | 0 |
| A0A0H3PB76 | | Keep | | Co-chaperone protein DnaJ | 2 | 0 |
| A0A0H3PB85 | + | Keep | +_+ | Transferase, hexapeptide repeat family | 3 | 0 |
| A0A0H3PBB0 | | Keep | | Uncharacterized protein | 1 | 0.0038119 |
| A0A0H3PBF4 | | Keep | | Flagellar assembly protein FliH, putative | 2 | 0 |
| A0A0H3PBL2 | + | Keep | +_+ | Peptide deformylase | 2 | 0 |
| A0A0H3PBV0 | | Keep | | Acetolactate synthase, small subunit | 6 | 0 |
| A0A0H3PBX6 | | Keep | | Putative, Chemotaxis protein MotB | 5 | 0 |
| A0A0H3PBY2 | + | Keep | +_+ | ThiF family protein | 3 | 0 |
| A0A0H3PCC6 | | Keep | | Cpp45 | 3 | 0 |
| A0A0H3PCI2 | + | Keep | +_+ | Uncharacterized protein | 4 | 0 |
| A0A0H3PCP8 | | Keep | | Putative, Lipoprotein | 1 | 0 |
| A0A0H3PCS4 | | Keep | | Riboflavin synthase, alpha subunit | 5 | 0 |
| A0A0H3PD29 | + | Keep | +_+ | NAD-dependent protein deacylase | 2 | 0 |
| A0A0H3PD33 | + | Keep | +_+ | Phosphohistidine phosphatase SixA | 3 | 0 |
| A0A0H3PDH6 | + | Keep | +_+ | 3-deoxy-D-manno-octulosonate cytidylyltransferase | 5 | 0 |
| A0A0H3PDN2 | + | Keep | +_+ | Putative methyltransferase | 7 | 0 |
| A0A0H3PDQ6 | | Keep | | Uncharacterized protein | 3 | 0 |
| A0A0H3PDZ8 | | Keep | | Formate dehydrogenase, iron-sulfur subunit | 3 | 0 |
| A0A0H3PE18 | + | Keep | +_+ | Imidazole glycerol phosphate synthase subunit HisF | 3 | 0 |
| A0A0H3PEI7 | + | Keep | +_+ | Dihydropteroate synthase | 3 | 0 |
| A0A0H3PER2 | | Keep | | Replicative DNA helicase | 8 | 0 |
| A0A0H3PER6 | + | Keep | +_+ | Transcription termination/antitermination protein NusG | 3 | 0 |
| A0A0H3PEX7 | + | Keep | +_+ | Uncharacterized protein | 4 | 0 |
| A0A0H3PEZ9 | | Keep | | Uncharacterized protein | 3 | 0 |
| A0A0H3PG98 | | Keep | | Cpp45 | 4 | 0 |
| A0A0H3PGN8 | + | Keep | +_+ | Pseudouridine synthase | 4 | 0 |
| A0A0H3PGQ1 | + | Keep | +_+ | RNA polymerase sigma factor, | 4 | 0 |

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|---|---|---|---|---|---|---|
| | | | | sigma-F | | |
| A0A0H3PH47 | + | Keep | +_+ | Uncharacterized protein | 15 | 0 |
| A0A0H3PH83 | + | Keep | +_+ | Single-stranded DNA-binding protein | 8 | 0 |
| A0A0H3PH92 | + | Keep | +_+ | Glycolate oxidase, subunit GlcD | 5 | 0 |
| A0A0H3PH94 | | Keep | | Guanylate kinase | 3 | 0 |
| A0A0H3PHE7 | | Keep | | Phospho-2-dehydro-3-deoxyheptonate aldolase | 10 | 0 |
| A0A0H3PHF3 | | Keep | | Peptidyl-prolyl cis-trans isomerase D, homolog | 6 | 0 |
| A0A0H3PIL4 | | Keep | | Histidinol dehydrogenase | 2 | 0 |
| A0A0H3PIR6 | | Keep | | Peptidase, M23/M37 family | 2 | 0 |
| A0A0H3PIV9 | | Keep | | Cation ABC transporter, periplasmic cation-binding protein | 1 | 0 |
| A0A0H3PIX1 | + | Keep | +_+ | Uncharacterized protein | 3 | 0 |
| A0A0H3PIZ2 | | Keep | | GatB/Yqey family protein | 5 | 0 |
| A0A0H3PJ30 | + | Keep | +_+ | Ribonucleoside-diphosphate reductase subunit beta | 3 | 0 |
| A0A0H3PJ41 | | Keep | | Response regulator/GGDEF domain protein | 5 | 0 |
| A0A0H3PJ93 | + | Keep | +_+ | Diaminopimelate decarboxylase | 5 | 0 |
| A1VXF1 | | Keep | | 3-dehydroquinate dehydratase | 1 | 0 |
| A1VXI9 | | Keep | | ATP synthase gamma chain | 2 | 0 |
| A1VXV5 | + | Keep | +_+ | Orotate phosphoribosyltransferase | 3 | 0 |
| A1VYA1 | + | Keep | +_+ | Orotidine 5'-phosphate decarboxylase | 7 | 0 |
| A1VYA3 | + | Keep | +_+ | 6,7-dimethyl-8-ribityllumazine synthase | 2 | 0 |
| A1VYG1 | | Keep | | Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha | 2 | 0 |
| A1VYI6 | + | Keep | +_+ | Elongation factor Tu | 16 | 0 |
| A1VYM4 | + | Keep | +_+ | Phosphoribosylaminoimidazole-succinocarboxamide synthase | 5 | 0 |
| A1VYR7 | | Keep | | Gamma-glutamyl phosphate reductase | 2 | 0 |
| A1VZ21 | | Keep | | ATP-dependent protease ATPase subunit HslU | 5 | 0 |
| A1VZ22 | | Keep | | ATP-dependent protease subunit HslV | 3 | 0 |
| A1VZB3 | | Keep | | Histidine--tRNA ligase | 6 | 0 |
| A1VZF0 | | Keep | | Cysteine--tRNA ligase | 6 | 0 |
| A1VZK1 | + | Keep | +_+ | UDP-N-acetylglucosamine 1-carboxyvinyltransferase | 5 | 0 |
| A1VZM9 | | Keep | | 3-phosphoshikimate 1-carboxyvinyltransferase | 5 | 0 |
| A1VZR4 | + | Keep | +_+ | Argininosuccinate lyase | 4 | 0 |
| A1VZV2 | | Keep | | 50S ribosomal protein L34 | 1 | 0 |
| A1VZY2 | + | Keep | +_+ | Ornithine carbamoyltransferase | 3 | 0 |
| A1W018 | | Keep | | Elongation factor 4 | 6 | 0 |
| A1W038 | + | Keep | +_+ | Succinyl-diaminopimelate desuccinylase | 5 | 0 |
| A1W043 | | Keep | | UDP-N-acetylmuramate--L-alanine ligase | 2 | 0 |
| A1W048 | + | Keep | +_+ | Glutamyl-tRNA(Gln) amidotransferase subunit A | 5 | 0 |
| A1W0K4 | + | Keep | +_+ | 60 kDa chaperonin | 13 | 0 |
| A1W0Z5 | + | Keep | +_+ | L-seryl-tRNA(Sec) selenium transferase | 3 | 0 |
| A1W1D0 | + | Keep | +_+ | Diaminopimelate epimerase | 1 | 0 |
| A1W1D6 | | Keep | | Acetyl-coenzyme A synthetase | 4 | 0 |
| A1W1H0 | + | Keep | +_+ | NADH-quinone oxidoreductase subunit I | 8 | 0 |
| A1W1J9 | | Keep | | ATP phosphoribosyltransferase | 8 | 0 |
| A1W1T3 | | Keep | | Biotin synthase | 4 | 0 |
| A1W1U3 | + | Keep | +_+ | 30S ribosomal protein S5 | 5 | 0 |
| A1W1U4 | | Keep | | 50S ribosomal protein L18 | 3 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| A1W1V3 | | Keep | | 50S ribosomal protein L16 | 4 | 0 |
| A1W1V5 | + | Keep | +_+ | 50S ribosomal protein L22 | 2 | 0 |
| A1W1W9 | + | Keep | +_+ | 3-isopropylmalate dehydratase small subunit | 2 | 0 |
| A1W1X2 | | Keep | | 2-isopropylmalate synthase | 6 | 0 |
| Q0Q7I4 | | Keep | | Putative, Molybdopterin biosynthesis MoeA protein | 2 | 0 |
| Q0Q7I7 | | Keep | | Aminodeoxyfutalosine synthase | 5 | 0 |
| Q29W27 | | Keep | | Capsular polysaccharide ABC transporter, periplasmic polysaccharide-binding protein | 5 | 0 |
| Q3I354 | + | Keep | +_+ | S-ribosylhomocysteine lyase | 2 | 0 |
| Q8GJE0 | + | Keep | +_+ | Single-stranded DNA-binding protein | 2 | 0 |

**Appendix 2A:** Significantly differentiated proteins in 81-176 in response to cholic acid (CA) 0.1%.

| | **Significantly downregulated proteins (Log2FC≤1)** | | | |
|---|---|---|---|---|
| **UniProt_Accession** | **Gene Name** | **Protein Function** | **logFC** | **P-Value** |
| A0A0H3PBL4 | *hypE* | Chaperone | -1.794878 | 1.18498E-05 |
| A0A0H3PJ30 | *nrdB* | DNA Replication | -1.017521 | 6.79936E-10 |
| A0A0H3PHE7 | *cjj81176_0739* | Metabolism | -1.23618 | 3.961E-06 |
| A1VYT7 | *queA* | Metabolism | -1.210831 | 0.01318142 |
| Q5QKR5 | *accB* | Metabolism | -1.007544 | 1.43728E-06 |
| A0A0H3P9J4 | *CJJ81176_0882* | Metabolism | -2.222816 | 8.40012E-10 |
| A1W1K3 | *hisA* | Metabolism | -1.166341 | 0.029595643 |
| A0A0H3PB93 | *modE* | Metabolism | -1.048745 | 0.26134271 |
| A0A0H3P9R1 | *pglJ* | Metabolism | -1.002706 | 0.196172497 |
| A1VXF1 | *aroQ* | Metabolism | -1.358573 | 0.104310674 |
| A1VXG4 | *cdtA* | Pathogenesis | -1.127145 | 0.034054452 |
| A0A0H3PBB3 | *rbfA* | Protein synthesis | -1.350807 | 0.01556003 |
| A1VXM1 | *rimP* | Protein synthesis | -1.34701 | 0.003010739 |
| A1VXI1 | *fmt* | Protein synthesis | -2.032699 | 0.00089605 |
| A0A0H3PD33 | *sixA* | Protein modification | -1.675605 | 0.074152534 |
| A0A0H3PA35 | *dsbA* | Stress Response | -2.156863 | 1.81536E-09 |
| A0A0H3PIS5 | *cmeA* | Transport | -1.038359 | 7.07783E-09 |
| A0A0H3PB85 | *CJJ81176_0254* | Uncharacterized protein | -1.794007 | 0.006516277 |
| A0A0H3PDG2 | *CJJ81176_0891* | Uncharacterized protein | -1.138389 | 0.002832467 |
| A0A0H3PCE6 | *CJJ81176_0935* | Uncharacterized protein | -1.667115 | 0.004379536 |
| A0A0H3P9L3 | *CJJ81176_0728* | Uncharacterized protein | -1.407008 | 5.31084E-05 |
| A0A0H3PH34 | *CJJ81176_1055* | Uncharacterized protein | -2.439112 | 0.004588877 |
| A0A0H3PIW6 | *CJJ81176_0547* | Uncharacterized protein | -2.115717 | 1.43821E-06 |
| A0A0H3P9W6 | *CJJ81176_1493* | Uncharacterized protein | -2.024154 | 1.45319E-05 |
| A0A0H3PAI3 | *CJJ81176_0586* | Uncharacterized protein | -1.726446 | 7.95784E-09 |
| A0A0H3P9E6 | *CJJ81176_1179* | Uncharacterized protein | -1.552508 | 2.56625E-08 |
| A0A0H3PBZ1 | *CJJ81176_0414* | Uncharacterized protein | -1.4829 | 0.001211042 |
| A0A0H3PEL5 | *CJJ81176_0280* | Uncharacterized protein | -1.455998 | 0.006771213 |
| A0A0H3PAF3 | *CJJ81176_0231* | Uncharacterized protein | -1.210513 | 5.11148E-06 |
| A0A0H3PAM9 | *CJJ81176_1458* | Uncharacterized protein | -1.181354 | 0.014101442 |
| A0A0H3PAA2 | *CJJ81176_0288* | Uncharacterized protein | -1.135368 | 0.000188526 |
| A0A0H3PHH8 | *CJJ81176_0888* | Uncharacterized protein | -1.108932 | 0.01060921 |
| A0A0H3PAA1 | *CJJ81176_1497* | Uncharacterized protein | -1.037728 | 2.42837E-06 |
| A0A0H3PAI8 | *CJJ81176_0626* | Uncharacterized protein | -1.172738 | 0.02275865 |
| A0A0H3P9T3 | *CJJ81176_1422* | Uncharacterized protein | -1.009229 | 0.028832285 |
| A0A0H3PAF1 | *CJJ81176_1363* | Uncharacterized protein | -1.179186 | 0.026217311 |
| A0A0H3P9I1 | *CJJ81176_0782* | Uncharacterized protein | -1.034632 | 1.46192E-05 |
| | | | | |

| | Significantly upregulated proteins (Log2FC≥1) | | | |
|---|---|---|---|---|
| **UniProt_Accession** | **Gene Name** | **Protein Function** | **logFC** | **P-Value** |
| A0A0H3P9C5 | *mapA* | Cell wall organization | 1.0756546 | 6.83826E-09 |
| A0A0H3PAN9 | *cjj81176_1205* | Chemotaxis | 1.1703392 | 2.2605E-07 |
| A0A0H3PEL1 | *cjj81176_0289* | Chemotaxis | 1.4390734 | 2.59977E-08 |
| A0A0H3PEF7 | *cjj81176_0180* | Chemotaxis | 1.6447823 | 1.24231E-10 |
| A0A0H3PA38 | *cydA* | Metabolism | 1.091054 | 0.018998472 |
| A0A0H3P9B7 | *cyf* | Metabolism | 1.2168399 | 0.000811782 |
| A0A0H3PI21 | *nrfH* | Metabolism | 1.3245518 | 0.011897963 |
| A0A0H3PEG0 | *lpxB* | Metabolism | 1.2598027 | 0.002577961 |
| A0A0H3PIF6 | *fliL* | Motility | 1.0680729 | 1.36475E-05 |
| A0A0H3PA17 | *putP* | Transport | 1.0605275 | 0.010760836 |
| A0A0H3PA76 | *cjj81176_1604* | Transport | 1.051132 | 0.000158674 |
| A0A0H3PD65 | *cjj81176_1037* | Transport | 1.1387525 | 4.93231E-10 |
| A0A0H3PEA5 | *CJJ81176_0635* | Transport | 1.3038455 | 0.015471587 |
| A0A0H3PD99 | *CJJ81176_0797* | Uncharacterized protein | 1.0754752 | 0.001106957 |
| A0A0H3PA50 | *CJJ81176_0126* | Uncharacterized protein | 1.0890366 | 0.000103921 |
| A0A0H3PCP8 | *CJJ81176_1045* | Uncharacterized protein | 1.3923631 | 3.07213E-05 |
| A0A0H3PEG8 | *CJJ81176_0642* | Uncharacterized protein | 1.3968882 | 0.00081135 |
| A0A0H3PGI9 | *CJJ81176_0987* | Uncharacterized protein | 1.98572 | 2.94023E-11 |
| A1VYL9 | *CJJ81176_0535* | Uncharacterized protein | 2.269712 | 0.0008154 |

**Appendix 2B:** Significantly differentiated proteins in 81-176 in response to deoxycholic acid (DCA) 0.05%.

| | **Significantly downregulated proteins** | | | |
|---|---|---|---|---|
| **UniProt_Accession** | **Gene name** | **Protein Function** | **logFC** | **P-Value** |
| A0A0H3P9D5 | *ftsH* | Cell division | -1.003282442 | 1.43426E-09 |
| A0A0H3PH83 | *ssb* | DNA Replication | -1.296003625 | 1.90198E-13 |
| A1VZM0 | *ftsK* | DNA Replication | -1.180538329 | 0.001622545 |
| A0A0H3PHL1 | *ubiX* | Metabolism | -2.14860732 | 1.01405E-09 |
| A1VXJ0 | *atpD* | Metabolism | -1.846577928 | 1.80567E-14 |
| A0A0H3PA38 | *cydA* | Metabolism | -1.572398315 | 8.01142E-10 |
| A0A0H3PAE3 | *hydA* | Metabolism | -1.560755539 | 1.19656E-14 |
| A0A0H3PCS4 | *ribE* | Metabolism | -1.538831636 | 5.65435E-10 |
| A0A0H3PEJ9 | *frdC* | Metabolism | -1.508509495 | 0.003042706 |
| A0A0H3PAJ7 | *hydB* | Metabolism | -1.412003652 | 2.9533E-14 |
| A0A0H3P9I8 | *speA* | Metabolism | -1.385389796 | 4.76079E-15 |
| A1W1S4 | *eno* | Metabolism | -1.35915011 | 9.48311E-08 |
| A0A0H3PIR1 | *fdhA* | Metabolism | -1.242889979 | 0.011511176 |
| A1VY43 | *ubiE* | Metabolism | -1.213975605 | 0.024749231 |
| Q29W37 | *panB* | Metabolism | -1.164425861 | 1.0781E-07 |
| Q5QKR5 | *accB* | Metabolism | -1.094693102 | 0.000518742 |
| A0A0H3PCR0 | *petB* | Metabolism | -1.084215285 | 3.17365E-08 |
| A0A0H3P9R9 | *ccoO* | Metabolism | -1.027969837 | 2.48918E-10 |
| A0A0H3PHB9 | *petA* | Metabolism | -1.008478573 | 2.18416E-08 |
| A0A0H3PBB6 | *trpE* | Metabolism | -1.265119151 | 4.30949E-13 |
| A1VYZ2 | *ilvC* | Metabolism | -1.964891277 | 6.16136E-18 |
| A0A0H3PHD6 | *glnA* | Metabolism | -1.921103036 | 1.11502E-16 |
| A0A0H3P9I4 | *purU* | Metabolism | -1.644891275 | 1.13108E-07 |
| A0A0H3P9R1 | *pglJ* | Metabolism | -1.371984336 | 0.128228077 |
| A1W085 | *pyrB* | Metabolism | -1.282202596 | 4.06965E-13 |
| A0A0H3PIT1 | *ftn* | Metabolism | -1.030556844 | 2.50532E-07 |
| A0A0H3PB49 | *CJJ81176_1548* | Motility | -1.766851698 | 0.001163017 |

| | | | | |
|---|---|---|---|---|
| A0A0H3PBX6 | *CJJ81176_0358* | Motility | -1.61665275 | 0.00495653 |
| A0A0H3PBG5 | *cjj81176_1338* | Motility | -1.450922078 | 2.19649E-13 |
| A0A0H3PAV1 | *CJJ81176_0359* | Motility | -1.019207272 | 3.78491E-08 |
| A0A0H3PIZ8 | *fliE* | Motility | -1.009479684 | 3.20393E-06 |
| A0A0H3P9F0 | *pglF* | Pathogenesis | -1.847223714 | 0.002523105 |
| A0A0H3PAD9 | *pglD* | Pathogenesis | -1.827601879 | 0.002517774 |
| A0A0H3PAL0 | *cadF* | Pathogenesis | -1.433202443 | 9.61348E-13 |
| A0A0H3PAF2 | *secD* | Pathogenesis | -1.041994876 | 2.03701E-08 |
| A0A0H3PAN7 | *secF* | Pathogenesis | -1.00272481 | 5.15858E-06 |
| Q2M5R2 | *flaA* | Pathogenesis | -1.391968401 | 1.71751E-14 |
| Q7X517 | *pseE* | Pathogenesis | -1.042454629 | 9.9974E-08 |
| A1VY30 | *rplY* | Protein synthesis | -2.42052398 | 2.6086E-17 |
| A1VYJ1 | *rplA* | Protein synthesis | -2.3954085 | 2.32701E-07 |
| A1W1V2 | *rpmC* | Protein synthesis | -1.744034513 | 6.11766E-17 |
| A0A0H3PCH6 | *rplC* | Protein synthesis | -1.528410607 | 3.35694E-15 |
| A1VXW7 | *rplT* | Protein synthesis | -1.495896933 | 2.58784E-13 |
| A1W1L3 | *rpsT* | Protein synthesis | -1.464048618 | 2.30234E-05 |
| A1VYI7 | *rpmG* | Protein synthesis | -1.405120571 | 0.01569719 |
| A0A0H3PGK7 | *pepA* | Protein synthesis | -1.91864899 | 1.32325E-12 |
| A0A0H3P9Q4 | *katA* | Stress Responce | -1.078220647 | 5.87997E-11 |
| Q3I354 | *luxS* | Stress Response | -1.560714685 | 0.003359118 |
| A1W0K4 | *groL* | Stress response | -2.220545819 | 6.53678E-19 |
| A0A0H3PA75 | *comEA* | Stress Response | -1.518431946 | 9.51326E-13 |
| A0A0H3PEV8 | *pbpA* | Stress Response | -1.224369902 | 0.000215077 |
| A0A0H3PBJ5 | *dsbD* | Stress Response | -1.036136095 | 0.02865686 |
| A0A0H3PIS5 | *cmeA* | Transport | -3.01479507 | 2.51617E-18 |
| A0A0H3PB79 | *cmeB* | Transport | -2.911680969 | 1.668E-13 |
| A0A0H3PAE4 | *cmeC* | Transport | -2.381525121 | 4.6268E-09 |
| A0A0H3PAW0 | *corA* | Transport | -1.078679114 | 0.014028751 |
| A0A0H3P9J7 | *CJJ81176_0137* | Transport | -1.064452729 | 0.204298534 |
| A0A0H3PAK6 | *chuA* | Transport | -1.003106417 | 1.97079E-06 |
| A0A0H3PB37 | *CJJ81176_1244* | Two-component regulatory system | -1.175074343 | 0.153535306 |
| A0A0H3PB43 | *CJJ81176_0637* | Uncharacterized protein | -1.27173741 | 0.013911469 |
| A0A0H3PDG2 | *CJJ81176_0891* | Uncharacterized protein | -1.529763099 | 0.001747932 |
| A0A0H3PB47 | *CJJ81176_1492* | Uncharacterized protein | -1.526101434 | 2.51631E-08 |
| A0A0H3P9J4 | *CJJ81176_0882* | Uncharacterized protein | -2.795499663 | 7.46144E-16 |
| A0A0H3PAI3 | *CJJ81176_0586* | Uncharacterized protein | -2.667730908 | 1.08082E-13 |
| A0A0H3PCI2 | *CJJ81176_0072* | Uncharacterized protein | -1.937374035 | 6.93187E-11 |
| A0A0H3PAI8 | *CJJ81176_0626* | Uncharacterized protein | -1.821859546 | 0.005038069 |
| A0A0H3P9W6 | *CJJ81176_1493* | Uncharacterized protein | -1.78587095 | 0.000165446 |
| A0A0H3PAC4 | *CJJ81176_1391* | Uncharacterized protein | -1.769026473 | 0.000262839 |
| A0A0H3P9D3 | *cjj81176_1210* | Uncharacterized protein | -1.70639506 | 1.37057E-16 |
| A0A0H3PH47 | *CJJ81176_1185* | Uncharacterized protein | -1.679381648 | 1.336E-10 |
| A0A0H3PCP8 | *CJJ81176_1045* | Uncharacterized protein | -1.58311534 | 5.55587E-09 |
| A0A0H3PBI5 | *CJJ81176_1639* | Uncharacterized protein | -1.5100786 | 0.007331941 |
| A0A0H3P9D1 | *CJJ81176_1051* | Uncharacterized protein | -1.420955427 | 1.22356E-14 |
| A0A0H3P9J0 | *CJJ81176_0912* | Uncharacterized protein | -1.378865295 | 0.001510567 |
| A0A0H3P9Y0 | *CJJ81176_1228* | Uncharacterized protein | -1.360148023 | 9.70451E-05 |
| A0A0H3PA15 | *cjj81176_0828* | Uncharacterized protein | -1.313904062 | 1.40255E-13 |
| A0A0H3PBE0 | *CJJ81176_0236* | Uncharacterized protein | -1.256737814 | 1.26504E-05 |
| A0A0H3PBF8 | *CJJ81176_1651* | Uncharacterized protein | -1.207473981 | 3.53578E-08 |
| A0A0H3P9N8 | *CJJ81176_0145* | Uncharacterized protein | -1.147788783 | 0.000482207 |
| A0A0H3P9U0 | *CJJ81176_1009* | Uncharacterized protein | -1.084569702 | 0.00070709 |
| Q29VV2 | *CJB1432c* | Uncharacterized protein | -1.00417327 | 0.050963459 |
| Q29VW3 | *CJB1421c* | Uncharacterized protein | -1.154507913 | 0.05201738 |
| A0A0H3PBU4 | *CJJ81176_0392* | Uncharacterized protein | -1.333309863 | 0.054582033 |
| A0A0H3PET5 | *cjj81176_0471* | Uncharacterized protein | -1.082223178 | 5.3541E-11 |
| A0A0H3PEU8 | *CJJ81176_0148* | Uncharacterized protein | -1.015567296 | 4.03599E-09 |

| | | | | |
|---|---|---|---|---|
| | **Significantly upregulated** | | | |
| **UniProt_Accession** | **Gene name** | **Protein Function** | **logFC** | **P-Value** |
| A0A0H3P9Z7 | *murE* | Cell cycle, Cell division | 1.072463265 | 3.95606E-06 |
| A1VXS1 | *tig* | Cell cycle, Cell division | 1.121080635 | 2.26186E-09 |
| A0A0H3P9H6 | *CJJ81176_0967* | Chaperone | 1.296706727 | 0.014071019 |
| A0A0H3PGG1 | *CJJ81176_pTet 0031* | DNA Replication | 1.309533578 | 0.000367427 |
| A0A0H3PF03 | *fabF* | Metabolism | 1.055285367 | 1.27193E-14 |
| A0A0H3P9J6 | *pta* | Metabolism | 1.010430855 | 3.02541E-10 |
| A0A0H3PJ06 | *mqnC* | Metabolism | 1.026456978 | 8.98215E-08 |
| A0A0H3PA20 | *dcd* | Metabolism | 1.034888831 | 3.02922E-07 |
| A0A0H3PBH7 | *nspC* | Metabolism | 1.054969328 | 0.006047851 |
| A0A0H3PAD5 | *lpxD* | Metabolism | 1.056038562 | 1.35346E-10 |
| A1VXV5 | *pyrE* | Metabolism | 1.05860604 | 0.002703813 |
| A1VYF9 | *acpP* | Metabolism | 1.087110203 | 1.204E-05 |
| Q0Q7I1 | *purM* | Metabolism | 1.108267274 | 9.89401E-06 |
| A0A0H3PEA7 | *oorB* | Metabolism | 1.109816217 | 2.45167E-12 |
| A1VZ01 | *nadK* | Metabolism | 1.113853817 | 0.001440012 |
| A0A0H3P9U4 | *hipO* | Metabolism | 1.117479144 | 3.06172E-07 |
| A1W1W9 | *leuD* | Metabolism | 1.162960387 | 3.01923E-05 |
| A0A0H3PB56 | *galU* | Metabolism | 1.177847726 | 4.23304E-07 |
| A1VYR7 | *proA* | Metabolism | 1.19586627 | 1.34306E-09 |
| A0A0H3PAD1 | *cjj81176_0466* | Metabolism | 1.214989403 | 6.52772E-06 |
| A1W1X0 | *leuC* | Metabolism | 1.216702934 | 0.000332366 |
| A0A0H3PAQ8 | *cjj81176_0337* | Metabolism | 1.224123876 | 8.23434E-07 |
| A0A0H3PHM5 | *mobB* | Metabolism | 1.245815218 | 0.011904394 |
| Q29VV6 | *fcl* | Metabolism | 1.250144552 | 1.83873E-10 |
| A0A0H3PAH7 | *oorA* | Metabolism | 1.287755907 | 3.41918E-15 |
| A1VZ24 | *argG* | Metabolism | 1.34841277 | 2.71942E-11 |
| A0A0H3PAP1 | *thiJ* | Metabolism | 1.373658913 | 9.25742E-07 |
| A0A0H3PA65 | *map* | Metabolism | 1.388867058 | 8.45382E-06 |
| A0A0H3PBQ2 | *sdhA* | Metabolism | 1.39834063 | 1.4503E-08 |
| A1W1K3 | *hisA* | Metabolism | 1.406014995 | 0.023248273 |
| A0A0H3PAG3 | *sdhC* | Metabolism | 1.427111021 | 4.31485E-08 |
| A0A0H3PAA8 | *cjj81176_0533* | Metabolism | 1.449295592 | 2.42379E-10 |
| A1VYB8 | *gatC* | Metabolism | 1.482305401 | 0.000746731 |
| A0A0H3PH15 | *thiD* | Metabolism | 1.584241938 | 3.83619E-08 |
| A0A0H3PBD0 | *bioA* | Metabolism | 1.600474141 | 0.001952673 |
| A0A0H3P9T6 | *ribA* | Metabolism | 1.711510283 | 0.000311869 |
| A1VZR0 | *apt* | Metabolism | 1.902204652 | 0.000154875 |
| A0A0H3P982 | *rpiB* | Metabolism | 1.955050403 | 2.11556E-09 |
| A0A0H3PAJ4 | *hisI* | Metabolism | 2.125278034 | 2.22733E-05 |
| A0A0H3PC31 | *hom* | Metabolism | 2.340793813 | 7.09804E-10 |
| A0A0H3PBK5 | *purS* | Metabolism | 2.496620564 | 0.000497449 |
| A1VYU1 | *rppH* | Metabolism | 1.153349736 | 0.000226588 |
| A0A0H3P9Z1 | *CJJ81176_1373* | Metabolism | 1.321839803 | 3.08931E-07 |
| A0A0H3PIZ2 | *CJJ81176_0601* | Metabolism | 1.024279332 | 0.014093347 |
| A0A0H3PB14 | *cjj81176_0397* | Metabolism | 1.145841906 | 6.88332E-11 |
| A0A0H3PDJ1 | *cjj81176_1533* | Metabolism | 1.18939729 | 2.32031E-13 |
| A0A0H3PBY2 | *CJJ81176_0318* | Metabolism | 1.206379152 | 0.001455795 |
| A0A0H3P9K9 | *cjj81176_0850* | Metabolism | 1.223247452 | 1.19386E-12 |
| A0A0H3PAM5 | *CJJ81176_0297* | Metabolism | 1.270286973 | 0.00094094 |
| A0A0H3P9Q8 | *CJJ81176_1286* | Metabolism | 1.31807062 | 4.98319E-12 |
| A0A0H3PGR5 | *cjj81176_0063* | Metabolism | 1.785672462 | 5.03196E-10 |
| A1VXA6 | *pyrG* | Metabolism | 1.115481592 | 1.08311E-09 |
| A1VZ41 | *ispG* | Metabolism | 1.120428427 | 1.82069E-06 |
| A0A0H3PJB7 | *sdhB* | Metabolism | 1.276694973 | 5.29169E-09 |

| | | | | |
|---|---|---|---|---|
| A1VZI4 | *fbp* | Metabolism | 1.280247721 | 3.46399E-13 |
| A1W0I5 | *metE* | Metabolism | 1.329340317 | 1.89466E-09 |
| A0A0H3PIL4 | *hisD* | Metabolism | 1.384865057 | 4.71104E-12 |
| A0A0H3P9P8 | *tkt* | Metabolism | 1.513389732 | 2.97151E-12 |
| A1VY36 | *hisC* | Metabolism | 1.659891461 | 2.59525E-08 |
| A1W0I0 | *gpsA* | Metabolism | 1.037472572 | 1.53007E-06 |
| A0A0H3PBV9 | *oorD* | Metabolism | 1.433702786 | 0.030515815 |
| A0A0H3PAV5 | *metC* | Metabolism | 1.185786388 | 8.57234E-10 |
| A0A0H3PA78 | *fliY* | Motility | 1.104913229 | 0.00072067 |
| A0A0H3PB06 | *cjj81176_08473* | Motility | 1.675317372 | 9.87858E-09 |
| A0A0H3PBF4 | *CJJ81176_0342* | Motility | 1.861436456 | 0.00575898 |
| A1W0U6 | *pseG* | Pathogenesis | 1.484075242 | 0.051016804 |
| Q5QKR7 | *pseC* | Pathogenesis | 1.206433894 | 1.91253E-06 |
| A0A0H3PA50 | *CJJ81176_0126* | Putative lipoprotein | 1.259487334 | 2.81378E-06 |
| Q939J8 | *pseI* | Pathogenesis | 1.368126421 | 8.08852E-09 |
| A1VYV6 | *cbf2 (peb4A)* | Pathogenesis | 2.23684879 | 1.18267E-16 |
| A1VZ23 | *rplI* | Protein synthesis | 1.141727127 | 2.31179E-11 |
| A1W1U6 | *rpsH* | Protein synthesis | 1.282637424 | 2.36465E-10 |
| A1VYQ2 | *proS* | Protein synthesis | 1.007294248 | 1.14643E-12 |
| A1VYL8 | *alaS* | Protein synthesis | 1.082008633 | 5.28935E-12 |
| A0A0H3P9S5 | *cysQ* | Protein synthesis | 1.146927871 | 1.84926E-07 |
| A0A0H3PAI4 | *ileS* | Protein synthesis | 1.171762016 | 7.63109E-15 |
| A1VZ00 | *aspS* | Protein synthesis | 1.198918127 | 8.89443E-12 |
| A0A0H3PDU5 | *tyrS* | Protein synthesis | 1.423238988 | 1.38102E-13 |
| A1W0I1 | *gatB* | Protein synthesis | 1.502373102 | 2.89614E-15 |
| A1W048 | *gatA* | Protein synthesis | 1.801909443 | 8.64309E-13 |
| A0A0H3PB64 | *trpS* | Protein synthesis | 2.163815364 | 7.26977E-11 |
| A0A0H3PBL2 | *def* | Protein synthesis | 2.847841384 | 5.58486E-05 |
| A0A0H3PAE1 | *CJJ81176_0192* | Protein synthesis | 1.306789539 | 0.012439928 |
| A0A0H3P9V7 | *CJJ81176_1101* | Stress Responce | 1.249758061 | 0.065954548 |
| A1VXQ2 | *sodB* | Stress Responce | 2.300096572 | 1.06222E-10 |
| A0A0H3PF18 | *cj81176_0446* | Transport | 1.042072018 | 5.6717E-07 |
| A0A0H3PA76 | *cjj81176_1604* | Transport | 1.270064626 | 1.65383E-07 |
| A0A0H3PIV9 | *CJJ81176_0179* | Transport | 1.632399678 | 4.12748E-07 |
| A0A0H3PJ16 | *modA* | Transport | 1.219265759 | 4.25999E-08 |
| A0A0H3PJ41 | *cj81176_0671* | Two-component regulatory system | 1.100369849 | 5.15873E-08 |
| A0A0H3PBN1 | *cjj81176_0379* | Two-component regulatory system | 1.27295878 | 4.55324E-07 |
| A1VYL9 | *CJJ81176_0535* | Uncharacterized protein | 1.983634398 | 0.016756019 |
| A0A0H3P9Z2 | *CJJ81176_0859* | Uncharacterized protein | 1.018360809 | 9.20584E-08 |
| A0A0H3PAV9 | *CJJ81176_1416* | Uncharacterized protein | 1.329443732 | 0.000119877 |
| A0A0H3PAH9 | *CJJ81176_1530* | Uncharacterized protein | 1.386238155 | 1.04067E-05 |
| Q2A947 | *Cj1451* | Uncharacterized protein | 1.608316593 | 0.00232559 |
| A0A0H3PBJ6 | *CJJ81176_0387* | Uncharacterized protein | 1.007066023 | 1.14793E-07 |
| Q2M5R0 | *Cj1342c* | Uncharacterized protein | 1.086563404 | 2.13651E-05 |
| A0A0H3PEN1 | *cjj81176_0292* | Uncharacterized protein | 1.124550252 | 6.26605E-13 |
| A1VY95 | *CJJ81176_0398* | Uncharacterized protein | 1.131728012 | 1.71841E-05 |
| A0A0H3PGI9 | *CJJ81176_0987* | Uncharacterized protein | 1.140553017 | 3.75212E-05 |
| A0A0H3PC13 | *CJJ81176_0374* | Uncharacterized protein | 1.222683574 | 2.00275E-11 |
| A0A0H3PI41 | *A0A0H3PI41* | Uncharacterized protein | 1.2519668 | 1.77677E-15 |
| A0A0H3P9Y5 | *CJJ81176_pTet 0016* | Uncharacterized protein | 1.253237438 | 0.003024591 |
| Q8GJE8 | *Cjp04* | Uncharacterized protein | 1.339577465 | 0.00497124 |
| A0A0H3PA63 | *CJJ81176_0729* | Uncharacterized protein | 1.393312514 | 1.07151E-12 |
| A0A0H3PJ75 | *CJJ81176_0306* | Uncharacterized protein | 1.434676924 | 0.00792529 |
| A0A0H3P991 | *CJJ81176_0018* | Uncharacterized protein | 1.509155115 | 7.02744E-08 |
| Q0Q7K3 | *CJJ81176_0779* | Uncharacterized protein | 1.532219075 | 1.43698E-05 |
| A0A0H3PDT4 | *CJJ81176_1617* | Uncharacterized protein | 1.618539679 | 0.002496734 |

| | | | | |
|---|---|---|---|---|
| A0A0H3PDV4 | *cj81176_1419* | Uncharacterized protein | 1.723137323 | 9.18852E-10 |
| A0A0H3PGW3 | *CJJ81176_1177* | Uncharacterized protein | 1.92854344 | 9.14616E-05 |
| A0A0H3PHX6 | *CJJ81176_1306* | Uncharacterized protein | 1.941674705 | 1.18219E-11 |
| A0A0H3P9A5 | *CJJ81176_0112* | Uncharacterized protein | 2.040097649 | 3.04708E-07 |
| A0A0H3PAU9 | *CJJ81176_0809* | Uncharacterized protein | 1.072446574 | 0.055552487 |
| A0A0H3PAA3 | *CJJ81176_1453* | Uncharacterized protein | 1.419151168 | 0.060189297 |
| A0A0H3PBJ6 | *CJJ81176_0387* | Uncharacterized protein | 1.007066023 | 1.14793E-07 |
| A0A0H3PHF9 | *CJJ81176_0723* | Uncharacterized protein | 2.34723418 | 4.91226E-07 |
| A0A0H3PBP8 | *CJJ81176_0462* | Uncharacterized protein | 2.890852381 | 3.07843E-08 |

**Appendix 2C:** Significantly differentiated proteins in 81-176 in response to lithocholic acid (LCA) 0.5%.

| | **Significantly downregulated** | | | |
|---|---|---|---|---|
| **UniProt_Accession** | **Gene Name** | **Protein Function** | **logFC** | **P-Value** |
| A1VXF1 | *aroQ* | Metabolism | -1.946233239 | 0.00672643 |
| A1VY40 | *dxs* | Metabolism | -1.250684216 | 0.00048007 |
| A0A0H3PA64 | *ggt* | Metabolism | -1.091364679 | 3.3679E-05 |
| A0A0H3P9B2 | *thiH* | Metabolism | -1.004468139 | 0.00025826 |
| A0A0H3P9P3 | *CJJ81176_1159* | Metabolism | -1.106306132 | 0.17610262 |
| A0A0H3PAM5 | *CJJ81176_0297* | Metabolism | -1.015163843 | 0.00065335 |
| A1VXM1 | *rimP* | Protein synthesis | -1.256610864 | 0.00590299 |
| A0A0H3PBL4 | *hypE* | Protein synthesis | -1.32782401 | 0.00013553 |
| A0A0H3PAT8 | *CJJ81176_1274* | Protein synthesis | -1.31828289 | 0.00721395 |
| A0A0H3PDE7 | *CJJ81176_0897* | Transport | -1.399468332 | 0.03190218 |
| A0A0H3PDG2 | *CJJ81176_0891* | Uncharacterized protein | -1.071933555 | 0.01268505 |
| A0A0H3PCE6 | *CJJ81176_0935* | Uncharacterized protein | -1.604045037 | 0.01445481 |
| Q0Q7K5 | *CJJ81176_0777* | Uncharacterized protein | -1.306389919 | 0.18973704 |
| A0A0H3PA59 | *CJJ81176_1259* | Uncharacterized protein | -1.155378161 | 0.15068155 |
| A0A0H3PBB0 | *CJJ81176_1666* | Uncharacterized protein | -1.124759762 | 0.06000636 |
| A0A0H3PB02 | *CJJ81176_0220* | Uncharacterized protein | -1.036522129 | 0.04630988 |
| A0A0H3PAT8 | *CJJ81176_1274* | Uncharacterized protein | -1.31828289 | 0.00721395 |
| A0A0H3P9L3 | *CJJ81176_0728* | Uncharacterized protein | -1.236774433 | 0.00190423 |
| A0A0H3P9A4 | *CJJ81176_0120* | Uncharacterized protein | -1.302763765 | 0.00056081 |
| A0A0H3PEL5 | *CJJ81176_0280* | Uncharacterized protein | -1.343015099 | 0.0116004 |
| | | | | |
| | **Significantly upregulated** | | | |
| **UniProt_Accession** | **Gene Name** | **Protein Function** | **logFC** | **P-Value** |
| A0A0H3PB49 | *CJJ81176_1548* | Chemotaxis | 1.265105214 | 0.01884375 |
| A0A0H3PEF7 | *cjj81176_0180* | Motility | 1.349493211 | 1.1433E-08 |
| A0A0H3PEE2 | *secG* | Pathogenesis | 1.149406064 | 0.06638875 |
| A0A0H3P9J0 | *CJJ81176_0912* | Transport | 1.784378504 | 0.01318131 |
| A0A0H3P971 | *CJJ81176_pTet0052* | Uncharacterized protein | 1.1326771 | 0.10868569 |
| A0A0H3PGI9 | *CJJ81176_0987* | Uncharacterized protein | 1.343722157 | 0.00104637 |
| A0A0H3PD99 | *CJJ81176_0797* | Uncharacterized protein | 1.435712271 | 7.1365E-06 |
| Q8GJA8 | *Cjp47* | Uncharacterized protein | 0.979140122 | 0.18488404 |

**Appendix 2D:** Significantly differentiated proteins in cj81-176 in response to taurocholic acid (TCA) 0.5%.

| | **Significantly downregulated** | | | |
|---|---|---|---|---|
| **UniProt_Accession** | **Gene Name** | **Protein Function** | **logFC** | **P-Value** |
| A0A0H3PA34 | *cheB* | Chemotaxis | -1.226602134 | 9.46978E-06 |
| A0A0H3PJ30 | *nrdB* | DNA Replication | -1.180021302 | 2.04063E-13 |
| A0A0H3P9R0 | *CJJ81176_1236* | DNA Response regulator | -1.092592157 | 3.45746E-07 |
| A1VXF1 | *aroQ* | Metabolism | -2.436742783 | 0.001101997 |
| A0A0H3PBF9 | *rpe* | Metabolism | -1.458789174 | 3.73715E-06 |
| Q5QKR5 | *accB* | Metabolism | -1.445814976 | 1.74741E-10 |
| A1W1K3 | *hisA* | Metabolism | -1.388482008 | 0.004050459 |
| A0A0H3P9S3 | *hydD* | Metabolism | -1.287175521 | 0.001189336 |
| A0A0H3PHM5 | *mobB* | Metabolism | -1.20117722 | 0.02038769 |
| A0A0H3PEI7 | *folP* | Metabolism | -1.189189468 | 0.00699004 |
| A0A0H3PCS4 | *ribE* | Metabolism | -1.085497094 | 8.09279E-06 |
| A0A0H3P9B2 | *thiH* | Metabolism | -1.039720885 | 8.69139E-05 |
| A0A0H3PD29 | *cobB* | Metabolism | -1.532800375 | 0.000279934 |
| A0A0H3PA64 | *ggt* | Metabolism | -1.166367573 | 4.24829E-06 |
| A0A0H3PHE7 | *cjj81176_0739* | Metabolism | -1.451360641 | 2.14432E-08 |
| A1VY40 | *dxs* | Metabolism | -1.268605424 | 0.000130626 |
| A0A0H3PAG6 | *tpiA* | Metabolism | -1.24298309 | 0.001418108 |
| A1W0R9 | *mqnA* | Metabolism | -1.174302311 | 0.116892298 |
| A1W0N3 | *guaA* | Metabolism | -1.170239402 | 9.84591E-08 |
| A1W062 | *fliW* | Motility | -1.624957263 | 0.029733566 |
| A0A0H3PAR2 | *fliI* | Motility | -1.143787081 | 0.014771905 |
| A1W0U6 | *pseG* | Pathogenesis | -1.152879834 | 0.024087801 |
| A0A0H3PD33 | *sixA* | Protein modification | -2.159988873 | 0.012063197 |
| A1W0R3 | *trmB* | Protein synthesis | -2.059288049 | 9.05339E-05 |
| A1VY31 | *pth* | Protein synthesis | -1.483064053 | 1.17701E-07 |
| A1VZW5 | *cmoB* | Protein synthesis | -1.293130493 | 0.000929833 |
| A0A0H3PBL4 | *hypE* | Protein synthesis | -2.082094284 | 4.99847E-08 |
| A1VXI1 | *fmt* | Protein synthesis | -1.665727249 | 0.019355779 |
| A0A0H3PA35 | *dsbA* | Stress Response | -3.364517181 | 3.08879E-14 |
| A0A0H3P9I9 | *ychF* | Stress Response | -1.187359961 | 3.33152E-10 |
| A0A0H3P9M1 | *napD* | Transport | -1.278147171 | 0.00425219 |
| A0A0H3PIS5 | *cmeA* | Transport | -1.428082684 | 2.9816E-12 |
| A0A0H3PAX0 | *tpx* | Uncharacterized protein | -1.037574676 | 1.38464E-05 |
| A0A0H3P9L3 | *CJJ81176_0728* | Uncharacterized protein | -1.250302129 | 0.007687957 |
| A0A0H3PB85 | *CJJ81176_0254* | Uncharacterized protein | -2.07207278 | 0.001137778 |
| A0A0H3P9J4 | *CJJ81176_0882* | Uncharacterized protein | -3.198992652 | 9.50327E-17 |
| A0A0H3PAI3 | *CJJ81176_0586* | Uncharacterized protein | -3.150110792 | 2.24208E-15 |
| A0A0H3P9T3 | *CJJ81176_1422* | Uncharacterized protein | -2.317175413 | 3.60752E-09 |
| A0A0H3PAF1 | *CJJ81176_1363* | Uncharacterized protein | -2.280876774 | 1.62761E-05 |
| A0A0H3PIW6 | *CJJ81176_0547* | Uncharacterized protein | -2.100410958 | 3.22962E-06 |
| A0A0H3P9W6 | *CJJ81176_1493* | Uncharacterized protein | -1.945433647 | 1.7386E-05 |
| A0A0H3PAT8 | *CJJ81176_1274* | Uncharacterized protein | -1.880296609 | 4.57567E-06 |
| A0A0H3PDW4 | *cjj81176_1424* | Uncharacterized protein | -1.669587613 | 0.000317528 |
| A0A0H3PHF5 | *CJJ81176_0907* | Uncharacterized protein | -1.561626275 | 1.88221E-09 |
| A0A0H3PHG6 | *CJJ81176_0854* | Uncharacterized protein | -1.559316859 | 0.018144143 |
| A0A0H3P9I1 | *CJJ81176_0782* | Uncharacterized protein | -1.556872277 | 2.53565E-09 |
| A0A0H3PAN1 | *cjj81176_1158* | Uncharacterized protein | -1.387338503 | 1.94861E-08 |
| A0A0H3PAA2 | *CJJ81176_0288* | Uncharacterized protein | -1.36130911 | 2.27899E-06 |
| A0A0H3P9A3 | *CJJ81176_0013* | Uncharacterized protein | -1.309926124 | 0.01642129 |
| A0A0H3P9E6 | *CJJ81176_1179* | Uncharacterized protein | -1.29672816 | 7.4028E-08 |
| A0A0H3PAA1 | *CJJ81176_1497* | Uncharacterized protein | -1.255004682 | 1.86926E-08 |
| A0A0H3P9A5 | *CJJ81176_0112* | Uncharacterized protein | -1.226860933 | 2.36353E-06 |
| A0A0H3PCA8 | *CJJ81176_pTet0048* | Uncharacterized protein | -1.186451631 | 5.7261E-10 |

| | | | | |
|---|---|---|---|---|
| A0A0H3PDS7 | *CJJ81176_1355* | Uncharacterized protein | -1.166619299 | 3.20708E-05 |
| A0A0H3PB78 | *CJJ81176_1414* | Uncharacterized protein | -1.163647029 | 9.32886E-10 |
| A0A0H3PAF3 | *CJJ81176_0231* | Uncharacterized protein | -1.145711317 | 5.16051E-06 |
| Q2M5Q6 | *cj81176_1318* | Uncharacterized protein | -1.13772247 | 3.59132E-05 |
| A0A0H3P9Z0 | *CJJ81176_pTet0010* | Uncharacterized protein | -1.118629421 | 0.000266962 |
| A0A0H3PBP0 | *CJJ81176_0442* | Uncharacterized protein | -1.070637671 | 4.23626E-12 |
| Q2M5Q3 | *Cj1305c* | Uncharacterized protein | -1.061759102 | 5.98995E-06 |
| A0A0H3P9A5 | *CJJ81176_0112* | Uncharacterized protein | -1.226860933 | 2.36353E-06 |
| A0A0H3PII9 | *CJJ81176_1327* | Uncharacterized protein | -1.219121522 | 0.049200803 |
| A0A0H3PAH4 | *CJJ81176_0565* | Uncharacterized protein | -1.162555964 | 0.051367488 |
| A0A0H3PBP0 | *CJJ81176_0442* | Uncharacterized protein | -1.070637671 | 4.23626E-12 |
| Q2M5Q3 | *Cj1305c* | Uncharacterized protein | -1.061759102 | 5.98995E-06 |
| A0A0H3PHF5 | *CJJ81176_0907* | Uncharacterized protein | -1.561626275 | 1.88221E-09 |
| A0A0H3PEL5 | *CJJ81176_0280* | Uncharacterized protein | -1.793989654 | 0.001350492 |
| A0A0H3PHH8 | *CJJ81176_0888* | Uncharacterized protein | -1.69475782 | 9.32964E-05 |
| | | | | |
| | | | | |
| | **Significantly upregulated** | | | |
| **UniProt_Accession** | **Gene Name** | **Protein Function** | **logFC** | **P-Value** |
| A0A0H3P9J9 | *cjj81176_0046* | Chemotaxis | 1.036335028 | 0.000173983 |
| A0A0H3PAG7 | *cheW* | Chemotaxis | 1.113400089 | 3.05581E-08 |
| A0A0H3PAN9 | *cjj81176_1205* | Chemotaxis | 1.117164062 | 4.12413E-07 |
| A0A0H3PEL1 | *cjj81176_0289* | Chemotaxis | 1.38527403 | 5.40841E-09 |
| A0A0H3PEF7 | *cjj81176_0180* | Chemotaxis | 1.663550865 | 2.6105E-10 |
| A0A0H3PDX5 | *rnc* | DNA Replication | 1.731695009 | 0.006182112 |
| A0A0H3PAG5 | *radA* | DNA Replication | 1.999618895 | 0.005959603 |
| A1VYR7 | *proA* | Metabolism | 1.009132668 | 3.53952E-10 |
| A0A0H3PGP1 | *lctP* | Metabolism | 1.34055812 | 0.012606899 |
| A0A0H3PI47 | *CJJ81176_1247* | Metabolism | 1.392403379 | 0.025089075 |
| A0A0H3PA51 | *napG* | Metabolism | 1.424011974 | 0.000283441 |
| A0A0H3PA38 | *cydA* | Metabolism | 1.659671162 | 0.001493063 |
| A1VYQ1 | *hemA* | Metabolism | 1.310968059 | 0.057115356 |
| A0A0H3PAD5 | *lpxD* | Metabolism | 1.729657515 | 5.2733E-12 |
| A0A0H3PIF6 | *fliL* | Motility | 1.347230808 | 4.00901E-08 |
| A1W0G0 | *tatA* | Pathogenesis | 1.250195315 | 4.94873E-05 |
| A0A0H3PA52 | *htrA* | Pathogenesis | 1.012383395 | 1.81185E-12 |
| A1VZQ5 | *peb1C* | Pathogenesis | 1.074665497 | 5.96012E-09 |
| A0A0H3PE81 | *CiaC* | Pathogenesis | 1.87174585 | 0.00011936 |
| A1W1L3 | *rpsT* | Protein synthesis | 1.019676169 | 0.00407909 |
| A1W1V3 | *rplP* | Protein synthesis | 1.027409942 | 3.17054E-10 |
| A1W1V4 | *rpsC* | Protein synthesis | 1.102830381 | 4.89003E-12 |
| A0A0H3PB64 | *trpS* | Protein synthesis | 1.616322759 | 2.21865E-07 |
| A1W165 | *truD* | Protein synthesis | 1.740343871 | 6.8171E-05 |
| A0A0H3PB76 | *dnaJ-1* | Stress Response | 1.136786135 | 0.008631516 |
| A0A0H3PBJ5 | *dsbD* | Stress Response | 1.028011072 | 0.102095583 |
| A0A0H3PCE2 | *cstA* | Stress Response | 1.302986402 | 2.76884E-07 |
| A0A0H3PA66 | *dcuB* | Transport | 1.089621325 | 2.75254E-05 |
| A0A0H3PA17 | *putP* | Transport | 1.878844877 | 0.000298502 |
| A0A0H3PA42 | *CJJ81176_0911* | Transport | 1.389768839 | 0.000397905 |
| Q0Q7I0 | *CJJ81176_1569* | Transport | 1.049186667 | 1.26849E-11 |
| A0A0H3P9J0 | *CJJ81176_0912* | Transport | 1.11729136 | 0.007246779 |
| A0A0H3P9C2 | *CJJ81176_1124* | Uncharacterized protein | 1.170249072 | 2.49245E-08 |
| A0A0H3PGG1 | *CJJ81176_pTet0031* | Uncharacterized protein | 1.39381591 | 1.43599E-11 |
| A0A0H3P9L7 | *CJJ81176_0128* | Uncharacterized protein | 1.008232908 | 0.000289218 |
| A0A0H3PCC6 | *CJJ81176_pTet0042* | Uncharacterized protein | 1.045925712 | 0.001508975 |
| A0A0H3PD99 | *CJJ81176_0797* | Uncharacterized protein | 1.047120111 | 0.000609426 |
| A0A0H3P9Y0 | *CJJ81176_1228* | Uncharacterized protein | 1.055185558 | 0.005322174 |
| A0A0H3PCF8 | *CJJ81176_0975* | Uncharacterized protein | 1.091090115 | 1.32648E-06 |

| | | | | |
|---|---|---|---|---|
| Q8GJA7 | *Cjp48* | Uncharacterized protein | 1.092112554 | 3.29008E-09 |
| A0A0H3PH37 | *CJJ81176_1222* | Uncharacterized protein | 1.115037771 | 0.000666304 |
| Q2M5Q9 | *CJJ81176_1315* | Uncharacterized protein | 1.131700017 | 2.97746E-06 |
| A1VY92 | *CJJ81176_0395* | Uncharacterized protein | 1.185734222 | 1.70747E-07 |
| A0A0H3PGE8 | *CJJ81176_pTet0018* | Uncharacterized protein | 1.221656562 | 1.88625E-10 |
| A0A0H3P9F6 | *CJJ81176_pTet0044* | Uncharacterized protein | 1.263478737 | 1.20815E-13 |
| A0A0H3PCP8 | *CJJ81176_1045* | Uncharacterized protein | 1.351483309 | 0.005830684 |
| A0A0H3PED7 | *CJJ81176_0477* | Uncharacterized protein | 1.364895946 | 0.000101422 |
| A0A0H3PAU3 | *CJJ81176_0159* | Uncharacterized protein | 1.517554583 | 0.000910309 |
| A0A0H3PEA5 | *CJJ81176_0635* | Uncharacterized protein | 1.673879027 | 0.009287528 |
| A0A0H3PGI9 | *CJJ81176_0987* | Uncharacterized protein | 1.705481603 | 7.42198E-06 |
| A0A0H3P9T9 | *CJJ81176_1157* | Uncharacterized protein | 1.93813911 | 0.003914575 |
| Q8GJA8 | *Cjp47* | Uncharacterized protein | 1.339364794 | 0.02680047 |
| A0A0H3PGX2 | *CJJ81176_1003* | Uncharacterized protein | 2.10407406 | 0.005728817 |
| A0A0H3PA50 | *CJJ81176_0126* | Uncharacterized protein | 2.265756552 | 5.52932E-08 |

**Appendix 2E:** Significantly differentiated proteins in 81-176 in response to chenodeoxycholic acid (CDCA) 0.05%.

| | **Significantly downregulated** | | | |
|---|---|---|---|---|
| **UniProt_Accession** | **Gene Name** | **Protein Function** | **logFC** | **P-Value** |
| A0A0H3PH83 | *ssb* | DNA Replication | -1.56799 | 3.8E-13 |
| A1VZM0 | *ftsK* | DNA Replication | -1.2322 | 0.00076 |
| A0A0H3PAE3 | *hydA* | Metabolism | -2.19851 | 4.2E-16 |
| A1VXF1 | *aroQ* | Metabolism | -2.03781 | 0.00791 |
| A0A0H3PHL1 | *ubiX* | Metabolism | -1.77652 | 9E-08 |
| A0A0H3PAJ7 | *hydB* | Metabolism | -1.77535 | 2.3E-14 |
| A0A0H3PEJ9 | *frdC* | Metabolism | -1.66138 | 0.00169 |
| A0A0H3PCS4 | *ribE* | Metabolism | -1.49456 | 1.4E-09 |
| A0A0H3PIT1 | *ftn* | Metabolism | -1.48032 | 2.9E-14 |
| A1W085 | *pyrB* | Metabolism | -1.45794 | 2.1E-11 |
| Q5QKR5 | *accB* | Metabolism | -1.37429 | 2.2E-07 |
| A1W1S4 | *eno* | Metabolism | -1.37324 | 6.8E-07 |
| A0A0H3PCR0 | *petB* | Metabolism | -1.27761 | 5.1E-11 |
| A0A0H3PAC7 | *nuoM* | Metabolism | -1.25239 | 0.00026 |
| A1VY43 | *ubiE* | Metabolism | -1.2238 | 0.00262 |
| A0A0H3PBB6 | *trpE* | Metabolism | -1.16377 | 8.2E-12 |
| A0A0H3PBU8 | *accD* | Metabolism | -1.03475 | 0.00083 |
| A0A0H3P9E8 | *petC* | Metabolism | -1.02676 | 2E-08 |
| A0A0H3P9T1 | *gapA* | Metabolism | -1.01979 | 2.7E-12 |
| A0A0H3PI37 | *nuoC* | Metabolism | -1.01048 | 3.8E-07 |
| A0A0H3PHD6 | *glnA* | Metabolism | -1.80965 | 8.3E-14 |
| A0A0H3P9I4 | *purU* | Metabolism | -1.7934 | 1.5E-06 |
| A1VYZ2 | *ilvC* | Metabolism | -1.56888 | 3.9E-15 |
| A0A0H3P9I8 | *speA* | Metabolism | -1.37572 | 3.5E-15 |
| A0A0H3P9M5 | *purB-1* | Metabolism | -1.12237 | 4.5E-15 |
| A0A0H3PET1 | *trpD* | Metabolism | -1.07006 | 3.8E-10 |
| A0A0H3PBG5 | *cjj81176_1338* | Motility | -1.35474 | 4.7E-12 |
| A0A0H3PIZ8 | *fliE* | Motility | -1.08333 | 1.1E-05 |
| A0A0H3PAN7 | *secF* | Pathogenesis | -1.00579 | 3E-05 |
| A0A0H3PAD9 | *pglD* | Pathogenesis | -2.1667 | 0.00024 |
| A0A0H3P9R1 | *pglJ* | Pathogenesis | -1.95631 | 0.01258 |
| A0A0H3P9F0 | *pglF* | Pathogenesis | -1.44391 | 0.01354 |
| Q7X517 | *pseE* | Pathogenesis | -1.03117 | 2.3E-07 |
| Q2M5R2 | *flaA* | Pathogenesis | -1.36689 | 4E-15 |
| A0A0H3PAL0 | *cadF* | Pathogenesis | -1.25318 | 3.2E-10 |
| A1VY30 | *rplY* | Protein synthesis | -2.2421 | 2.9E-16 |

| | | | | |
|---|---|---|---|---|
| A1VYJ1 | *rplA* | Protein synthesis | -1.97717 | 2.6E-06 |
| A1VYI7 | *rpmG* | Protein synthesis | -1.51249 | 0.00924 |
| A1W1V2 | *rpmC* | Protein synthesis | -1.46742 | 2.6E-14 |
| A1VXW7 | *rplT* | Protein synthesis | -1.36643 | 2E-11 |
| A0A0H3PCH6 | *rplC* | Protein synthesis | -1.31708 | 3.6E-14 |
| A1VYJ0 | *rplK* | Protein synthesis | -1.28281 | 1.5E-13 |
| A0A0H3PI52 | *rplO* | Protein synthesis | -1.08329 | 5.4E-11 |
| A1VYJ2 | *rplJ* | Protein synthesis | -1.04452 | 1.4E-11 |
| A0A0H3PGK7 | *pepA* | Protein synthesis | -1.93379 | 5.1E-12 |
| A0A0H3P9B1 | *yajC* | Protein transport | -1.1643 | 9.2E-06 |
| A1W0K4 | *groL* | Stress response | -1.93314 | 1.1E-16 |
| A0A0H3PA75 | *comEA* | Stress Response | -1.55786 | 5.2E-12 |
| A0A0H3PB76 | *dnaJ-1* | Stress Response | -1.02103 | 0.0035 |
| A0A0H3PIS5 | *cmeA* | Transport | -3.08849 | 4.6E-18 |
| A0A0H3PB79 | *cmeB* | Transport | -3.06466 | 3.2E-13 |
| A0A0H3PAE4 | *cmeC* | Transport | -2.36633 | 1.1E-09 |
| A1VXJ0 | *atpD* | Transport | -1.68961 | 4.5E-13 |
| A1VXI7 | *atpH* | Transport | -1.4116 | 0.00437 |
| A1VXI9 | *atpG* | Transport | -1.06101 | 1.1E-09 |
| A1VXJ1 | *atpC* | Transport | -1.02116 | 0.00015 |
| A0A0H3PDG2 | *CJJ81176_0891* | Uncharacterized protein | -1.04461 | 0.00171 |
| A0A0H3PCE6 | *CJJ81176_0935* | Uncharacterized protein | -2.11684 | 0.00094 |
| A0A0H3PA15 | *cjj81176_0828* | Uncharacterized protein | -1.19494 | 2.4E-10 |
| A0A0H3P9U0 | *CJJ81176_1009* | Uncharacterized protein | -1.10052 | 0.00046 |
| A0A0H3P9J4 | *CJJ81176_0882* | Uncharacterized protein | -2.73346 | 1E-14 |
| A0A0H3PAI3 | *CJJ81176_0586* | Uncharacterized protein | -3.75876 | 1.5E-16 |
| Q9KIS1 | *CJJ81176_pVir0002* | Uncharacterized protein | -2.81982 | 0.00242 |
| A0A0H3PB49 | *CJJ81176_1548* | Uncharacterized protein | -2.20177 | 0.00018 |
| A0A0H3PBI5 | *CJJ81176_1639* | Uncharacterized protein | -1.64819 | 0.0013 |
| A0A0H3P9D3 | *cjj81176_1210* | Uncharacterized protein | -1.59431 | 5.3E-15 |
| A0A0H3PCI2 | *CJJ81176_0072* | Uncharacterized protein | -1.58186 | 1.1E-08 |
| A0A0H3PA02 | *CJJ81176_0826* | Uncharacterized protein | -1.55944 | 6.9E-11 |
| A0A0H3PH47 | *CJJ81176_1185* | Uncharacterized protein | -1.4049 | 1.2E-09 |
| A0A0H3P9D1 | *CJJ81176_1051* | Uncharacterized protein | -1.3945 | 3E-14 |
| A0A0H3P9J0 | *CJJ81176_0912* | Uncharacterized protein | -1.32296 | 0.00248 |
| A0A0H3P9M2 | *CJJ81176_0734* | Uncharacterized protein | -1.32066 | 0.01387 |
| A0A0H3P9W6 | *CJJ81176_1493* | Uncharacterized protein | -1.25253 | 0.00989 |
| A0A0H3P9N8 | *CJJ81176_0145* | Uncharacterized protein | -1.13124 | 0.00088 |
| A0A0H3PBE0 | *CJJ81176_0236* | Uncharacterized protein | -1.09599 | 0.00926 |
| A0A0H3PHJ5 | *CJJ81176_0726* | Uncharacterized protein | -1.08397 | 0.00157 |
| A0A0H3P9Y0 | *CJJ81176_1228* | Uncharacterized protein | -1.03956 | 0.0048 |
| A0A0H3PAQ1 | *CJJ81176_0849* | Uncharacterized protein | -1.0156 | 1.1E-05 |
| A0A0H3PB47 | *CJJ81176_1492* | Uncharacterized protein | -1.88091 | 6.2E-09 |
| A0A0H3P9V0 | *CJJ81176_1433* | Uncharacterized protein | -1.32626 | 0.0632 |
| A0A0H3PBZ1 | *CJJ81176_0414* | Uncharacterized protein | -1.00483 | 0.0185 |
| | | | | |
| | **Significantly upregulated** | | | |
| **UniProt_Accession** | **Gene Name** | **Protein Function** | **logFC** | **P-Value** |
| A0A0H3PB06 | *cjj81176_08473* | Chemotaxis | 1.37991 | 4.9E-08 |
| Q8GJE2 | *topA* | DNA Replication | 1.03856 | 3.5E-05 |
| A0A0H3PGG1 | *CJJ81176_pTet0031* | DNA Replication | 1.06298 | 1.3E-09 |
| A1VZ44 | *ackA* | Metabolism | 1.00921 | 2E-10 |
| A1VZJ8 | *folD* | Metabolism | 1.03725 | 8.8E-07 |
| A0A0H3PAD5 | *lpxD* | Metabolism | 1.04267 | 7.7E-11 |
| A1VZ24 | *argG* | Metabolism | 1.04925 | 1.5E-11 |
| A1VY36 | *hisC* | Metabolism | 1.06536 | 9.5E-07 |
| A0A0H3P9P8 | *tkt* | Metabolism | 1.0755 | 2.1E-09 |
| A0A0H3P9U4 | *hipO* | Metabolism | 1.08018 | 3.9E-07 |

| | | | | |
|---|---|---|---|---|
| A1W0I0 | *gpsA* | Metabolism | 1.08052 | 2.8E-08 |
| A0A0H3PAP1 | *thiJ* | Metabolism | 1.08057 | 0.0005 |
| A1VZI4 | *fbp* | Metabolism | 1.08593 | 2.1E-11 |
| A0A0H3PH94 | *gmk* | Metabolism | 1.0895 | 0.00974 |
| Q29VW1 | *gmhA-2* | Metabolism | 1.14757 | 0.00122 |
| A0A0H3PHM5 | *mobB* | Metabolism | 1.1507 | 0.02014 |
| A0A0H3P9T0 | *gmhA-1* | Metabolism | 1.15201 | 0.00029 |
| A1W0I5 | *metE* | Metabolism | 1.16243 | 2.8E-10 |
| A0A0H3PE18 | *hisF-2* | Metabolism | 1.18045 | 0.00025 |
| A1W1X0 | *leuC* | Metabolism | 1.19265 | 0.00231 |
| A0A0H3PJB7 | *sdhB* | Metabolism | 1.22214 | 1.1E-08 |
| A1VZF4 | *dapA* | Metabolism | 1.22408 | 6.9E-12 |
| A1VZ01 | *nadK* | Metabolism | 1.25749 | 0.0006 |
| A0A0H3PBQ2 | *sdhA* | Metabolism | 1.27037 | 1.6E-07 |
| A0A0H3PB56 | *galU* | Metabolism | 1.28866 | 6.7E-05 |
| A1VYG9 | *thiC* | Metabolism | 1.34049 | 4.9E-07 |
| A0A0H3PEG0 | *lpxB* | Metabolism | 1.34727 | 0.01274 |
| A0A0H3PC31 | *hom* | Metabolism | 1.35845 | 8.8E-06 |
| A0A0H3PAG3 | *sdhC* | Metabolism | 1.41291 | 4.3E-07 |
| A1VZR0 | *apt* | Metabolism | 1.56291 | 5.7E-05 |
| A0A0H3PBD0 | *bioA* | Metabolism | 1.58592 | 0.01147 |
| A0A0H3PBK5 | *purS* | Metabolism | 1.61628 | 0.00037 |
| A0A0H3PCK6 | *ansA* | Metabolism | 1.83226 | 2.8E-10 |
| A0A0H3PAJ4 | *hisI* | Metabolism | 2.19192 | 6.2E-05 |
| A0A0H3P982 | *rpiB* | Metabolism | 2.57291 | 2.5E-08 |
| A1VYU1 | *rppH* | Metabolism | 1.21526 | 0.00045 |
| A1W1K3 | *hisA* | Metabolism | 1.17107 | 0.07795 |
| A0A0H3P9T6 | *ribA* | Metabolism | 1.10782 | 0.03354 |
| A0A0H3PID6 | *CJJ81176_1304* | Metabolism | 1.00951 | 5.2E-09 |
| A0A0H3P9Q8 | *CJJ81176_1286* | Metabolism | 1.1543 | 2.3E-13 |
| A0A0H3PAQ8 | *cjj81176_0337* | Metabolism | 1.06729 | 2E-05 |
| A0A0H3PB14 | *cjj81176_0397* | Metabolism | 1.08221 | 6.5E-12 |
| A0A0H3PDJ1 | *cjj81176_1533* | Metabolism | 1.0936 | 2E-14 |
| A0A0H3PB89 | *CJJ81176_1237* | Metabolism | 1.32453 | 0.01013 |
| A0A0H3PBF4 | *CJJ81176_0342* | Motility | 2.30781 | 1.2E-05 |
| Q939J8 | *pseI* | Pathogenesis | 1.43849 | 3.2E-10 |
| A0A0H3PCP5 | *cdtC* | Pathogenesis | 1.06123 | 0.12352 |
| A1VYV6 | *cbf2 (peb4A)* | Pathogenesis | 1.71426 | 4.7E-08 |
| A0A0H3PA65 | *map* | Protein modification | 1.17363 | 5.5E-12 |
| A0A0H3PAI4 | *ileS* | Protein synthesis | 1.09221 | 1.5E-14 |
| A1W048 | *gatA* | Protein synthesis | 1.37637 | 2.5E-10 |
| A1W0I1 | *gatB* | Protein synthesis | 1.50893 | 9.7E-10 |
| A0A0H3PDU5 | *tyrS* | Protein synthesis | 1.52475 | 1.7E-12 |
| A0A0H3PB64 | *trpS* | Protein synthesis | 1.95546 | 7E-10 |
| A1VXQ2 | *sodB* | Stress Responce | 2.0572 | 7.2E-11 |
| A0A0H3PJ16 | *modA* | Transport | 1.42437 | 3.9E-09 |
| Q0Q7H5 | *CJJ81176_1574* | Transport | 1.80897 | 0.00022 |
| A0A0H3PIV9 | *CJJ81176_0179* | Transport | 1.48166 | 1.4E-09 |
| A0A0H3PA76 | *cjj81176_1604* | Transport | 1.2868 | 2.1E-08 |
| A0A0H3PED0 | *CJJ81176_0391* | Two-component regulatory system | 1.07716 | 0.00675 |
| A0A0H3PBN1 | *cjj81176_0379* | Two-component regulatory system | 1.14678 | 9.8E-08 |
| A0A0H3P9Z2 | *CJJ81176_0859* | Uncharacterized protein | 1.03678 | 3.6E-08 |
| A1VYL9 | *CJJ81176_0535* | Uncharacterized protein | 3.13746 | 6.9E-05 |
| Q2A947 | *CJJ81176_1444* | Uncharacterized protein | 1.23078 | 4.6E-13 |
| A0A0H3PEN1 | *cjj81176_0292* | Uncharacterized protein | 1.3502 | 1.2E-12 |
| A0A0H3PHT3 | *CJJ81176_1375* | Uncharacterized protein | 1.068 | 4E-10 |
| A0A0H3PBP8 | *CJJ81176_0462* | Uncharacterized protein | 1.01638 | 0.00432 |
| A0A0H3PA30 | *CJJ81176_0922* | Uncharacterized protein | 1.02573 | 0.02259 |
| A0A0H3PDV4 | *cj81176_1419* | Uncharacterized protein | 1.02871 | 1.4E-12 |

| | | | | |
|---|---|---|---|---|
| A0A0H3PA27 | *CJJ81176_0713* | Uncharacterized protein | 1.03061 | 0.00013 |
| Q2M5R0 | *CJJ81176_1341* | Uncharacterized protein | 1.03396 | 0.00017 |
| A0A0H3PAR1 | *napL* | Uncharacterized protein | 1.06586 | 0.007 |
| A0A0H3PB55 | *CJJ81176_0474* | Uncharacterized protein | 1.0933 | 1.7E-06 |
| A0A0H3PBE5 | *cjj81176_0430* | Uncharacterized protein | 1.11025 | 5.5E-10 |
| A0A0H3PIY1 | *CJJ81176_0564* | Uncharacterized protein | 1.1291 | 0.01215 |
| A0A0H3PA63 | *CJJ81176_0729* | Uncharacterized protein | 1.1305 | 5.5E-12 |
| A0A0H3PAD1 | *cjj81176_0466* | Uncharacterized protein | 1.1315 | 8.4E-05 |
| A0A0H3PDB4 | *CJJ81176_0917* | Uncharacterized protein | 1.20457 | 3.3E-10 |
| A0A0H3PGW3 | *CJJ81176_1177* | Uncharacterized protein | 1.242 | 0.00166 |
| Q6QNL7 | *CJJ81176_1356* | Uncharacterized protein | 1.24768 | 0.00056 |
| A0A0H3P991 | *CJJ81176_0018* | Uncharacterized protein | 1.27929 | 2.9E-06 |
| A0A0H3PBJ6 | *CJJ81176_0387* | Uncharacterized protein | 1.28678 | 3.3E-07 |
| Q0Q7K3 | *CJJ81176_0779* | Uncharacterized protein | 1.30061 | 1.2E-05 |
| A0A0H3PAG9 | *CJJ81176_0672* | Uncharacterized protein | 1.31495 | 7.1E-09 |
| A0A0H3PAI2 | *CJJ81176_1230* | Uncharacterized protein | 1.31576 | 0.00024 |
| A0A0H3PHX6 | *CJJ81176_1306* | Uncharacterized protein | 1.34117 | 2.7E-08 |
| A0A0H3PEW9 | *CJJ81176_0659* | Uncharacterized protein | 1.38772 | 0.02264 |
| A1VY95 | *CJJ81176_0398* | Uncharacterized protein | 1.5365 | 5.1E-07 |
| A1VZY1 | *CJJ81176_1011* | Uncharacterized protein | 1.55314 | 0.0023 |
| A0A0H3PAJ5 | *CJJ81176_1107* | Uncharacterized protein | 1.60859 | 0.01119 |
| A0A0H3PE85 | *CJJ81176_1618* | Uncharacterized protein | 1.11372 | 0.04286 |
| A0A0H3PI41 | *CJJ81176_1600* | Uncharacterized protein | 1.63972 | 5E-17 |
| A0A0H3PDT4 | *CJJ81176_1617* | Uncharacterized protein | 1.69538 | 0.00146 |

**Appendix 2F:** Significantly differentiated proteins in 81-176 in response to ursodeoxycholic acid (UDCA) 0.5%.

| | | | | |
|---|---|---|---|---|
| **UniProt_Accession** | **Gene** | **Protein Function** | **logFC** | **P-Value** |
| A1W0W6 | *mobA* | Metabolism | -1.398854703 | 0.012429197 |
| A0A0H3P9T3 | *CJJ81176_1422* | Uncharacterized protein | -1.341740555 | 4.79977E-05 |
| A0A0H3PBZ1 | *CJJ81176_0414* | Uncharacterized protein | -1.181643952 | 0.005227225 |
| A0A0H3PB39 | *CJJ81176_1673* | Uncharacterized protein | -1.18289333 | 0.020056362 |
| A0A0H3P9G9 | *CJJ81176_pTet0032* | Uncharacterized protein | -1.13543441 | 0.160051784 |
| A0A0H3PCZ7 | *CJJ81176_1082* | Uncharacterized protein | -1.499240598 | 0.151252219 |
| A0A0H3PIW6 | *CJJ81176_0547* | Uncharacterized protein | -1.096335133 | 0.007491295 |
| | | | | |
| | | | | |
| **UniProt_Accession** | **Gene** | **Protein Function** | **logFC** | **P-Value** |
| A0A0H3PI47 | *CJJ81176_1247* | Metabolism | 2.059418551 | 0.008336997 |
| A0A0H3PE81 | *CiaC* | Pathogenesis | 1.809005206 | 8.95667E-08 |

**Appendix 2G**: Significantly differentiated proteins in 81-176 in response to glycocholic acid (GCA) 0.4%.

| | **Significantly downregulated** | | | |
|---|---|---|---|---|
| **UniProt_Accession** | **Gene Name** | **Protein Function** | **logFC** | **P-Value** |
| A0A0H3PJ30 | *nrdB* | DNA Replication | -1.143835257 | 2.18709E-10 |
| A0A0H3PAK5 | *rpoD* | DNA Transcription | -1.110678025 | 0.145086211 |
| A0A0H3P9J4 | *CJJ81176_0882* | Metabolism | -1.589490775 | 4.05121E-07 |
| A0A0H3P9Y9 | *ldh* | Metabolism | -1.085995767 | 7.14287E-10 |
| A0A0H3P9X6 | *CJJ81176_1083* | Metabolism | -1.375508203 | 0.023936195 |
| A0A0H3PA02 | *CJJ81176_0826* | Metabolism | -1.048547551 | 5.21099E-08 |
| A0A0H3PBG5 | *cjj81176_1338* | Motility | -1.467081064 | 2.76046E-05 |

| | | | | |
|---|---|---|---|---|
| A0A0H3PIZ8 | *fliE* | Motility | -1.014704626 | 0.006325288 |
| Q2M5R2 | *flaA* | Pathogenesis | -1.274627167 | 9.48533E-05 |
| A0A0H3PD33 | *sixA* | Protein modification | -2.346764883 | 0.013496177 |
| A1W1V8 | *rplW* | Protein synthesis | -2.263809691 | 2.12135E-06 |
| A1VYI7 | *rpmG* | Protein synthesis | -1.513581593 | 0.01881341 |
| A1W1L3 | *rpsT* | Protein synthesis | -1.210796415 | 0.00607638 |
| A0A0H3PA35 | *dsbA* | Stress Response | -2.400093188 | 1.45848E-07 |
| A0A0H3PIS5 | *cmeA* | Transport | -1.207371022 | 1.437E-06 |
| A0A0H3PAE4 | *cmeC* | Transport | -1.162826559 | 7.21125E-05 |
| A0A0H3PAI3 | *CJJ81176_0586* | Uncharacterized protein | -3.179907441 | 5.07141E-12 |
| A0A0H3PIW6 | *CJJ81176_0547* | Uncharacterized protein | -1.525318713 | 0.001084511 |
| A0A0H3PAF3 | *A0A0H3PAF3* | Uncharacterized protein | -1.222891049 | 6.07848E-05 |
| A0A0H3P9S8 | *CJJ81176_1184* | Uncharacterized protein | -1.20184073 | 1.43633E-05 |
| A0A0H3P9S8 | *CJJ81176_1184* | Uncharacterized protein | -1.20184073 | 1.43633E-05 |
| A0A0H3PHH2 | *CJJ81176_0808* | Uncharacterized protein | -1.047309078 | 0.005280099 |
| A0A0H3P9I1 | *CJJ81176_0782* | Uncharacterized protein | -1.029495338 | 9.2641E-06 |
| | | | | |
| | | | | |
| | **Significantly upregulated** | | | |
| **UniProt_Accession** | **Gene** | **Protein Function** | **logFC** | **P-Value** |
| A1W043 | *murC* | Cell cycle, Cell division | 1.30638759 | 2.14799E-07 |
| A0A0H3P9Z7 | *murE* | Cell cycle, Cell division | 1.171759808 | 5.85253E-10 |
| A0A0H3P9J1 | *yidC* | Chaperone | 1.159957255 | 0.008568378 |
| A0A0H3PAG7 | *cheW* | Chemotaxis | 1.027041862 | 0.000219843 |
| A0A0H3P9T7 | *cj81176_1498* | Chemotaxis | 1.046157988 | 0.003569922 |
| A0A0H3P9P7 | *cjj81176_1128* | Chemotaxis | 1.054926312 | 2.61337E-09 |
| A0A0H3PAM0 | *cheA* | Chemotaxis | 1.078586196 | 2.21771E-05 |
| A0A0H3PAN9 | *cjj81176_1205* | Chemotaxis | 1.091109732 | 0.013167756 |
| A0A0H3PB06 | *cjj81176_08473* | Chemotaxis | 1.139291369 | 4.87666E-06 |
| A0A0H3PEF7 | *cjj81176_0180* | Chemotaxis | 1.221795884 | 0.004841876 |
| A0A0H3P9J9 | *cjj81176_0046* | Chemotaxis | 1.474069681 | 0.000442611 |
| A0A0H3PEL1 | *cjj81176_0289* | Chemotaxis | 1.638007468 | 0.005497677 |
| A0A0H3PCL7 | *nrdA* | DNA Replication | 1.062431781 | 5.56015E-05 |
| A0A0H3PB11 | *CJJ81176_1474* | DNA Replication | 1.477321213 | 6.60977E-09 |
| Q8GJE2 | *topA* | DNA Replication | 1.57332997 | 5.25586E-05 |
| A0A0H3PEP2 | *polA* | DNA Replication | 1.850885202 | 6.32125E-09 |
| A0A0H3PBJ8 | *CJJ81176_0612* | DNA Replication | 1.119513949 | 0.007981402 |
| A0A0H3PED7 | *CJJ81176_0477* | DNA Replication | 1.491543742 | 0.000775788 |
| A0A0H3P9R8 | *cjj81176_1043* | DNA Transcription | 1.428616588 | 6.52245E-07 |
| A0A0H3P9G3 | *rho* | DNA Transcription | 1.059025918 | 3.56854E-06 |
| A0A0H3PAP2 | *pgi* | Metabolism | 1.012691635 | 0.000278505 |
| A0A0H3PAC7 | *nuoM* | Metabolism | 1.010236576 | 0.029368607 |
| A0A0H3PH15 | *thiD* | Metabolism | 1.056040027 | 0.000103225 |
| A0A0H3PA89 | *pyrD* | Metabolism | 1.09491081 | 9.72442E-06 |
| A0A0H3PJ78 | *CJJ81176_0401* | Metabolism | 1.197293268 | 0.000250901 |
| A0A0H3PCT8 | *cjj81176_1032* | Metabolism | 1.202727544 | 0.000370224 |
| A1W1X2 | *leuA* | Metabolism | 1.239537156 | 1.74485E-06 |
| A0A0H3PAJ4 | *hisI* | Metabolism | 1.38823583 | 0.001173788 |
| A0A0H3P9Q2 | *nrfA* | Metabolism | 1.52794861 | 3.72101E-07 |
| A0A0H3PHG1 | *coaBC* | Metabolism | 1.695279363 | 4.72584E-05 |
| A0A0H3PBH6 | *cj81176_1322* | Metabolism | 1.725931639 | 8.11058E-09 |
| A0A0H3PI21 | *nrfH* | Metabolism | 1.753766277 | 4.27635E-05 |
| A1VYR7 | *proA* | Metabolism | 1.851721081 | 1.54993E-06 |
| A1VZR0 | *apt* | Metabolism | 2.257265786 | 7.49402E-05 |
| A0A0H3PAD5 | *lpxD* | Metabolism | 2.451256332 | 3.29872E-08 |
| A1W1D6 | *acsA* | Metabolism | 1.022475599 | 0.000262376 |
| A1W0W6 | *mobA* | Metabolism | 1.318705744 | 0.056775699 |
| Q29VV6 | *fcl* | Metabolism | 1.111559635 | 7.93591E-12 |

| | | | | |
|---|---|---|---|---|
| A1W0I0 | *gpsA* | Metabolism | 1.408498417 | 6.76347E-10 |
| A0A0H3P9H5 | *serA* | Metabolism | 1.290937906 | 2.60918E-08 |
| A0A0H3PJH9 | *ctpA* | Metabolism | 1.149951956 | 2.28101E-07 |
| A0A0H3PB53 | *cjj81176_1596* | Metabolism | 1.466195445 | 9.26049E-06 |
| A1VYU1 | *rppH* | Metabolism | 1.652548227 | 0.003120588 |
| A0A0H3PCI0 | *cjj81176* | Metabolism | 1.193667448 | 0.001078924 |
| A0A0H3PAW0 | *corA* | Metabolism | 2.092660358 | 0.010257307 |
| A0A0H3PB89 | *CJJ81176_1237* | Metabolism | 1.106292834 | 0.044823262 |
| A0A0H3P9Y0 | *CJJ81176_1228* | Metabolism | 1.051574173 | 0.002356033 |
| A0A0H3P9T9 | *CJJ81176_1157* | Metabolism | 1.442731017 | 5.77388E-05 |
| A0A0H3PEX3 | *CJJ81176_0544* | Metabolism | 1.945370193 | 0.011501981 |
| A0A0H3PEJ9 | *frdC* | Metabolism | 1.244114655 | 0.052599159 |
| A1VYM4 | *purC* | Metabolism | 1.120206962 | 7.50334E-06 |
| A0A0H3PA78 | *fliY* | Motility | 1.047934508 | 0.012086825 |
| A0A0H3PIF6 | *fliL* | Motility | 1.087571319 | 0.014373096 |
| A0A0H3P9L2 | *fliM* | Motility | 2.378593386 | 0.003061909 |
| A1VZQ5 | *peb1C* | Pathogenesis | 1.128861745 | 0.00063624 |
| A0A0H3PAY0 | *tatB* | Pathogenesis | 1.163206973 | 0.024829563 |
| A0A0H3PE81 | *CiaC* | Pathogenesis | 1.339773352 | 0.000354885 |
| A0A0H3PAC3 | *CJJ81176_1161* | Pathogenesis | 1.864722951 | 1.32845E-09 |
| A1VXT6 | *infC* | Protein modification | 1.523398384 | 7.77475E-06 |
| A1W1V4 | *rpsC* | Protein synthesis | 1.02473971 | 6.01156E-06 |
| A1VZ23 | *rplI* | Protein synthesis | 1.660099792 | 5.82011E-12 |
| A1VZ59 | *glyQ* | Protein synthesis | 1.149476182 | 1.16337E-07 |
| A0A0H3PAZ6 | *hypB* | Protein synthesis | 1.363303109 | 0.000148318 |
| A0A0H3PHD8 | *valS* | Protein synthesis | 1.052169564 | 3.38209E-10 |
| A1VYL8 | *alaS* | Protein synthesis | 1.223687733 | 5.12978E-11 |
| A0A0H3PBL2 | *def* | Protein synthesis | 1.224371613 | 0.012570718 |
| A1VZN1 | *pheS* | Protein synthesis | 1.314439796 | 0.000110534 |
| A1VYQ2 | *proS* | Protein synthesis | 1.430432833 | 4.89263E-10 |
| A0A0H3PHR2 | *pheT* | Protein synthesis | 1.450255078 | 7.88255E-08 |
| A0A0H3PID1 | *glyS* | Protein synthesis | 1.540443645 | 1.81652E-13 |
| A0A0H3P9K7 | *metS* | Protein synthesis | 1.541667562 | 9.21481E-08 |
| A1W165 | *truD* | Protein synthesis | 1.641922345 | 0.000566212 |
| A0A0H3PDU5 | *tyrS* | Protein synthesis | 1.933866117 | 1.80471E-10 |
| A0A0H3PB64 | *trpS* | Protein synthesis | 2.713166919 | 8.45629E-08 |
| A0A0H3PDX5 | *rnc* | Protein synthesis | 1.268587632 | 0.015096371 |
| A0A0H3PCJ0 | *CJJ81176_0101* | Protein synthesis | 1.354291925 | 1.03627E-07 |
| A1VY44 | *xseA* | Stress Response | 1.917866767 | 0.002666005 |
| A0A0H3PEB4 | *nth* | Stress Response | 2.079993069 | 5.37591E-05 |
| A1VYU6 | *ligA* | Stress Response | 1.129567362 | 1.70097E-05 |
| A0A0H3PBY8 | *AhpC/Tsa* | Stress Response | 1.007801231 | 0.000255865 |
| A1VYN0 | *htpG* | Stress response | 1.270831186 | 0.000826664 |
| A0A0H3PEV8 | *pbpA* | Stress Response | 1.028057706 | 0.014984185 |
| A0A0H3PCE2 | *cstA* | Stress Response | 1.061589091 | 0.007125932 |
| A0A0H3PJI4 | *recN* | Stress Response | 1.284566677 | 0.035303827 |
| A0A0H3PAG5 | *radA* | Stress Response | 1.14732027 | 0.055603586 |
| A0A0H3PHF3 | *cjj81176_0717* | Stress Response | 1.035873548 | 1.91342E-05 |
| A0A0H3PHE3 | *metN* | Transport | 1.100717694 | 0.000642589 |
| A0A0H3P9L8 | *atpF* | Transport | 1.683509237 | 0.001492146 |
| A0A0H3PF18 | *cj81176_0446* | Transport | 1.116102467 | 0.00192143 |
| A0A0H3PA66 | *dcuB* | Transport | 1.293582352 | 1.72574E-05 |
| A0A0H3PA60 | *dcuA* | Transport | 1.709820081 | 1.39941E-09 |
| A0A0H3P9B1 | *yajC* | Transport | 1.433773825 | 0.025248299 |
| A0A0H3PA76 | *cjj81176_1604* | Transport | 1.760880095 | 2.22661E-05 |
| A0A0H3PJB3 | *CJJ81176_0263* | Transport | 1.420042717 | 0.018000274 |
| A0A0H3PAQ2 | *CJJ81176_0494* | Transport | 2.206134019 | 0.000871678 |
| A0A0H3PBF3 | *cj81176_1241* | Two-component regulatory system | 1.361431748 | 9.08388E-05 |
| A0A0H3PED0 | *CJJ81176_0391* | Two-component regulatory system | 1.941070876 | 0.000951954 |

| | | | | |
|---|---|---|---|---|
| A0A0H3PJ41 | *cj81176_0671* | Two-component regulatory system | 1.087017298 | 1.39294E-10 |
| A1VYL9 | *CJJ81176_0535* | Uncharacterized | 1.998863847 | 0.000651394 |
| Q6QNL8 | *Cj1356c* | Uncharacterized | 2.213285665 | 1.26517E-05 |
| A0A0H3PAV3 | *CJJ81176_0846* | Uncharacterized | 1.164012416 | 5.89684E-05 |
| A0A0H3P9Z2 | *CJJ81176_0859* | Uncharacterized | 2.080132578 | 3.86704E-09 |
| A0A0H3PBF4 | *CJJ81176_0342* | Uncharacterized | 1.444326928 | 0.012033914 |
| A0A0H3PI86 | *CJJ81176_1476* | Uncharacterized | 1.099729357 | 0.005302534 |
| A0A0H3PH37 | *CJJ81176_1222* | Uncharacterized | 1.302400463 | 8.67774E-05 |
| A0A0H3PA50 | *CJJ81176_0126* | Uncharacterized | 1.57224041 | 2.52682E-05 |
| A0A0H3PD99 | *CJJ81176_0797* | Uncharacterized | 1.041439818 | 0.007054014 |
| Q2M5R0 | *CJJ81176_1341* | Uncharacterized | 1.065593133 | 0.015840807 |
| A0A0H3PIL0 | *CJJ81176_1513* | Uncharacterized | 1.077566378 | 0.020973691 |
| A0A0H3PA08 | *CJJ81176_0742* | Uncharacterized | 1.094047949 | 0.012374995 |
| A0A0H3PC19 | *CJJ81176_0428* | Uncharacterized | 1.128295837 | 0.000296915 |
| A0A0H3PA18 | *CJJ81176_0942* | Uncharacterized | 1.148716667 | 0.002232561 |
| A0A0H3P9Z9 | *CJJ81176_0708* | Uncharacterized | 1.165436685 | 0.000393889 |
| A0A0H3PB67 | *CJJ81176_1452* | Uncharacterized | 1.167488132 | 0.007383591 |
| A0A0H3PAU3 | *CJJ81176_0159* | Uncharacterized | 1.169892818 | 0.004826395 |
| A0A0H3PAI2 | *CJJ81176_1230* | Uncharacterized | 1.177114669 | 0.002549107 |
| A0A0H3PCC6 | *CJJ81176_pTet0042* | Uncharacterized | 1.209258671 | 0.000100791 |
| Q1HG73 | *CJJ81176_0034* | Uncharacterized | 1.23406311 | 4.86216E-08 |
| A0A0H3PHT8 | *CJJ81176_1541* | Uncharacterized | 1.236412863 | 0.000280977 |
| Q8GJE8 | *Cjp04* | Uncharacterized | 1.244925612 | 0.00066129 |
| A0A0H3PHX6 | *CJJ81176_1306* | Uncharacterized | 1.275876656 | 5.00632E-06 |
| A0A0H3PAJ5 | *CJJ81176_1107* | Uncharacterized | 1.282709201 | 0.006616104 |
| A0A0H3PBE2 | *CJJ81176_0543* | Uncharacterized | 1.309783337 | 0.007152666 |
| A0A0H3PA98 | *CJJ81176_1344* | Uncharacterized | 1.363458043 | 1.01647E-06 |
| A0A0H3PDN2 | *CJJ81176_1418* | Uncharacterized | 1.424887664 | 2.03865E-06 |
| A0A0H3PBM4 | *CJJ81176_0677* | Uncharacterized | 1.440113545 | 0.001116171 |
| A0A0H3PJ65 | *CJJ81176_0275* | Uncharacterized | 1.493134748 | 9.86817E-07 |
| A0A0H3PBR7 | *CJJ81176_0420* | Uncharacterized | 1.49857783 | 0.016232645 |
| A0A0H3PIU3 | *CJJ81176_0188* | Uncharacterized | 1.507397539 | 0.00015927 |
| A0A0H3PJK4 | *CJJ81176_0436* | Uncharacterized | 1.520818826 | 1.49833E-06 |
| A0A0H3PDK8 | *CJJ81176_1475* | Uncharacterized | 1.52484768 | 1.24297E-08 |
| A0A0H3PAS5 | *CJJ81176_0840* | Uncharacterized | 1.557240634 | 0.000943813 |
| A0A0H3PCF8 | *CJJ81176_0975* | Uncharacterized | 1.581746809 | 0.003542892 |
| Q0Q7K3 | *CJJ81176_0779* | Uncharacterized | 1.643495037 | 3.22952E-07 |
| A0A0H3PGX2 | *CJJ81176_1003* | Uncharacterized | 1.678544949 | 0.006049199 |
| A0A0H3P991 | *CJJ81176_0018* | Uncharacterized | 1.695804477 | 0.009658057 |
| A0A0H3PA27 | *CJJ81176_0713* | Uncharacterized | 1.699192913 | 3.39798E-07 |
| A0A0H3PHF9 | *CJJ81176_0723* | Uncharacterized | 1.700744095 | 0.011289348 |
| Q2M5Q7 | *CJJ81176_1317* | Uncharacterized | 1.709578567 | 0.000752027 |
| A0A0H3PCA0 | *CJJ81176_pTet0008* | Uncharacterized | 1.731028923 | 6.59507E-07 |
| A0A0H3PC13 | *CJJ81176_0374* | Uncharacterized | 1.842944074 | 3.08318E-12 |
| A0A0H3PCN0 | *CJJ81176_0127* | Uncharacterized | 1.870282445 | 1.15494E-09 |
| A0A0H3PAS8 | *CJJ81176_0740* | Uncharacterized | 1.881068607 | 0.000394551 |
| A0A0H3PE85 | *CJJ81176_1618* | Uncharacterized | 2.027660503 | 0.000426093 |
| Q0Q7K1 | *cj0760* | Uncharacterized | 2.245727233 | 0.003470769 |
| A0A0H3PGL0 | *CJJ81176_1732* | Uncharacterized | 2.532902078 | 0.003326745 |
| A0A0H3P9J3 | *CJJ81176_0988* | Uncharacterized | 1.066400865 | 0.033590784 |
| A0A0H3PA31 | *CJJ81176_0693* | Uncharacterized | 1.163798268 | 0.117526572 |
| A0A0H3PB39 | *CJJ81176_1673* | Uncharacterized | 1.186264918 | 0.081739944 |
| A0A0H3PBU4 | *CJJ81176_0392* | Uncharacterized | 1.248619432 | 0.167949515 |
| A0A0H3PJC9 | *CJJ81176_0518* | Uncharacterized | 1.333414313 | 0.040484982 |
| A0A0H3PAS3 | *CJJ81176_0705* | Uncharacterized | 1.389532681 | 0.046693518 |
| A0A0H3P9T9 | *CJJ81176_1157* | Uncharacterized | 1.442731017 | 5.77388E-05 |
| A0A0H3PE25 | *CJJ81176_1654* | Uncharacterized | 1.55429974 | 0.052886054 |
| A0A0H3PCE6 | *CJJ81176_0935* | Uncharacterized | 1.161217324 | 0.100954548 |
| Q2M5Q9 | *CJJ81176_1315* | Uncharacterized | 2.749047464 | 7.83127E-06 |

**Appendix 3**: Significantly differentiated proteins between 81-176 cultured in CDB supplemented with DCA 0.05% at 37$^{o}$C for 12h and 24h

| | **Significantly downregulated** | | | |
|---|---|---|---|---|
| **UniProt_Accession** | **Gene Name** | **Protein function** | **logFC** | **P.Value** |
| A0A0H3PB06 | *TlpC* | Chemotaxis | -1.534630721 | 1.11004E-08 |
| A1W0A5 | *cheY* | Chemotaxis | -1.072770056 | 2.27169E-07 |
| A0A0H3PGG1 | *CJJ81176_pTet0031* | DNA Replication | -2.176509223 | 6.05531E-05 |
| A0A0H3P989 | *recJ* | DNA Replication | -1.144633329 | 0.000310315 |
| A0A0H3PHM5 | *mobB* | Metabolism | -2.289105969 | 3.63172E-09 |
| A0A0H3PAM5 | *CJJ81176_0297* | Metabolism | -2.225155586 | 6.10595E-07 |
| A0A0H3PD29 | *cobB* | Metabolism | -2.061733731 | 0.000227175 |
| A0A0H3PGR5 | *cjj81176_0063* | Metabolism | -1.951654431 | 2.18894E-13 |
| A0A0H3PC31 | *hom* | Metabolism | -1.949448199 | 7.65525E-11 |
| A1W1X0 | *leuC* | Metabolism | -1.94754045 | 2.50273E-10 |
| A0A0H3P9B2 | *thiH* | Metabolism | -1.931403378 | 5.08353E-11 |
| A0A0H3P9A4 | *CJJ81176_0120* | Metabolism | -1.84724992 | 2.96764E-06 |
| A0A0H3PAJ4 | *hisI* | Metabolism | -1.779587205 | 1.07128E-13 |
| A0A0H3PBD0 | *bioA* | Metabolism | -1.769616625 | 0.008901011 |
| A1VXL7 | *thrB* | Metabolism | -1.767063041 | 2.48131E-05 |
| A1VZR0 | *apt* | Metabolism | -1.734767843 | 8.47807E-12 |
| A0A0H3P982 | *rpiB* | Metabolism | -1.713717284 | 0.000193393 |
| A0A0H3PAG3 | *sdhC* | Metabolism | -1.678460176 | 5.61472E-12 |
| A0A0H3PB56 | *galU* | Metabolism | -1.61044587 | 2.42396E-10 |
| A0A0H3PBK5 | *purS* | Metabolism | -1.575394653 | 0.000890006 |
| A0A0H3P9E4 | *pepD* | Metabolism | -1.558173863 | 0.000213678 |
| A0A0H3P9Z1 | *CJJ81176_1373* | Metabolism | -1.53105753 | 1.61516E-06 |
| A1VYU1 | *rppH* | Metabolism | -1.50051283 | 2.73047E-05 |
| Q0Q7I1 | *purM* | Metabolism | -1.496089294 | 2.22828E-08 |
| A1W1K3 | *hisA* | Metabolism | -1.464787013 | 1.33985E-05 |
| A1W1W9 | *leuD* | Metabolism | -1.426655645 | 6.4391E-10 |
| A0A0H3PJB7 | *sdhB* | Metabolism | -1.418049631 | 2.33294E-14 |
| A0A0H3PEI7 | *folP* | Metabolism | -1.416812645 | 0.000125525 |
| A1VXP5 | *moaA* | Metabolism | -1.384868147 | 2.24678E-07 |
| A0A0H3PBQ2 | *sdhA* | Metabolism | -1.383788401 | 3.9996E-12 |
| A0A0H3PB89 | *CJJ81176_1237* | Metabolism | -1.38237189 | 0.0241455 |
| A1VZJ8 | *folD* | Metabolism | -1.351022602 | 4.68043E-05 |
| A0A0H3P9J6 | *pta* | Metabolism | -1.321680589 | 6.74251E-14 |
| A0A0H3P9B6 | *thiF* | Metabolism | -1.314800614 | 0.00041964 |
| A1VZI4 | *fbp* | Metabolism | -1.310330523 | 2.48258E-14 |
| A1VXV5 | *pyrE* | Metabolism | -1.302260891 | 0.000752522 |
| A1VZ41 | *ispG* | Metabolism | -1.300968817 | 1.92483E-06 |
| A0A0H3PE58 | *CJJ81176_1470* | Metabolism | -1.295945237 | 0.003131783 |
| A0A0H3PIZ2 | *CJJ81176_0601* | Metabolism | -1.291103226 | 3.19107E-07 |
| A0A0H3PH94 | *gmk* | Metabolism | -1.291085541 | 0.004938315 |
| A0A0H3P9R4 | *sdaA* | Metabolism | -1.28828577 | 7.18377E-08 |
| A0A0H3PH73 | *pglE* | Metabolism | -1.281261867 | 1.88466E-09 |
| A1VZ44 | *ackA* | Metabolism | -1.262409482 | 7.70713E-11 |
| A0A0H3PJF7 | *coaX* | Metabolism | -1.23483052 | 0.020671446 |
| A0A0H3PBS3 | *fabG* | Metabolism | -1.227079887 | 2.56008E-13 |
| A1VZM9 | *aroA* | Metabolism | -1.219767158 | 5.74613E-06 |
| A1VYB8 | *gatC* | Metabolism | -1.211406746 | 3.49823E-10 |
| A0A0H3PE18 | *hisF-2* | Metabolism | -1.207889272 | 1.88098E-07 |
| A0A0H3P9T0 | *gmhA-1* | Metabolism | -1.206919932 | 9.82001E-05 |
| A0A0H3PB33 | *CJJ81176_0291* | Metabolism | -1.179805682 | 5.72858E-07 |
| A0A0H3PJ06 | *mqnC* | Metabolism | -1.174422411 | 5.60488E-07 |
| A0A0H3PAD3 | *fdhD* | Metabolism | -1.173277982 | 4.14031E-11 |
| A0A0H3PHL6 | *CJJ81176_0799* | Metabolism | -1.168451704 | 9.44047E-06 |

| | | | | |
|---|---|---|---|---|
| A0A0H3P9S5 | *cysQ* | Metabolism | -1.168216915 | 0.000116334 |
| A1VZY2 | *argF* | Metabolism | -1.164517176 | 0.000309723 |
| A1VZZ8 | *tgt* | Metabolism | -1.142980114 | 0.005863137 |
| A0A0H3PAH1 | *tyrA* | Metabolism | -1.136133556 | 5.07923E-05 |
| A1VY47 | *fabH* | Metabolism | -1.13422335 | 4.41177E-06 |
| A1VZF4 | *dapA* | Metabolism | -1.116745028 | 3.41227E-09 |
| A1W0W6 | *mobA* | Metabolism | -1.105859675 | 0.020254191 |
| A0A0H3P9G7 | *hemN* | Metabolism | -1.102795793 | 2.46272E-09 |
| A0A0H3P9M4 | *aspC* | Metabolism | -1.101069446 | 6.65544E-08 |
| A0A0H3PBF9 | *rpe* | Metabolism | -1.097938349 | 0.000184067 |
| A0A0H3P9K7 | *metS* | Metabolism | -1.088022463 | 2.57612E-11 |
| A1VY36 | *hisC* | Metabolism | -1.087038366 | 7.64178E-10 |
| A1VYR7 | *proA* | Metabolism | -1.083293554 | 2.35355E-09 |
| A1W0U8 | *hisF2* | Metabolism | -1.081876993 | 0.059827283 |
| A0A0H3PCM5 | *metX* | Metabolism | -1.079159296 | 1.40402E-09 |
| A1VX91 | *ilvD* | Metabolism | -1.078272971 | 1.85463E-09 |
| A0A0H3PF06 | *CJJ81176_0186* | Metabolism | -1.070281228 | 1.40566E-10 |
| A0A0H3P9M7 | *acnB* | Metabolism | -1.05519857 | 1.14214E-12 |
| Q5QKR7 | *pseC* | Metabolism | -1.03813732 | 3.71904E-08 |
| A0A0H3PF03 | *fabF* | Metabolism | -1.037174478 | 4.18632E-10 |
| Q939J8 | *pseI* | Metabolism | -1.036392088 | 6.47106E-07 |
| A0A0H3PCK6 | *ansA* | Metabolism | -1.032726469 | 7.26567E-06 |
| A0A0H3PIV6 | *ribB* | Metabolism | -1.028543383 | 1.07019E-06 |
| A1W0I5 | *metE* | Metabolism | -1.02577443 | 2.088E-10 |
| Q2M5Q2 | *pseF* | Metabolism | -1.011511723 | 0.000507276 |
| A1W035 | *thiG* | Metabolism | -1.007640233 | 6.05704E-10 |
| A0A0H3PB14 | *cjj81176_0397* | Metabolism | -1.004241639 | 9.40176E-11 |
| A1VYF9 | *acpP* | Metabolism | -1.003103607 | 6.3537E-07 |
| A1W062 | *fliW* | Motility | -1.793716999 | 0.009987511 |
| A1W0U6 | *pseG* | Pathogenesis | -1.757353754 | 0.000385104 |
| A0A0H3PD33 | *sixA* | Protein modification | -3.042123656 | 6.11302E-06 |
| A0A0H3PA65 | *map* | Protein modification | -1.254302055 | 1.29781E-08 |
| A0A0H3PB64 | *trpS* | Protein synthesis | -2.052688853 | 4.94296E-14 |
| A1W162 | *rimO* | Protein synthesis | -1.709971097 | 1.41232E-06 |
| A0A0H3PBB3 | *rbfA* | Protein synthesis | -1.578918451 | 0.070189606 |
| A1W048 | *gatA* | Protein synthesis | -1.366271092 | 7.80038E-11 |
| A1W0I1 | *gatB* | Protein synthesis | -1.298013236 | 1.59092E-09 |
| A0A0H3PAE1 | *CJJ81176_0192* | Protein synthesis | -1.260081481 | 0.022658867 |
| A1VZI8 | *gltX1* | Protein synthesis | -1.197827538 | 9.42759E-07 |
| A1VYC1 | *lysS* | Protein synthesis | -1.175353636 | 2.79091E-12 |
| A0A0H3PDU5 | *tyrS* | Protein synthesis | -1.151533665 | 5.04525E-13 |
| A0A0H3PAI4 | *ileS* | Protein synthesis | -1.1132896 | 7.71337E-10 |
| A1W1L4 | *prfA* | Protein synthesis | -1.015069632 | 7.51996E-09 |
| A1W1U6 | *rpsH* | Protein synthesis | -1.012713367 | 2.17938E-08 |
| A0A0H3PBL2 | *def* | Protein synthesis | -1.010121188 | 0.054905098 |
| Q3I354 | *luxS* | Stress Response | -1.687624386 | 0.011716769 |
| A0A0H3P9Q3 | *csrA* | Stress Response | -1.61842485 | 0.007022795 |
| Q0Q7K8 | *grpE* | Stress Response | -1.365429908 | 1.5009E-07 |
| A0A0H3P9V7 | *CJJ81176_1101* | Stress Response | -1.319815791 | 0.010561711 |
| A0A0H3PBL4 | *hypE* | Stress Response | -1.286790385 | 8.88023E-09 |
| A0A0H3PAC3 | *CJJ81176_1161* | Stress Response | -1.283249823 | 5.10693E-10 |
| A1VXQ2 | *sodB* | Stress Response | -1.083981333 | 4.16353E-06 |
| Q0Q7H5 | *CJJ81176_1574* | Transport | -2.904280895 | 1.28308E-13 |
| A0A0H3PIV9 | *CJJ81176_0179* | Transport | -1.415883294 | 7.75629E-10 |
| A0A0H3PJ16 | *modA* | Transport | -1.334639945 | 1.35986E-09 |
| A0A0H3PBL7 | *fur* | Transport | -1.236790271 | 0.068498131 |
| A0A0H3PAG9 | *CJJ81176_0672* | Transport | -1.069939498 | 3.6333E-09 |
| A0A0H3PA76 | *cjj81176_1604* | Transport | -1.040698247 | 6.26529E-06 |
| A0A0H3PED0 | *CJJ81176_0391* | Two-component | -1.053551844 | 9.66291E-08 |

| | | | | |
|---|---|---|---|---|
| | | regulatory system | | |
| A0A0H3PAS8 | *CJJ81176_0740* | Uncharacterized | -3.395499634 | 0.000125724 |
| A0A0H3PHH8 | *CJJ81176_0888* | Uncharacterized | -2.273851563 | 0.00055882 |
| A0A0H3PGW3 | *CJJ81176_1177* | Uncharacterized | -2.124432478 | 0.002463034 |
| A0A0H3P9I1 | *CJJ81176_0782* | Uncharacterized | -2.034013837 | 9.50885E-09 |
| A0A0H3P9L3 | *CJJ81176_0728* | Uncharacterized | -1.846362151 | 0.000681756 |
| A0A0H3P9G9 | *CJJ81176_pTet0032* | Uncharacterized | -1.832741674 | 0.001704366 |
| A0A0H3PI41 | *CJJ81176_1600* | Uncharacterized | -1.734572848 | 5.54876E-14 |
| A0A0H3PAF1 | *CJJ81176_1363* | Uncharacterized | -1.707873526 | 0.000712319 |
| A0A0H3PB96 | *CJJ81176_0611* | Uncharacterized | -1.682630282 | 0.006326735 |
| Q0Q7K1 | *cj0760* | Uncharacterized | -1.671608069 | 0.005405484 |
| A0A0H3PHG6 | *CJJ81176_0854* | Uncharacterized | -1.654926939 | 0.000469686 |
| A0A0H3PAT8 | *CJJ81176_1274* | Uncharacterized | -1.650434727 | 0.000376957 |
| A0A0H3PHF5 | *CJJ81176_0907* | Uncharacterized | -1.638369373 | 1.84875E-12 |
| A0A0H3PA59 | *CJJ81176_1259* | Uncharacterized | -1.611467114 | 4.29174E-07 |
| A0A0H3P9Y5 | *CJJ81176_pTet0016* | Uncharacterized | -1.576465209 | 0.000410273 |
| A0A0H3PBJ6 | *CJJ81176_0387* | Uncharacterized | -1.57120965 | 5.15548E-11 |
| A0A0H3PHU2 | *CJJ81176_1517* | Uncharacterized | -1.561956351 | 1.1584E-08 |
| A0A0H3P9T3 | *CJJ81176_1422* | Uncharacterized | -1.546128793 | 3.03535E-05 |
| A0A0H3PIY1 | *CJJ81176_0564* | Uncharacterized | -1.539276312 | 1.26619E-07 |
| A0A0H3PEL5 | *CJJ81176_0280* | Uncharacterized | -1.535329791 | 0.000695997 |
| A0A0H3PH34 | *CJJ81176_1055* | Uncharacterized | -1.530772415 | 0.019927418 |
| A0A0H3PDG2 | *CJJ81176_0891* | Uncharacterized | -1.53059867 | 0.019346873 |
| A0A0H3PAA1 | *CJJ81176_1497* | Uncharacterized | -1.490702487 | 2.61848E-10 |
| A0A0H3PEG8 | *CJJ81176_0642* | Uncharacterized | -1.486781416 | 6.9234E-05 |
| A0A0H3PIX1 | *CJJ81176_0650* | Uncharacterized | -1.483651219 | 4.07685E-05 |
| A0A0H3PB55 | *CJJ81176_0474* | Uncharacterized | -1.471043488 | 1.43002E-07 |
| A0A0H3PC13 | *CJJ81176_0374* | Uncharacterized | -1.451690112 | 4.2979E-14 |
| Q2A947 | *CJJ81176_1444* | Uncharacterized | -1.432051832 | 9.2821E-12 |
| A0A0H3PD80 | *CJJ81176_0830* | Uncharacterized | -1.394971406 | 0.000642855 |
| A0A0H3PJA2 | *CJJ81176_0520* | Uncharacterized | -1.357218846 | 0.000976082 |
| A0A0H3PEN1 | *CJJ81176_0292* | Uncharacterized | -1.314662722 | 2.28087E-10 |
| A0A0H3PA08 | *CJJ81176_0742* | Uncharacterized | -1.295589597 | 2.48463E-05 |
| A0A0H3PB85 | *CJJ81176_0254* | Uncharacterized | -1.290201949 | 0.147090706 |
| A0A0H3PJC9 | *CJJ81176_0518* | Uncharacterized | -1.266945882 | 0.061688801 |
| A0A0H3PAG4 | *CJJ81176_1510* | Uncharacterized | -1.266882381 | 1.65188E-07 |
| A0A0H3PA31 | *CJJ81176_0693* | Uncharacterized | -1.249801916 | 0.063061913 |
| A0A0H3PBB5 | *CJJ81176_0693* | Uncharacterized | -1.238206891 | 5.66522E-09 |
| A0A0H3PB58 | *CJJ81176_0610* | Uncharacterized | -1.229926949 | 3.09426E-08 |
| A0A0H3PDJ1 | *CJJ81176_1533* | Uncharacterized | -1.202103169 | 7.3123E-11 |
| A0A0H3P9A5 | *CJJ81176_0112* | Uncharacterized | -1.187920712 | 6.96931E-10 |
| A0A0H3PAD1 | *CJJ81176_0466* | Uncharacterized | -1.187338638 | 3.05249E-06 |
| A0A0H3PAK7 | *CJJ81176_0681* | Uncharacterized | -1.18385986 | 7.49103E-08 |
| A0A0H3PCP5 | *cdtC* | Uncharacterized | -1.182000198 | 0.017584303 |
| A0A0H3PHE7 | *CJJ81176_0739* | Uncharacterized | -1.155225574 | 1.49838E-05 |
| A0A0H3P9J3 | *CJJ81176_0988* | Uncharacterized | -1.14196251 | 1.60798E-05 |
| Q0Q7K3 | *CJJ81176_0779* | Uncharacterized | -1.139419855 | 9.00761E-06 |
| A0A0H3P9K9 | *cjj81176_0850* | Uncharacterized | -1.129985874 | 6.0963E-13 |
| A0A0H3PAQ8 | *CJJ81176_0337* | Uncharacterized | -1.119511761 | 2.10814E-10 |
| A0A0H3PGL6 | *CJJ81176_0030* | Uncharacterized | -1.113400773 | 0.00884966 |
| A0A0H3P9Q8 | *CJJ81176_1286* | Uncharacterized | -1.10726362 | 5.93067E-12 |
| A0A0H3PHP5 | *CJJ81176_0887* | Uncharacterized | -1.106241806 | 0.000305572 |
| A0A0H3PBQ0 | *CJJ81176_0195* | Uncharacterized | -1.081195667 | 0.007115605 |
| A0A0H3PAR1 | *napL* | Uncharacterized | -1.067540614 | 8.28608E-05 |
| A0A0H3PAI2 | *CJJ81176_1230* | Uncharacterized | -1.060892094 | 1.06996E-06 |
| A0A0H3PA98 | *CJJ81176_1344* | Uncharacterized | -1.042897512 | 8.70453E-12 |
| Q0Q7K6 | *CJJ81176_0776* | Uncharacterized | -1.028796474 | 3.7577E-07 |
| A0A0H3PAM8 | *CJJ81176_1076* | Uncharacterized | -1.003509512 | 7.39095E-08 |
| | | | | |

| | Significantly upregulated | | | |
|---|---|---|---|---|
| **UniProt_Accession** | **Gene Name** | **Protein function** | **logFC** | **P.Value** |
| A0A0H3P9D5 | *ftsH* | Cell cycle, cell division | 2.173071713 | 3.52151E-10 |
| A1VZM0 | *ftsK* | Cell cycle, cell division | 1.206609253 | 6.203E-05 |
| A0A0H3PEV8 | *pbpA* | Cell cycle, cell division | 2.606146452 | 0.000107464 |
| A0A0H3PJ47 | *bamA* | Cell wall organization | 1.547012526 | 3.51273E-12 |
| A0A0H3PI91 | *porA* | Cell wall organization | 1.640234516 | 6.81376E-12 |
| A0A0H3P9C5 | *mapA* | Cell wall organization | 1.773310002 | 4.45543E-12 |
| A0A0H3PB07 | *lptD* | Cell wall organization | 1.82184033 | 0.017863088 |
| A0A0H3PAG7 | *cheW* | Chemotaxis | 1.06966514 | 3.12795E-07 |
| A0A0H3P9C4 | *CJJ81176_1204* | Chemotaxis | 1.392566591 | 4.52485E-10 |
| A0A0H3PAM0 | *cheA* | Chemotaxis | 1.406382402 | 1.32746E-10 |
| A0A0H3P9T7 | *cj81176_1498* | Chemotaxis | 2.236791754 | 1.03158E-10 |
| A0A0H3PB49 | *CJJ81176_1548* | Chemotaxis | 2.823808768 | 0.000505134 |
| A0A0H3PEL1 | *cjj81176_0289* | Chemotaxis | 2.886471206 | 1.2738E-08 |
| A0A0H3PAN9 | *cjj81176_1205* | Chemotaxis | 3.027306659 | 3.3978E-14 |
| A0A0H3P9J9 | *CJJ81176_0046* | Chemotaxis | 3.095805589 | 1.23666E-10 |
| A0A0H3PEF7 | *CJJ81176_0180* | Chemotaxis | 3.395299458 | 1.72665E-14 |
| A0A0H3PIS5 | *cmeA* | Transport | 3.866187869 | 2.02331E-12 |
| A0A0H3PH83 | *ssb* | DNA replication | 1.210711091 | 7.32275E-11 |
| A0A0H3P9U0 | *CJJ81176_1009* | Metabolism | 1.0114573 | 5.66619E-05 |
| Q7X517 | *pseE* | Metabolism | 1.011534365 | 2.67661E-06 |
| A1VY43 | *ubiE* | Metabolism | 1.034007976 | 0.004552774 |
| A0A0H3PAD9 | *pglD* | Metabolism | 1.042328173 | 0.012131383 |
| Q5QKR5 | *accB* | Metabolism | 1.085260353 | 1.71291E-05 |
| A0A0H3PAC1 | *nuoG* | Metabolism | 1.095531654 | 1.99603E-08 |
| A0A0H3P9Q2 | *nrfA* | Metabolism | 1.174567178 | 8.24316E-07 |
| A0A0H3PET1 | *trpD* | Metabolism | 1.180370506 | 1.2593E-10 |
| A0A0H3PI47 | *CJJ81176_1247* | Metabolism | 1.203414777 | 0.061402896 |
| A0A0H3PI21 | *nrfH* | Metabolism | 1.224664948 | 0.009156254 |
| A0A0H3PHD6 | *glnA* | Metabolism | 1.258567077 | 3.39388E-12 |
| A0A0H3PCS4 | *ribE* | Metabolism | 1.273775212 | 4.93874E-11 |
| A1W085 | *pyrB* | Metabolism | 1.273882434 | 5.22552E-13 |
| A0A0H3PI37 | *nuoC* | Metabolism | 1.28676503 | 7.08186E-05 |
| A0A0H3PAJ7 | *hydB* | Metabolism | 1.290463488 | 3.50573E-10 |
| A0A0H3PIT1 | *ftn* | Metabolism | 1.306756165 | 1.28904E-14 |
| A0A0H3PHL1 | *ubiX* | Metabolism | 1.341669585 | 0.003022641 |
| A1W1H0 | *nuoI* | Metabolism | 1.399191001 | 9.041E-08 |
| A0A0H3P9R9 | *ccoO* | Metabolism | 1.453583933 | 6.45613E-11 |
| A0A0H3PBB6 | *trpE* | Metabolism | 1.472737067 | 6.52535E-14 |
| A0A0H3P9T9 | *CJJ81176_1157* | Metabolism | 1.493144143 | 4.61332E-06 |
| A1VXS2 | *folE* | Metabolism | 1.51352937 | 0.073046349 |
| A0A0H3P9R1 | *pglJ* | Metabolism | 1.586503085 | 0.009810915 |
| A1VYZ2 | *ilvC* | Metabolism | 1.606074532 | 2.12654E-16 |
| A0A0H3PB93 | *CJJ81176_1499* | Metabolism | 1.774882994 | 0.04524904 |
| A0A0H3PHB9 | *petA* | Metabolism | 1.806541516 | 1.33916E-14 |
| A0A0H3P9J4 | *CJJ81176_0882* | Metabolism | 1.810384085 | 2.17323E-11 |
| A0A0H3PCR0 | *petB* | Metabolism | 1.897178779 | 7.37089E-08 |
| A0A0H3P9F0 | *pglF* | Metabolism | 2.030092135 | 0.000280704 |
| A0A0H3PCT8 | *cjj81176_1032* | Metabolism | 2.060543568 | 2.40615E-09 |
| A1VZQ5 | *peb1C* | Metabolism | 2.077310837 | 1.18718E-08 |
| A0A0H3P9Y0 | *CJJ81176_1228* | Metabolism | 2.092152219 | 2.61481E-07 |
| A0A0H3P9I4 | *purU* | Metabolism | 2.11822664 | 1.39929E-08 |
| A0A0H3PCI0 | *cjj81176* | Metabolism | 2.1908747 | 6.67048E-12 |
| A0A0H3P9E8 | *petC* | Metabolism | 2.210383226 | 5.44201E-10 |
| A0A0H3PAL0 | *cadF* | Cell wall organization | 2.273982894 | 2.73592E-12 |
| A0A0H3PDM3 | *sdaC* | Metabolism | 2.332013205 | 1.02622E-10 |
| A0A0H3PAW0 | *corA* | Metabolism | 2.491184165 | 0.0009348 |

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|---|---|---|---|---|
| A0A0H3PAE3 | *hydA* | Metabolism | 2.866348588 | 7.75829E-12 |
| A0A0H3PAQ1 | *CJJ81176_0849* | Metabolism | 2.908527642 | 3.38968E-05 |
| A0A0H3PA38 | *cydA* | Metabolism | 3.022213319 | 6.16298E-07 |
| A0A0H3PEX3 | *CJJ81176_0544* | Metabolism | 3.06025843 | 3.24908E-06 |
| A0A0H3PIZ8 | *fliE* | Motility | 1.338098791 | 0.021614333 |
| A0A0H3PF34 | *fliF* | Motility | 1.882282736 | 6.95475E-10 |
| Q2M5R2 | *flaA* | Motility | 1.921724784 | 1.40944E-18 |
| A0A0H3PBG5 | *cjj81176_1338* | Motility | 2.039822623 | 1.16673E-17 |
| A0A0H3PIF6 | *fliL* | Motility | 2.275821132 | 8.12241E-11 |
| A0A0H3PI52 | *rplO* | Protein synthesis | 1.064371649 | 6.25159E-13 |
| A1VXH9 | *obg* | Protein synthesis | 1.111162021 | 0.002148676 |
| A1W1U4 | *rplR* | Protein synthesis | 1.114760357 | 1.68697E-10 |
| A1VYJ6 | *rpsL* | Protein synthesis | 1.172090759 | 0.163837268 |
| A1W1U3 | *rpsE* | Protein synthesis | 1.185601705 | 7.48658E-05 |
| A1W1V5 | *rplV* | Protein synthesis | 1.206633281 | 1.05308E-12 |
| A1VXW7 | *rplT* | Protein synthesis | 1.209336085 | 1.24733E-10 |
| A1VYJ1 | *rplA* | Protein synthesis | 1.239341767 | 2.49062E-12 |
| A1W1V2 | *rpmC* | Protein synthesis | 1.258787285 | 7.93274E-13 |
| A1W1L3 | *rpsT* | Protein synthesis | 1.277591245 | 0.007747614 |
| A0A0H3PCH6 | *rplC* | Protein synthesis | 1.3066351 | 1.18475E-14 |
| A0A0H3PA47 | *rnj* | Protein synthesis | 1.327749013 | 5.70135E-10 |
| A1VYI7 | *rpmG* | Protein synthesis | 1.362601965 | 0.001026053 |
| A1W1J3 | *rpmJ* | Protein synthesis | 1.37457629 | 0.323756354 |
| A1W0J3 | *rny* | Protein synthesis | 1.880780239 | 1.62554E-09 |
| A0A0H3PGK7 | *pepA* | Protein synthesis | 1.936446829 | 2.94373E-13 |
| A1VY30 | *rplY* | Protein synthesis | 2.358075658 | 7.616E-20 |
| A0A0H3P9Q4 | *katA* | Stress Response | 1.005356501 | 4.66151E-08 |
| A1W0P5 | *dnaJ* | Stress Response | 1.013013275 | 0.00053212 |
| A0A0H3PIS8 | *clpX* | Stress Response | 1.020097139 | 5.43587E-07 |
| A0A0H3PB76 | *dnaJ-1* | Stress Response | 1.306834132 | 0.006246595 |
| A0A0H3PA35 | *dsbA* | Stress Response | 1.319781243 | 0.001734695 |
| A1W0K4 | *groL* | Stress Response | 1.364946809 | 9.48545E-16 |
| A0A0H3PHF3 | *cjj81176_0717* | Stress Response | 1.563331999 | 2.63829E-08 |
| A0A0H3P9J1 | *yidC* | Cell wall organization | 1.580794826 | 7.54068E-09 |
| A0A0H3PBJ5 | *dsbD* | Stress Response | 1.904425219 | 0.00619071 |
| A0A0H3PA75 | *comEA* | Stress Response | 1.914335994 | 2.08722E-12 |
| A0A0H3PE81 | *CiaC* | Metabolism | 1.931146363 | 1.97855E-06 |
| A0A0H3PCE2 | *cstA* | Stress Response | 2.365352403 | 3.75519E-12 |
| Q29W27 | *kpsD* | Transport | 1.085338779 | 2.64635E-10 |
| A0A0H3PEA5 | *CJJ81176_0635* | Transport | 1.168364064 | 0.033999043 |
| A1VXI7 | *atpH* | Transport | 1.226584565 | 0.033513218 |
| A0A0H3PGP1 | *lctP* | Transport | 1.236518175 | 0.024524006 |
| A0A0H3PA60 | *dcuA* | Transport | 1.25434967 | 1.7428E-05 |
| A0A0H3PEE2 | *secG* | Transport | 1.394747584 | 1.99684E-05 |
| A0A0H3PAK6 | *chuA* | Transport | 1.400975447 | 1.80343E-08 |
| A0A0H3P9N4 | *kdpB* | Transport | 1.417416867 | 0.000901219 |
| A0A0H3PA66 | *dcuB* | Transport | 1.536759955 | 5.23718E-05 |
| A1VXI9 | *atpG* | Transport | 1.541047665 | 4.0942E-08 |
| A0A0H3PHE3 | *metN* | Transport | 1.719892587 | 1.78983E-08 |
| A0A0H3P9B1 | *yajC* | Transport | 1.736855269 | 4.83408E-09 |
| A0A0H3PA42 | *CJJ81176_0911* | Transport | 1.748312971 | 0.000148306 |
| A0A0H3PAB7 | *CJJ81176_1434* | Transport | 1.751248681 | 5.30611E-09 |
| A0A0H3PE25 | *CJJ81176_1654* | Transport | 1.752945144 | 1.10125E-09 |
| A1VXI8 | *atpA* | Transport | 1.756857943 | 2.14743E-11 |
| A0A0H3PBD1 | *ccoP* | Transport | 1.773251843 | 1.23263E-08 |
| A1VXJ1 | *atpC* | Transport | 1.855245074 | 1.1599E-08 |
| A0A0H3PBJ1 | *kpsE* | Transport | 1.970657173 | 7.62848E-10 |
| A0A0H3PAF2 | *secD* | Transport | 2.338629998 | 1.91572E-11 |
| A0A0H3PAN7 | *secF* | Transport | 2.350826676 | 7.89427E-11 |

| | | | | |
|---|---|---|---|---|
| A0A0H3P9L8 | *atpF* | Transport | 2.638521164 | 0.00023113 |
| A1VXJ0 | *atpD* | Transport | 2.719165948 | 7.78808E-17 |
| A0A0H3PA17 | *putP* | Transport | 2.750590717 | 3.36109E-05 |
| A0A0H3P9J0 | *CJJ81176_0912* | Transport | 3.013920027 | 7.41051E-05 |
| A0A0H3PAE4 | *cmeC* | Transport | 3.155469016 | 5.26297E-12 |
| A0A0H3PB79 | *cmeB* | Transport | 3.473684545 | 3.89008E-13 |
| A0A0H3PB37 | *CJJ81176_1244* | Two-component regulatory system | 1.06422278 | 0.094260688 |
| A0A0H3P9T5 | *CJJ81176_1649* | Uncharacterized | 1.013796755 | 0.056675903 |
| A1VY92 | *CJJ81176_0395* | Uncharacterized | 1.035424134 | 1.20356E-05 |
| A0A0H3PIU3 | *CJJ81176_0188* | Uncharacterized | 1.082731981 | 0.001072951 |
| A0A0H3PD61 | *CJJ81176_1193* | Uncharacterized | 1.098124637 | 7.91067E-07 |
| A0A0H3PA88 | *CJJ81176_0125* | Uncharacterized | 1.118968966 | 4.78171E-09 |
| Q8GJA7 | *Cjp48* | Uncharacterized | 1.123805003 | 3.54599E-07 |
| Q29VV3 | *CJJ81176_1431* | Uncharacterized | 1.127757554 | 0.009811353 |
| A0A0H3PB43 | *CJJ81176_0637* | Uncharacterized | 1.165255782 | 8.72122E-07 |
| A0A0H3P9D3 | *cjj81176_1210* | Uncharacterized | 1.185324937 | 1.00405E-09 |
| Q0Q7J3 | *cj1355* | Uncharacterized | 1.229534636 | 6.55363E-08 |
| A0A0H3PES2 | *CJJ81176_0377* | Uncharacterized | 1.240296272 | 0.001386495 |
| A0A0H3PCX2 | *CJJ81176_1232* | Uncharacterized | 1.245569399 | 1.31379E-05 |
| A0A0H3ADZ7 | *hup* | Uncharacterized | 1.264887744 | 6.21819E-09 |
| A0A0H3P9C2 | *CJJ81176_1124* | Uncharacterized | 1.31653347 | 7.36561E-08 |
| A0A0H3P9J8 | *cjaC* | Uncharacterized | 1.36470193 | 3.33963E-09 |
| A0A0H3P9V0 | *CJJ81176_1433* | Uncharacterized | 1.367020908 | 8.85194E-05 |
| A0A0H3P9B9 | *cjaA* | Uncharacterized | 1.411504315 | 1.65405E-08 |
| A0A0H3PGL0 | *CJJ81176_1732* | Uncharacterized | 1.436179708 | 1.68278E-09 |
| A0A0H3PHJ5 | *CJJ81176_0726* | Uncharacterized | 1.452301773 | 1.8819E-12 |
| A0A0H3P9D1 | *CJJ81176_1051* | Uncharacterized | 1.481422634 | 1.99861E-11 |
| A0A0H3PGE8 | *CJJ81176_pTet0018* | Uncharacterized | 1.548734895 | 1.43136E-09 |
| A0A0H3PB67 | *CJJ81176_1452* | Uncharacterized | 1.563369294 | 1.35156E-07 |
| A0A0H3PC19 | *CJJ81176_0428* | Uncharacterized | 1.566718528 | 3.27714E-08 |
| A0A0H3PAL8 | *CJJ81176_1027* | Uncharacterized | 1.573424266 | 0.012441403 |
| Q6QNL8 | *Cj1356c* | Uncharacterized | 1.590681529 | 9.32244E-07 |
| A0A0H3PDT4 | *CJJ81176_1617* | Uncharacterized | 1.657154082 | 0.071166488 |
| A0A0H3PBI5 | *CJJ81176_1639* | Uncharacterized | 1.662964872 | 0.006063851 |
| A0A0H3PAU3 | *CJJ81176_0159* | Uncharacterized | 1.671975882 | 0.007456854 |
| A0A0H3PH37 | *CJJ81176_1222* | Uncharacterized | 1.682314971 | 3.33243E-05 |
| A0A0H3PET5 | *cjj81176_0471* | Uncharacterized | 1.705488763 | 1.9425E-10 |
| A0A0H3PIR6 | *CJJ81176_0166* | Uncharacterized | 1.715585118 | 7.88298E-07 |
| A0A0H3PAI8 | *CJJ81176_0626* | Uncharacterized | 1.718786651 | 4.74753E-06 |
| A0A0H3PAA5 | *CJJ81176_0419* | Uncharacterized | 1.811679797 | 7.37043E-11 |
| A0A0H3PBU4 | *CJJ81176_0392* | Uncharacterized | 1.852776167 | 3.57379E-05 |
| A0A0H3P994 | *CJJ81176_0144* | Uncharacterized | 1.881489245 | 1.23235E-09 |
| A0A0H3P9M2 | *CJJ81176_0734* | Uncharacterized | 1.893375893 | 1.12249E-09 |
| A0A0H3PBE0 | *CJJ81176_0236* | Uncharacterized | 1.901333108 | 1.17762E-12 |
| A0A0H3PBT4 | *cj81176_0295* | Uncharacterized | 1.951355007 | 2.88554E-11 |
| A0A0H3PA18 | *CJJ81176_0942* | Uncharacterized | 2.081688219 | 2.7245E-11 |
| A0A0H3PJB3 | *CJJ81176_0263* | Uncharacterized | 2.113533523 | 0.000383216 |
| A0A0H3PI86 | *CJJ81176_1476* | Uncharacterized | 2.115314941 | 2.97708E-10 |
| A0A0H3PBF8 | *CJJ81176_1651* | Uncharacterized | 2.141941675 | 7.73802E-08 |
| A0A0H3PCF8 | *CJJ81176_0975* | Uncharacterized | 2.143141581 | 0.000455117 |
| A0A0H3PH47 | *CJJ81176_1185* | Uncharacterized | 2.149501166 | 1.39504E-16 |
| A0A0H3PEU8 | *CJJ81176_0148* | Uncharacterized | 2.271650979 | 3.27023E-11 |
| Q29VV2 | *CJB1432c* | Uncharacterized | 2.287195504 | 6.1859E-06 |
| A0A0H3PBX6 | *CJJ81176_0358* | Uncharacterized | 2.330585662 | 5.17289E-05 |
| A0A0H3PD99 | *CJJ81176_0797* | Uncharacterized | 2.397486078 | 1.06425E-09 |
| A0A0H3P9N8 | *CJJ81176_0145* | Uncharacterized | 2.407440325 | 2.94726E-09 |
| A0A0H3PBE2 | *CJJ81176_0543* | Uncharacterized | 2.470750781 | 8.11207E-09 |
| A0A0H3PAV1 | *CJJ81176_0359* | Uncharacterized | 2.490335773 | 1.65436E-10 |

| | | | | |
|---|---|---|---|---|
| A0A0H3PI11 | *CJJ81176_1608* | Uncharacterized | 2.683389403 | 2.60281E-08 |
| A0A0H3PCI2 | *CJJ81176_0072* | Uncharacterized | 2.714764272 | 8.55926E-08 |
| Q9KIS1 | *virB9* | Uncharacterized | 2.94560032 | 0.000146357 |
| A0A0H3PB47 | *CJJ81176_1492* | Uncharacterized | 3.208264906 | 5.38793E-14 |
| A0A0H3PAI3 | *CJJ81176_0586* | Uncharacterized | 3.584385466 | 4.75641E-14 |
| A0A0H3PCP8 | *CJJ81176_1045* | Uncharacterized | 4.070407695 | 8.69715E-08 |

**Appendix 4**: Significantly differentiated proteins between 81-176 cultured in CDM at 37$^{o}$C for 12h and 24h

| | **Significantly downregulated** | | | |
|---|---|---|---|---|
| **UniProt_Accession** | **Gene Name** | **Protein function** | **logFC** | **P.Value** |
| A0A0H3PDA2 | *ftsZ* | Cell cycle, cell division | -1.124378927 | 0.001024381 |
| A0A0H3PA34 | *cheB* | Chemotaxis | -1.023509806 | 0.000200879 |
| A0A0H3PAK5 | *rpoD* | DNA Transcription | -1.14153773 | 0.004831498 |
| A1VXF1 | *aroQ* | Metabolism | -1.929614046 | 0.010971065 |
| A0A0H3P9B2 | *thiH* | Metabolism | -1.619986513 | 3.41504E-07 |
| A0A0H3PAH1 | *tyrA* | Metabolism | -1.25838715 | 4.73533E-06 |
| A0A0H3P9A4 | *CJJ81176_0120* | Metabolism | -1.232835504 | 0.000439309 |
| A0A0H3PEI7 | *folP* | Metabolism | -1.18645333 | 0.012832494 |
| A0A0H3P9B6 | *thiF* | Metabolism | -1.163561192 | 0.00110726 |
| A0A0H3PHM5 | *mobB* | Metabolism | -1.162065042 | 0.013164189 |
| A1W1K3 | *hisA* | Metabolism | -1.157719247 | 0.024016809 |
| A1VY40 | *dxs* | Metabolism | -1.156809817 | 0.002101453 |
| A1W1X0 | *leuC* | Metabolism | -1.14404468 | 0.000591185 |
| A1W0W6 | *mobA* | Metabolism | -1.133492511 | 0.040430667 |
| A1VYQ4 | *hemC* | Metabolism | -1.127312453 | 1.15528E-05 |
| A1W0R9 | *mqnA* | Metabolism | -1.122639313 | 0.1533829 |
| A0A0H3PD29 | *cobB* | Metabolism | -1.111902205 | 0.010031723 |
| Q5QKR5 | *accB* | Metabolism | -1.093490891 | 3.26702E-06 |
| A0A0H3PHE7 | *CJJ81176_0739* | Metabolism | -1.089224516 | 1.52624E-05 |
| A0A0H3PEL5 | *CJJ81176_0280* | Metabolism | -1.060459486 | 0.047857789 |
| A1VZZ8 | *tgt* | Metabolism | -1.053973776 | 0.017731153 |
| A0A0H3PAG6 | *tpiA* | Metabolism | -1.052270977 | 0.006153097 |
| A1W062 | *fliW* | Motility | -1.544033778 | 0.057487147 |
| A0A0H3PEY5 | *fliS* | Motility | -1.084384063 | 0.022819905 |
| A0A0H3PD33 | *sixA* | Protein modification | -3.955299163 | 3.98644E-05 |
| A0A0H3PBB3 | *rbfA* | Protein synthesis | -1.452643764 | 0.056937696 |
| A1VYB8 | *gatC* | Protein synthesis | -1.219847877 | 8.15132E-08 |
| A1VYJ1 | *rplA* | Protein synthesis | -1.125911081 | 0.000634579 |
| A1VXM1 | *rimP* | Protein synthesis | -1.100409553 | 0.014747744 |
| A1VZW5 | *cmoB* | Protein synthesis | -1.074223258 | 0.006817062 |
| A0A0H3P9Q3 | *csrA* | Stress Response | -1.887225663 | 0.000449004 |
| A0A0H3PBL4 | *hypE* | Stress Response | -1.705200182 | 4.13701E-06 |
| Q3I354 | *luxS* | Stress Response | -1.395374976 | 0.022762402 |
| A0A0H3PGY0 | *ppiB* | Stress Response | -1.389256161 | 6.73735E-09 |
| A0A0H3P9M1 | *napD* | Stress Response | -1.313522895 | 0.010590837 |
| A0A0H3PAD9 | *pglD* | Stress Response | -1.1200156 | 0.07668226 |
| A1W0U6 | *pseG* | Stress Response | -1.055892337 | 0.048973283 |
| A0A0H3P9V7 | *CJJ81176_1101* | Stress Response | -1.003509865 | 0.047452082 |
| A0A0H3PDE7 | *CJJ81176_0897* | Transport | -1.173489552 | 0.101776228 |
| A0A0H3PDG2 | *CJJ81176_0891* | Uncharacterized | -2.806590388 | 0.000119465 |
| A0A0H3P9L3 | *CJJ81176_0728* | Uncharacterized | -2.147459285 | 1.6983E-06 |
| A0A0H3PHH8 | *CJJ81176_0888* | Uncharacterized | -1.889548153 | 0.004592447 |
| A0A0H3PB85 | *CJJ81176_0254* | Uncharacterized | -1.882613568 | 0.046580897 |
| A0A0H3PA59 | *CJJ81176_1259* | Uncharacterized | -1.872267063 | 0.01599769 |
| A0A0H3PH34 | *CJJ81176_1055* | Uncharacterized | -1.748157285 | 0.042847874 |

| | | | | |
|---|---|---|---|---|
| A0A0H3PB96 | *CJJ81176_0611* | Uncharacterized | -1.67477184 | 0.003428618 |
| Q8GJE8 | *Cjp04* | Uncharacterized | -1.636640091 | 0.005393802 |
| A0A0H3PHG6 | *CJJ81176_0854* | Uncharacterized | -1.589807192 | 0.012027374 |
| A0A0H3PIW6 | *CJJ81176_0547* | Uncharacterized | -1.562823986 | 4.8314E-05 |
| A0A0H3P9W6 | *A0A0H3P9W6* | Uncharacterized | -1.50085694 | 0.001973334 |
| A0A0H3P973 | *CJJ81176_pTet0021* | Uncharacterized | -1.412111873 | 1.83897E-05 |
| A0A0H3PAM9 | *CJJ81176_1458* | Uncharacterized | -1.367532524 | 0.001646516 |
| A0A0H3P9T3 | *CJJ81176_1422* | Uncharacterized | -1.35349802 | 0.000144934 |
| A0A0H3PDW4 | *CJJ81176_1424* | Uncharacterized | -1.340779726 | 0.003872782 |
| A0A0H3PAF1 | *CJJ81176_1363* | Uncharacterized | -1.322005508 | 0.009022957 |
| A0A0H3P9G9 | *CJJ81176_pTet0032* | Uncharacterized | -1.275777679 | 0.120526867 |
| A0A0H3PAT8 | *CJJ81176_1274* | Uncharacterized | -1.253360799 | 0.004097231 |
| A0A0H3P9J7 | *CJJ81176_0137* | Uncharacterized | -1.232835713 | 0.078405912 |
| Q0Q7K5 | *CJJ81176_0777* | Uncharacterized | -1.167199356 | 0.238795241 |
| A0A0H3PB39 | *CJJ81176_1673* | Uncharacterized | -1.154944735 | 0.01552709 |
| Q2M5Q6 | *CJJ81176_1318* | Uncharacterized | -1.114579632 | 0.00018542 |
| A0A0H3PAA2 | *CJJ81176_0288* | Uncharacterized | -1.087996906 | 0.000122953 |
| A0A0H3PB78 | *CJJ81176_1414* | Uncharacterized | -1.046853272 | 1.22934E-08 |
| A0A0H3P9M8 | *CJJ81176_0136* | Uncharacterized | -1.037985947 | 4.66345E-06 |
| A0A0H3PHF5 | *CJJ81176_0907* | Uncharacterized | -1.030620907 | 1.61063E-07 |
| | | | | |
| | **Significantly upregulated** | | | |
| **UniProt_Accession** | **Gene Name** | **Protein function** | **logFC** | **P.Value** |
| A0A0H3PEV8 | *pbpA* | Cell cycle, cell division | 1.438319346 | 0.026404304 |
| A0A0H3PB07 | *lptD* | Cell wall organization | 1.343297362 | 0.007515595 |
| A0A0H3PAG7 | *cheW* | Chemotaxis | 1.13073066 | 6.38554E-08 |
| A0A0H3PAN9 | *cjj81176_1205* | Chemotaxis | 1.752430038 | 2.9729E-09 |
| A0A0H3P9T7 | *TlpA* | Chemotaxis | 2.414797738 | 5.47608E-11 |
| A0A0H3PEL1 | *TlpA* | Chemotaxis | 2.439369086 | 1.63873E-07 |
| A0A0H3P9J9 | *TlpB* | Chemotaxis | 2.456105483 | 2.45604E-08 |
| A0A0H3PEF7 | *TlpA* | Chemotaxis | 2.722413392 | 5.15972E-12 |
| A0A0H3P9B7 | *cyf* | Metabolism | 1.003588353 | 0.001256929 |
| A0A0H3PCT8 | *cjj81176_1032* | Metabolism | 1.008996375 | 3.18937E-05 |
| A0A0H3P9T9 | *CJJ81176_1157* | Metabolism | 1.044683339 | 0.000223874 |
| A0A0H3P9E8 | *petC* | Metabolism | 1.131114329 | 7.43666E-06 |
| A0A0H3PI37 | *nuoC* | Metabolism | 1.202740944 | 4.57652E-05 |
| A0A0H3PCI0 | *cjj81176* | Metabolism | 1.266299832 | 2.40967E-08 |
| A1VXS2 | *folE* | Metabolism | 1.306524137 | 0.12522755 |
| A1W0W2 | *dxr* | Metabolism | 1.332629987 | 0.01775308 |
| A0A0H3PI47 | *CJJ81176_1247* | Metabolism | 1.41392327 | 0.024564539 |
| A0A0H3PI21 | *nrfH* | Metabolism | 1.46794995 | 0.001771608 |
| A0A0H3PHB9 | *petA* | Metabolism | 1.503579164 | 3.52021E-12 |
| A0A0H3PAQ1 | *CJJ81176_0849* | Metabolism | 1.740715498 | 0.005142159 |
| A0A0H3PAW0 | *corA* | Metabolism | 1.764708303 | 0.006096685 |
| A0A0H3PA38 | *cydA* | Metabolism | 2.279570537 | 3.1208E-05 |
| A0A0H3PA70 | *proB* | Metabolism | 2.376846173 | 2.0936E-05 |
| A0A0H3PF34 | *fliF* | Motility | 1.250050418 | 1.93049E-07 |
| A0A0H3PIZ8 | *fliE* | Motility | 1.798950115 | 0.00094453 |
| A0A0H3PIF6 | *fliL* | Motility | 1.926432008 | 6.62207E-09 |
| A0A0H3PAL0 | *cadF* | Pathogenesis | 1.058846306 | 1.85629E-07 |
| A1VZQ5 | *peb1C* | Pathogenesis | 1.132613153 | 4.28571E-05 |
| A0A0H3P9C5 | *mapA* | Pathogenesis | 1.17678743 | 2.87743E-10 |
| A0A0H3PCL3 | *cdtB* | Pathogenesis | 1.046402995 | 4.92885E-08 |
| A0A0H3PE81 | *CiaC* | Pathogenesis | 1.542108368 | 6.5569E-05 |
| A0A0H3PEI3 | *rlpA* | Protein synthesis | 1.017383375 | 2.02356E-06 |
| A1W1L3 | *rpsT* | Protein synthesis | 1.036451932 | 0.040642106 |
| A1W1J3 | *rpmJ* | Protein synthesis | 3.248334486 | 0.004122131 |
| A1VYJ6 | *rpsL* | Protein synthesis | 7.436026834 | 4.23303E-12 |

| | | | | |
|---|---|---|---|---|
| A0A0H3PCE2 | *cstA* | Stress Response | 1.238271042 | 2.45139E-07 |
| A1VYZ7 | *msrA* | Stress Response | 1.365016476 | 0.042110313 |
| A1VXG9 | *ung* | Stress Response | 1.496340666 | 0.000362753 |
| A0A0H3PB79 | *cmeB* | Transport | 1.087544498 | 0.000107416 |
| A0A0H3PGP1 | *lctP* | Transport | 1.117746513 | 0.010813319 |
| A0A0H3PBJ1 | *kpsE* | Transport | 1.119839185 | 6.23036E-07 |
| A0A0H3PAF2 | *secD* | Transport | 1.139555465 | 1.03512E-06 |
| A0A0H3PDM3 | *sdaC* | Transport | 1.239908755 | 3.0713E-07 |
| A0A0H3PAK6 | *chuA* | Transport | 1.283893641 | 9.72796E-07 |
| A0A0H3P9J0 | *CJJ81176_0912* | Transport | 1.294025258 | 0.082409608 |
| A0A0H3PA17 | *putP* | Transport | 1.29619928 | 0.029325149 |
| A0A0H3PA66 | *dcuB* | Transport | 1.310823641 | 5.4483E-05 |
| A0A0H3PD65 | *cjj81176_1037* | Transport | 1.328393494 | 6.58228E-12 |
| A0A0H3PAQ2 | *CJJ81176_0494* | Transport | 1.474653722 | 0.007750911 |
| A0A0H3PE25 | *CJJ81176_1654* | Transport | 1.556359341 | 3.76178E-07 |
| A0A0H3P9L8 | *atpF* | Transport | 1.957270123 | 0.003460327 |
| A0A0H3PA88 | *CJJ81176_0125* | Uncharacterized | 1.007615008 | 2.84793E-10 |
| A0A0H3PB47 | *CJJ81176_1492* | Uncharacterized | 1.040958078 | 2.54501E-05 |
| A0A0H3PAJ5 | *CJJ81176_1107* | Uncharacterized | 1.066477033 | 0.092462427 |
| A0A0H3PAA5 | *CJJ81176_0419* | Uncharacterized | 1.088116518 | 2.08148E-07 |
| Q8GJA7 | *Cjp48* | Uncharacterized | 1.094037765 | 2.63401E-07 |
| A0A0H3P9N8 | *CJJ81176_0145* | Uncharacterized | 1.10055266 | 0.003208365 |
| A0A0H3PAU3 | *CJJ81176_0159* | Uncharacterized | 1.131457356 | 0.031081302 |
| A0A0H3P9C2 | *CJJ81176_1124* | Uncharacterized | 1.147900845 | 1.09697E-06 |
| A0A0H3PJB3 | *CJJ81176_0263* | Uncharacterized | 1.155979091 | 0.027133791 |
| A0A0H3PEU8 | *CJJ81176_0148* | Uncharacterized | 1.174445194 | 1.17727E-06 |
| Q6QNL8 | *Cj1356c* | Uncharacterized | 1.20674499 | 1.64137E-07 |
| A0A0H3PA42 | *CJJ81176_0911* | Uncharacterized | 1.210730979 | 0.00239252 |
| A0A0H3PET5 | *CJJ81176_0471* | Uncharacterized | 1.215470958 | 1.05198E-07 |
| A0A0H3PA18 | *CJJ81176_0942* | Uncharacterized | 1.25558517 | 6.61143E-08 |
| A0A0H3PBE2 | *CJJ81176_0543* | Uncharacterized | 1.277031475 | 0.000654412 |
| Q0Q7J3 | *cj1355* | Uncharacterized | 1.300113084 | 2.01075E-09 |
| A0A0H3PBF8 | *CJJ81176_1651* | Uncharacterized | 1.33944721 | 5.67728E-05 |
| A0A0H3PI11 | *CJJ81176_1608* | Uncharacterized | 1.342418745 | 0.000145276 |
| A0A0H3PH47 | *CJJ81176_1185* | Uncharacterized | 1.345239856 | 3.87877E-09 |
| A0A0H3P971 | *CJJ81176_pTet0052* | Uncharacterized | 1.396062068 | 0.056915305 |
| A0A0H3P9S8 | *CJJ81176_1184* | Uncharacterized | 1.446222273 | 1.23807E-10 |
| A0A0H3PH37 | *CJJ81176_1222* | Uncharacterized | 1.500548794 | 4.0374E-05 |
| A0A0H3PD99 | *CJJ81176_0797* | Uncharacterized | 1.515129925 | 2.64072E-05 |
| A0A0H3PDT4 | *CJJ81176_1617* | Uncharacterized | 1.586314195 | 0.085306271 |
| A0A0H3PAV1 | *CJJ81176_0359* | Uncharacterized | 1.594697044 | 5.61714E-08 |
| Q29VV2 | *CJB1432c* | Uncharacterized | 1.649001098 | 0.005167644 |
| A0A0H3PBB0 | *CJJ81176_1666* | Uncharacterized | 1.889649076 | 0.041203729 |
| A0A0H3PA50 | *CJJ81176_0126* | Uncharacterized | 2.055779415 | 2.58884E-05 |
| A0A0H3PEX3 | *CJJ81176_0544* | Uncharacterized | 2.121120765 | 0.000346329 |
| A0A0H3PGI9 | *CJJ81176_0987* | Uncharacterized | 2.151605665 | 1.24803E-06 |
| A0A0H3PCP8 | *CJJ81176_1045* | Uncharacterized | 2.646095737 | 6.90185E-05 |
| A0A0H3PCF8 | *CJJ81176_0975* | Uncharacterized | 2.831999088 | 1.72009E-06 |

**Appendix 5**: Significantly differentiated proteins in 81-176 cultured in CDB at 37$^{o}$C for 12h

| UniProt_Accession | Gene Name | Protein function | logFC | P.Value |
|---|---|---|---|---|
| A0A0H3PB49 | *CJJ81176_1548* | Chemotaxis | -1.012627601 | 0.129285813 |
| A0A0H3PGG1 | *CJJ81176_pTet0031* | DNA Replication | -1.265683704 | 1.16584E-11 |
| A0A0H3PAK5 | *rpoD* | DNA Transcription | -1.08887503 | 0.120385054 |
| A1W1D6 | *acsA* | Metabolism | -2.253249172 | 2.73796E-14 |
| A0A0H3PHJ0 | *CJJ81176_0738* | Metabolism | -1.555074314 | 1.86197E-11 |
| A0A0H3PD90 | *purE* | Metabolism | -1.542966646 | 5.50823E-05 |
| A0A0H3P9X6 | *CJJ81176_1083* | Metabolism | -1.530750598 | 0.004182213 |
| A0A0H3PAL3 | *fldA* | Metabolism | -1.436151362 | 3.08469E-08 |
| A0A0H3PEL5 | *CJJ81176_0280* | Metabolism | -1.392750562 | 0.035546827 |
| A0A0H3PBI3 | *CJJ81176_1495* | Metabolism | -1.254957503 | 2.00519E-12 |
| A0A0H3P9N5 | *cjj81176_0075* | Metabolism | -1.175341326 | 2.91935E-11 |
| A0A0H3PAT0 | *cysK* | Metabolism | -1.114177273 | 2.10646E-09 |
| A0A0H3PHN8 | *cjj81176_0836* | Metabolism | -1.113971675 | 4.28145E-12 |
| A0A0H3PA64 | *ggt* | Metabolism | -1.031197599 | 6.92921E-06 |
| A0A0H3PHL6 | *CJJ81176_0799* | Pathogenesis | -1.345093647 | 1.82945E-07 |
| A1W1L3 | *rpsT* | Protein synthesis | -1.871963559 | 1.69947E-06 |
| A1W1V8 | *rplW* | Protein synthesis | -1.024849048 | 0.006390824 |
| A0A0H3P9Q4 | *katA* | Stress Response | -1.430300186 | 1.0351E-13 |
| Q0Q7K8 | *grpE* | Stress Response | -1.050450015 | 7.35255E-13 |
| A1W0K4 | *groL* | Stress Response | -1.029703387 | 8.24797E-16 |
| Q0Q7K7 | *dnaK* | Stress Response | -1.001716295 | 1.61803E-12 |
| A0A0H3PA76 | *cjj81176_1604* | Transport | -1.884449664 | 8.53952E-16 |
| A0A0H3PAK6 | *chuA* | Transport | -1.751857182 | 4.38503E-09 |
| A0A0H3PEW2 | *CJJ81176_0211* | Transport | -1.541574233 | 3.59883E-14 |
| A0A0H3PAU0 | *cjj81176_1525* | Transport | -1.461206947 | 1.51123E-15 |
| Q0Q7I0 | *CJJ81176_1569* | Transport | -1.387397935 | 1.38141E-14 |
| A0A0H3PE25 | *CJJ81176_1654* | Transport | -1.185587582 | 2.05211E-06 |
| Q8GJC5 | *Cjp29* | Uncharacterized | -2.247060614 | 3.43519E-05 |
| A0A0H3PA01 | *CJJ81176_1650* | Uncharacterized | -2.180824058 | 3.51434E-15 |
| A0A0H3PI41 | *CJJ81176_1600* | Uncharacterized | -1.664964927 | 2.61358E-16 |
| A0A0H3PEG8 | *CJJ81176_0642* | Uncharacterized | -1.50973595 | 1.767E-07 |
| A0A0H3PAX0 | *tpx* | Uncharacterized | -1.451871548 | 2.02398E-06 |
| A0A0H3P9S8 | *CJJ81176_1184* | Uncharacterized | -1.410283174 | 1.71184E-12 |
| A0A0H3PBF8 | *CJJ81176_1651* | Uncharacterized | -1.393540848 | 5.37837E-10 |
| A0A0H3PET5 | *CJJ81176_0471* | Uncharacterized | -1.376462522 | 2.11491E-12 |
| A0A0H3ADZ7 | *hup* | Uncharacterized | -1.328744595 | 1.40326E-05 |
| A0A0H3PAI8 | *CJJ81176_0626* | Uncharacterized | -1.26833221 | 0.015688389 |
| Q0Q7J3 | *cj1355* | Uncharacterized | -1.143875516 | 2.83942E-10 |
| A0A0H3PBB0 | *CJJ81176_1666* | Uncharacterized | -1.135400508 | 0.161195318 |
| A0A0H3PHG6 | *CJJ81176_0854* | Uncharacterized | -1.12694493 | 0.153348366 |
| A0A0H3PCC6 | *CJJ81176_pTet0042* | Uncharacterized | -1.09835524 | 4.70774E-09 |
| A0A0H3P9G9 | *CJJ81176_pTet0032* | Uncharacterized | -1.030780809 | 0.181887663 |
| A0A0H3PH34 | *CJJ81176_1055* | Uncharacterized | -1.02691582 | 0.202688409 |
| | | | | |
| **UniProt_Accession** | **Gene Name** | **Protein function** | **logFC** | **P.Value** |
| A0A0H3PDK8 | *CJJ81176_1475* | Uncharacterized | 1.002159309 | 0.00019233 |
| A1W043 | *murC* | Cell cycle,cell division | 1.009311232 | 7.20424E-12 |
| A0A0H3PAM2 | *mreB* | Cell cycle,cell division | 1.035714445 | 1.06369E-05 |
| A1VXS1 | *tig* | Cell cycle,cell division | 1.196284841 | 1.10735E-13 |
| A0A0H3PE69 | *murI* | Cell wall organization | 1.185132327 | 1.066E-08 |
| A0A0H3PB07 | *lptD* | Cell wall organization | 1.268709341 | 0.000151021 |
| A1VZK1 | *murA* | Cell wall organization | 1.820028278 | 3.01587E-13 |
| A0A0H3P9P7 | *cjj81176_1128* | Chemotaxis | 1.00326504 | 2.87938E-14 |
| A0A0H3P9J9 | *TlpB* | Chemotaxis | 1.424856911 | 0.000770438 |

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|---|---|---|---|---|
| A0A0H3PAN9 | *cjj81176_1205* | Chemotaxis | 1.457940607 | 4.88379E-07 |
| A0A0H3PB06 | *TlpC* | Chemotaxis | 1.677891777 | 5.73937E-09 |
| A0A0H3P9C4 | *CJJ81176_1204* | Chemotaxis | 1.937705384 | 1.06476E-06 |
| A0A0H3P989 | *recJ* | DNA Replication | 1.135241488 | 0.000681861 |
| A0A0H3PB11 | *CJJ81176_1474* | DNA Replication | 1.209454757 | 6.30302E-11 |
| A0A0H3PH83 | *ssb* | DNA Replication | 1.233668598 | 5.216E-12 |
| A0A0H3PGQ1 | *fliA* | DNA Transcription | 1.099249421 | 0.053608625 |
| A0A0H3P9R8 | *CJJ81176_1043* | DNA Transcription | 1.127930017 | 7.78845E-06 |
| A0A0H3P9Q7 | *mfd* | DNA Transcription | 1.965001499 | 2.59213E-09 |
| A1W091 | *ispE* | Metabolism | 1.025009458 | 0.141946627 |
| A0A0H3PBB5 | *purD* | Metabolism | 1.025110164 | 0.000182275 |
| A0A0H3PHG1 | *coaBC* | Metabolism | 1.041583025 | 5.18985E-08 |
| A0A0H3P9S3 | *hydD* | Metabolism | 1.052207753 | 0.098924033 |
| A1VY69 | *trpB* | Metabolism | 1.056789278 | 6.7184E-08 |
| A1VYG9 | *thiC* | Metabolism | 1.059826855 | 0.000497563 |
| A0A0H3P9P8 | *tkt* | Metabolism | 1.071442652 | 2.41399E-07 |
| A1W0I0 | *gpsA* | Metabolism | 1.072933807 | 1.95494E-08 |
| A0A0H3PF31 | *CJJ81176_0427* | Metabolism | 1.074899212 | 0.046731037 |
| A1W068 | *thiE* | Metabolism | 1.080845641 | 0.004797753 |
| Q29VW1 | *gmhA-2* | Metabolism | 1.09507679 | 1.32445E-07 |
| A1VZU6 | *purL* | Metabolism | 1.09873941 | 2.43639E-10 |
| Q1HG74 | *gltB* | Metabolism | 1.102381848 | 1.17519E-08 |
| A0A0H3PH15 | *thiD* | Metabolism | 1.110413003 | 0.001052757 |
| Q0Q7I1 | *purM* | Metabolism | 1.112791882 | 2.80747E-06 |
| A0A0H3PC48 | *purQ* | Metabolism | 1.119470852 | 8.67324E-05 |
| A0A0H3PHM5 | *mobB* | Metabolism | 1.122899672 | 0.019986126 |
| A0A0H3PA89 | *pyrD* | Metabolism | 1.146977601 | 1.05017E-05 |
| A0A0H3PAC7 | *nuoM* | Metabolism | 1.168756148 | 0.006396825 |
| A0A0H3PEF4 | *frdA* | Metabolism | 1.185823798 | 3.59285E-13 |
| A0A0H3PBF9 | *rpe* | Metabolism | 1.199859352 | 0.002954458 |
| A0A0H3PH92 | *glcD* | Metabolism | 1.199922796 | 8.92353E-07 |
| A0A0H3PEJ1 | *ilvB* | Metabolism | 1.250608836 | 3.59495E-07 |
| A0A0H3PAA8 | *CJJ81176_0533* | Metabolism | 1.255604242 | 2.55083E-08 |
| A0A0H3PBA0 | *carA* | Metabolism | 1.25831499 | 9.87768E-14 |
| A0A0H3PA66 | *dcuB* | Metabolism | 1.265216732 | 6.3025E-06 |
| A1W0U9 | *hisH1* | Metabolism | 1.266775528 | 0.019291062 |
| A0A0H3P9T9 | *CJJ81176_1157* | Metabolism | 1.302265843 | 0.000901493 |
| A0A0H3PA90 | *cj81176_0571* | Metabolism | 1.312373136 | 2.45643E-08 |
| A1VZR0 | *apt* | Metabolism | 1.320194604 | 3.56573E-10 |
| A0A0H3PBV9 | *oorD* | Metabolism | 1.321433112 | 0.005068317 |
| A0A0H3PCH2 | *rbr* | Metabolism | 1.357062113 | 1.93881E-14 |
| A0A0H3P9Q8 | *CJJ81176_1286* | Metabolism | 1.358014208 | 1.04547E-12 |
| A0A0H3PBC8 | *CJJ81176_1415* | Metabolism | 1.368268037 | 0.00222284 |
| A0A0H3P9V8 | *CJJ81176_1527* | Metabolism | 1.385245158 | 0.001501757 |
| A1W0I5 | *metE* | Metabolism | 1.407347114 | 2.13111E-08 |
| A0A0H3PAD5 | *lpxD* | Metabolism | 1.411517078 | 1.01067E-14 |
| A0A0H3PC31 | *hom* | Metabolism | 1.419874613 | 6.69863E-05 |
| A1VY70 | *trpA* | Metabolism | 1.516153864 | 8.50919E-08 |
| A1VXA6 | *pyrG* | Metabolism | 1.519666462 | 8.42707E-11 |
| A1W0N8 | *pnp* | Metabolism | 1.526016742 | 7.95408E-08 |
| A0A0H3PBH6 | *cj81176_1322* | Metabolism | 1.560431383 | 1.74836E-06 |
| A1VYG1 | *accA* | Metabolism | 1.582313627 | 5.06598E-05 |
| Q29VV6 | *fcl* | Metabolism | 1.593665031 | 4.89E-18 |
| A0A0H3PEZ1 | *frdB* | Metabolism | 1.626765275 | 4.47613E-08 |
| A0A0H3PI21 | *nrfH* | Metabolism | 1.627475968 | 0.000108993 |
| A1VY44 | *xseA* | Metabolism | 1.656806261 | 0.000741322 |
| A1VYU1 | *rppH* | Metabolism | 1.689949219 | 1.08795E-05 |
| A1VXZ8 | *lpxA* | Metabolism | 1.700508093 | 5.65527E-13 |
| Q2M5Q4 | *accP* | Metabolism | 1.70534272 | 5.52559E-14 |

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|---|---|---|---|---|
| A0A0H3P982 | *rpiB* | Metabolism | 1.72540155 | 1.56788E-06 |
| A1VZ41 | *ispG* | Metabolism | 1.745965219 | 1.87345E-06 |
| A1VXH9 | *obg* | Metabolism | 1.811983562 | 1.21995E-05 |
| A1W1J4 | *rpsM* | Metabolism | 1.825328487 | 2.12438E-05 |
| Q0Q7I7 | *mqnE* | Metabolism | 1.826698062 | 7.20563E-14 |
| A0A0H3PCK6 | *ansA* | Metabolism | 1.838291378 | 1.1417E-14 |
| A1W0W2 | *dxr* | Metabolism | 1.845344029 | 0.001200837 |
| A1VYL8 | *alaS* | Metabolism | 1.918137352 | 1.05528E-10 |
| A1VZF8 | *nadE* | Metabolism | 1.973929954 | 4.49443E-06 |
| A0A0H3PJ93 | *lysA* | Metabolism | 2.106400649 | 3.57213E-15 |
| A0A0H3P9Q2 | *nrfA* | Metabolism | 2.155978903 | 1.86374E-11 |
| A0A0H3PJ06 | *mqnC* | Metabolism | 2.199970247 | 1.1854E-06 |
| A0A0H3PBR9 | *ccpA-2* | Metabolism | 2.302530319 | 1.43161E-20 |
| A0A0H3PBD0 | *bioA* | Metabolism | 2.306506813 | 0.000255391 |
| A0A0H3PGM1 | *aspA* | Metabolism | 2.732611783 | 5.06594E-16 |
| A0A0H3PGR5 | *cjj81176_0063* | Metabolism | 2.857473624 | 2.78229E-09 |
| A0A0H3PAG3 | *sdhC* | Metabolism | 2.960350146 | 1.8716E-09 |
| A0A0H3PJB7 | *sdhB* | Metabolism | 3.610409082 | 2.96957E-15 |
| A0A0H3PBQ2 | *sdhA* | Metabolism | 4.009521599 | 1.06359E-15 |
| A0A0H3PDD9 | *flaC* | Motility | 1.203037594 | 0.000527575 |
| A0A0H3P9L2 | *fliM* | Motility | 1.653968736 | 0.004056254 |
| A0A0H3PBK9 | *flaG* | Motility | 2.404790948 | 0.000472864 |
| A0A0H3PDU5 | *tyrS* | Protein synthesis | 1.026906445 | 3.75533E-12 |
| A1VYR0 | *efp* | Protein synthesis | 1.058895312 | 9.31708E-07 |
| A1W0I1 | *gatB* | Protein synthesis | 1.094938585 | 2.2664E-07 |
| A1VZB3 | *hisS* | Protein synthesis | 1.095597171 | 1.24734E-10 |
| A1VYH4 | *miaB* | Protein synthesis | 1.106075376 | 0.000386451 |
| A1W0R3 | *trmB* | Protein synthesis | 1.138274912 | 0.030815194 |
| A0A0H3PB64 | *trpS* | Protein synthesis | 1.140703337 | 6.65002E-05 |
| A0A0H3P9K7 | *metS* | Protein synthesis | 1.155656945 | 9.85715E-09 |
| A1VYB8 | *gatC* | Protein synthesis | 1.295690129 | 0.015177806 |
| A0A0H3PDV7 | *selB* | Protein synthesis | 1.315073659 | 0.022819021 |
| A1W0J3 | *rny* | Protein synthesis | 1.346451112 | 8.97237E-05 |
| A0A0H3PAI4 | *ileS* | Protein synthesis | 1.354098576 | 3.11121E-15 |
| A0A0H3PCJ0 | *CJJ81176_0101* | Protein synthesis | 1.421402969 | 9.60517E-07 |
| A1VZ20 | *era* | Protein synthesis | 1.743381929 | 0.01070401 |
| A0A0H3P9L9 | *rpsA* | Protein synthesis | 1.779468629 | 3.40088E-15 |
| A1W165 | *truD* | Protein synthesis | 1.846810707 | 2.05732E-05 |
| A0A0H3PJI4 | *recN* | Stress Response | 1.115555263 | 0.007606851 |
| A1VYU6 | *ligA* | Stress Response | 1.117436601 | 2.79765E-06 |
| A0A0H3PEV1 | *xth* | Stress Response | 1.135087322 | 1.99535E-11 |
| A1VXS0 | *clpP* | Stress Response | 1.255454361 | 7.18782E-11 |
| A1W0P5 | *dnaJ* | Stress Response | 1.369575933 | 0.003826991 |
| A0A0H3PAC3 | *CJJ81176_1161* | Stress Response | 1.493826187 | 9.8901E-11 |
| A0A0H3PEB4 | *nth* | Stress Response | 1.541948153 | 2.57175E-11 |
| A0A0H3PAG5 | *radA* | Stress Response | 1.678139032 | 0.00326215 |
| A1W0U6 | *pseG* | Stress Response | 1.984735749 | 0.011269854 |
| A0A0H3PIA1 | *CJJ81176_1539* | Stress Response | 2.043396392 | 3.13386E-07 |
| A0A0H3PE81 | *CiaC* | Stress Response | 2.094853922 | 2.72258E-07 |
| A0A0H3P9D2 | *CJJ81176_1077* | Stress Response | 2.102450016 | 3.06109E-06 |
| A0A0H3PAU2 | *CJJ81176_1538* | Stress Response | 2.108432847 | 0.000200835 |
| A0A0H3PHE3 | *metN* | Transport | 1.004496196 | 0.014042284 |
| A0A0H3PAG9 | *CJJ81176_0672* | Transport | 1.064360876 | 4.29364E-07 |
| A1W0G0 | *tatA* | Transport | 1.096684068 | 0.012163065 |
| A1VXI7 | *atpH* | Transport | 1.273941307 | 0.030916536 |
| A1VZC8 | *napA* | Transport | 1.450701825 | 6.02982E-16 |
| A0A0H3PEE2 | *secG* | Transport | 1.496929357 | 0.021056653 |
| A0A0H3PA74 | *napB* | Transport | 1.619367939 | 2.8065E-12 |
| A0A0H3PA51 | *napG* | Transport | 1.971268346 | 1.27661E-05 |

| | | | | |
|---|---|---|---|---|
| Q0Q7H5 | *CJJ81176_1574* | Transport | 2.414816468 | 5.1207E-09 |
| A0A0H3PA60 | *dcuA* | Transport | 3.827759148 | 4.07846E-07 |
| A0A0H3PJ41 | *cj81176_0671* | Two-component regulatory system | 1.112289782 | 1.13262E-10 |
| A0A0H3P9R0 | *CJJ81176_1236* | Two-component regulatory system | 1.244439439 | 1.01919E-05 |
| A1VYV6 | *Putative cbf2* | Uncharacterized | 1.009478847 | 3.35689E-10 |
| A0A0H3PAU3 | *CJJ81176_0159* | Uncharacterized | 1.009592083 | 0.002276221 |
| Q2M5Q0 | *CJJ81176_1314* | Uncharacterized | 1.011356299 | 0.056700533 |
| Q0Q7K6 | *CJJ81176_0776* | Uncharacterized | 1.018914955 | 0.000384764 |
| A0A0H3PBU4 | *CJJ81176_0392* | Uncharacterized | 1.044219258 | 0.25580587 |
| A0A0H3PDW4 | *CJJ81176_1424* | Uncharacterized | 1.067121445 | 0.025373282 |
| A0A0H3PJ75 | *CJJ81176_0306* | Uncharacterized | 1.074796292 | 0.006979027 |
| Q2M5Q7 | *CJJ81176_1317* | Uncharacterized | 1.088798871 | 0.005949668 |
| A0A0H3PJA2 | *CJJ81176_0520* | Uncharacterized | 1.090483613 | 0.005199429 |
| A0A0H3P994 | *CJJ81176_0144* | Uncharacterized | 1.101162111 | 0.11512465 |
| A0A0H3P991 | *CJJ81176_0018* | Uncharacterized | 1.113943091 | 9.13358E-08 |
| A0A0H3PED7 | *CJJ81176_0477* | Uncharacterized | 1.114024196 | 0.001672857 |
| A0A0H3PEC2 | *CJJ81176_0189* | Uncharacterized | 1.114989621 | 0.046841276 |
| A0A0H3PB96 | *CJJ81176_0611* | Uncharacterized | 1.139074389 | 0.037100637 |
| A0A0H3PBE5 | *CJJ81176_0430* | Uncharacterized | 1.174311961 | 2.41486E-09 |
| A0A0H3PEN5 | *CJJ81176_0484* | Uncharacterized | 1.181155926 | 0.004957225 |
| A0A0H3PAB0 | *CJJ81176_0107* | Uncharacterized | 1.184171512 | 1.34449E-09 |
| A0A0H3P9Z9 | *CJJ81176_0708* | Uncharacterized | 1.213623076 | 0.003118232 |
| A0A0H3PC19 | *CJJ81176_0428* | Uncharacterized | 1.250734737 | 1.72127E-06 |
| A0A0H3PED0 | *CJJ81176_0391* | Uncharacterized | 1.280371266 | 0.002103489 |
| A0A0H3PAA3 | *CJJ81176_1453* | Uncharacterized | 1.301847945 | 0.05792168 |
| A0A0H3PCA8 | *CJJ81176_pTet0048* | Uncharacterized | 1.305945565 | 2.91411E-09 |
| A0A0H3PEH2 | *CJJ81176_0532* | Uncharacterized | 1.312207438 | 3.11278E-08 |
| A0A0H3PJE6 | *CJJ81176_0622* | Uncharacterized | 1.324036519 | 0.000236212 |
| Q2A947 | *CJJ81176_1444* | Uncharacterized | 1.326513791 | 2.85148E-13 |
| A0A0H3PAJ5 | *CJJ81176_1107* | Uncharacterized | 1.342943253 | 0.008375076 |
| A0A0H3PAS5 | *CJJ81176_0840* | Uncharacterized | 1.343673306 | 0.006966963 |
| A0A0H3PB78 | *CJJ81176_1414* | Uncharacterized | 1.344692686 | 0.005851801 |
| A0A0H3PJB0 | *CJJ81176_0403* | Uncharacterized | 1.358818649 | 0.000125312 |
| A0A0H3PCA0 | *CJJ81176_pTet0008* | Uncharacterized | 1.377305354 | 3.99301E-10 |
| A0A0H3PD99 | *CJJ81176_0797* | Uncharacterized | 1.400011602 | 0.00039195 |
| A0A0H3PBF4 | *CJJ81176_0342* | Uncharacterized | 1.403953147 | 0.0071964 |
| A0A0H3PJ11 | *CJJ81176_0153* | Uncharacterized | 1.413071245 | 3.12823E-08 |
| A0A0H3PGI9 | *CJJ81176_0987* | Uncharacterized | 1.420494898 | 0.016346357 |
| A0A0H3P9P2 | *CJJ81176_1326* | Uncharacterized | 1.433162751 | 2.24489E-07 |
| A0A0H3P968 | *CJJ81176_pTet0026* | Uncharacterized | 1.459991767 | 0.000224254 |
| A0A0H3PBZ1 | *CJJ81176_0414* | Uncharacterized | 1.472582137 | 0.005697302 |
| A0A0H3PGX2 | *CJJ81176_1003* | Uncharacterized | 1.493744709 | 0.019478149 |
| A0A0H3PAV9 | *CJJ81176_1416* | Uncharacterized | 1.50551169 | 3.78854E-07 |
| A0A0H3PAS3 | *CJJ81176_0705* | Uncharacterized | 1.518960907 | 0.022170492 |
| Q29VV4 | *CJJ81176_1430* | Uncharacterized | 1.530775757 | 3.34176E-15 |
| A0A0H3PAL1 | *CJJ81176_1102* | Uncharacterized | 1.537334839 | 9.37329E-07 |
| Q0Q7K3 | *CJJ81176_0779* | Uncharacterized | 1.557817115 | 2.12575E-05 |
| A0A0H3PGL0 | *CJJ81176_1732* | Uncharacterized | 1.569838789 | 0.013555967 |
| A0A0H3PJC9 | *CJJ81176_0518* | Uncharacterized | 1.572293153 | 0.002180475 |
| Q6QNL8 | *Cj1356c* | Uncharacterized | 1.625331653 | 2.57189E-08 |
| Q1HG73 | *CJJ81176_0034* | Uncharacterized | 1.6344356 | 6.21578E-11 |
| A0A0H3PGW3 | *CJJ81176_1177* | Uncharacterized | 1.63860112 | 0.004211886 |
| A0A0H3P9I1 | *CJJ81176_0782* | Uncharacterized | 1.641085272 | 8.88753E-06 |
| A0A0H3PAF3 | *CJJ81176_0231* | Uncharacterized | 1.726887946 | 1.41038E-06 |
| A0A0H3PB47 | *CJJ81176_1492* | Uncharacterized | 1.742259894 | 4.26179E-06 |
| A0A0H3PBN8 | *CJJ81176_0437* | Uncharacterized | 1.749721868 | 1.84363E-08 |
| A0A0H3PJK4 | *CJJ81176_0436* | Uncharacterized | 1.768446654 | 2.407E-08 |

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|---|---|---|---|---|
| A0A0H3PAI7 | *CJJ81176_1559* | Uncharacterized | 1.790286229 | 1.85218E-13 |
| A0A0H3PEW6 | *CJJ81176_0447* | Uncharacterized | 1.891985563 | 1.56241E-09 |
| A0A0H3PDH2 | *CJJ81176_0856* | Uncharacterized | 1.94630326 | 0.002983055 |
| A1VYL9 | *CJJ81176_0535* | Uncharacterized | 1.947651838 | 0.004899868 |
| A0A0H3PHT8 | *CJJ81176_1541* | Uncharacterized | 1.954109344 | 5.73972E-05 |
| A0A0H3PIQ2 | *CJJ81176_1657* | Uncharacterized | 1.983313638 | 0.0023077 |
| A0A0H3PIU3 | *CJJ81176_0188* | Uncharacterized | 2.041955298 | 1.64234E-05 |
| A0A0H3PAS8 | *CJJ81176_0740* | Uncharacterized | 2.095112507 | 0.000356817 |
| A0A0H3PBM5 | *CJJ81176_0187* | Uncharacterized | 2.171819171 | 1.43559E-05 |
| Q2M5Q9 | *CJJ81176_1315* | Uncharacterized | 2.210649706 | 2.25513E-09 |
| A0A0H3PAE1 | *CJJ81176_0192* | Uncharacterized | 2.240578454 | 0.003079777 |
| Q2M5R0 | *CJJ81176_1341* | Uncharacterized | 2.310441987 | 1.57019E-12 |
| A0A0H3PB91 | *CJJ81176_0355* | Uncharacterized | 2.33854159 | 2.36564E-15 |
| A0A0H3PAR1 | *napL* | Uncharacterized | 2.448651857 | 6.4748E-05 |
| A0A0H3P9D8 | *CJJ81176_1104* | Uncharacterized | 2.841286981 | 6.69656E-08 |
| A0A0H3PDT4 | *CJJ81176_1617* | Uncharacterized | 3.38488425 | 5.88243E-07 |

**Appendix 6**: Significantly differentiated proteins in 81-176 cultured in CDB at 37$^{o}$C for 24h

| **UniProt_Accession** | **Gene Name** | **Protein function** | **logFC** | **P.Value** |
|---|---|---|---|---|
| A0A0H3P9D5 | *ftsH* | Cell cycle, cell division | -1.074010067 | 4.80113E-06 |
| A0A0H3PEL1 | *TlpA* | Chemotaxis | -2.092142211 | 5.55915E-06 |
| A0A0H3PEF7 | *TlpA* | Chemotaxis | -2.049395887 | 1.02916E-08 |
| A0A0H3P9T7 | *TlpA* | Chemotaxis | -1.953210557 | 3.556E-09 |
| A0A0H3PB49 | *CJJ81176_1548* | Chemotaxis | -1.475173173 | 0.091313167 |
| A0A0H3P9J9 | *TlpB* | Chemotaxis | -1.031248572 | 0.016788485 |
| A0A0H3PGG1 | *CJJ81176_pTet0031* | DNA Replication | -1.409694119 | 2.93398E-13 |
| A0A0H3PA38 | *cydA* | Metabolism | -3.170368376 | 3.69671E-07 |
| A1VXS2 | *folE* | Metabolism | -2.214052574 | 0.01497923 |
| A1W1D6 | *acsA* | Metabolism | -1.980626235 | 5.88399E-12 |
| A0A0H3PAQ1 | *CJJ81176_0849* | Metabolism | -1.894068483 | 0.003074014 |
| A0A0H3PCI0 | *cjj81176* | Metabolism | -1.666789967 | 1.83549E-10 |
| A0A0H3P9N5 | *cjj81176_0075* | Metabolism | -1.651421119 | 1.31432E-14 |
| A0A0H3PD90 | *purE* | Metabolism | -1.628850422 | 1.38436E-05 |
| A0A0H3PA70 | *proB* | Metabolism | -1.554173328 | 1.29829E-05 |
| A0A0H3PHN8 | *cjj81176_0836* | Metabolism | -1.364416576 | 9.97899E-14 |
| A0A0H3PHB9 | *petA* | Metabolism | -1.361258878 | 2.11835E-09 |
| A0A0H3P9B7 | *cyf* | Metabolism | -1.160540717 | 0.001297993 |
| A0A0H3PBI3 | *CJJ81176_1495* | Metabolism | -1.160306918 | 1.14093E-13 |
| A0A0H3PHJ0 | *CJJ81176_0738* | Metabolism | -1.145940421 | 7.37491E-13 |
| A0A0H3PI47 | *CJJ81176_1247* | Metabolism | -1.112489041 | 0.086275419 |
| A0A0H3PAT0 | *cysK* | Metabolism | -1.110579684 | 8.8231E-10 |
| A0A0H3PAW0 | *corA* | Metabolism | -1.102312553 | 0.065214629 |
| A0A0H3PIR1 | *fdhA* | Metabolism | -1.021237654 | 0.048466976 |
| A0A0H3PIF6 | *fliL* | Motility | -1.792554519 | 4.86158E-09 |
| A0A0H3PED8 | *CJJ81176_0315* | Pathogenesis | -1.358677973 | 2.8684E-11 |
| A0A0H3PHL6 | *CJJ81176_0799* | Pathogenesis | -1.182353344 | 5.2266E-07 |
| A0A0H3PAL0 | *cadF* | Pathogenesis | -1.084642831 | 3.08722E-07 |
| A1VYJ6 | *rpsL* | Protein synthesis | -6.983238833 | 1.42492E-11 |
| A1W1J3 | *rpmJ* | Protein synthesis | -3.420706882 | 0.002827918 |
| A1W1L3 | *rpsT* | Protein synthesis | -2.908415491 | 1.34696E-06 |
| A0A0H3PA47 | *rnj* | Protein synthesis | -1.03913326 | 6.60317E-08 |
| A0A0H3PCE2 | *cstA* | Stress Response | -1.842407065 | 7.51471E-10 |
| A0A0H3P9Q4 | *katA* | Stress Response | -1.619495824 | 2.78853E-12 |
| A0A0H3PA75 | *comEA* | Stress Response | -1.266246242 | 6.18711E-10 |

| | | | | |
|---|---|---|---|---|
| A0A0H3PAP0 | *trx* | Stress Response | -1.060108272 | 1.33863E-12 |
| A0A0H3PAK6 | *chuA* | Transport | -3.035750823 | 4.65926E-13 |
| A0A0H3PA76 | *cjj81176_1604* | Transport | -2.864476221 | 1.86683E-17 |
| A0A0H3PE25 | *CJJ81176_1654* | Transport | -2.741946923 | 3.97144E-13 |
| A0A0H3PEW2 | *CJJ81176_0211* | Transport | -2.125939559 | 1.35993E-17 |
| Q0Q7I0 | *CJJ81176_1569* | Transport | -2.055410854 | 2.2467E-15 |
| A0A0H3PAU0 | *cjj81176_1525* | Transport | -1.846171823 | 7.37057E-17 |
| A0A0H3PAE4 | *cmeC* | Transport | -1.682616742 | 1.57873E-09 |
| A0A0H3PA17 | *putP* | Transport | -1.628286068 | 0.050433426 |
| A0A0H3P9L8 | *atpF* | Transport | -1.402977331 | 0.026803117 |
| A0A0H3PD65 | *cjj81176_1037* | Transport | -1.359734649 | 4.03189E-14 |
| A0A0H3PIS5 | *cmeA* | Transport | -1.244798369 | 6.38925E-05 |
| A0A0H3PB79 | *cmeB* | Transport | -1.20679488 | 0.000200058 |
| A0A0H3PJ16 | *modA* | Transport | -1.125669413 | 2.48076E-07 |
| A0A0H3PAY0 | *tatB* | Transport | -1.082929869 | 0.017605787 |
| A0A0H3PCQ6 | *CJJ81176_1038* | Transport | -1.020547801 | 7.45264E-11 |
| A0A0H3PA01 | *CJJ81176_1650* | Uncharacterized | -3.120222479 | 1.55616E-17 |
| A0A0H3PBB0 | *CJJ81176_1666* | Uncharacterized | -3.025049584 | 0.00248412 |
| A0A0H3P9S8 | *CJJ81176_1184* | Uncharacterized | -2.856505447 | 1.93146E-15 |
| A0A0H3PBF8 | *CJJ81176_1651* | Uncharacterized | -2.732988059 | 2.15093E-09 |
| A0A0H3PET5 | *CJJ81176_0471* | Uncharacterized | -2.59193348 | 5.50167E-13 |
| Q0Q7J3 | *cj1355* | Uncharacterized | -2.4439886 | 1.15092E-14 |
| A0A0H3PCF8 | *CJJ81176_0975* | Uncharacterized | -2.326110762 | 4.85016E-05 |
| A0A0H3PCP8 | *CJJ81176_1045* | Uncharacterized | -2.218606712 | 0.000729609 |
| Q8GJC5 | *Cjp29* | Uncharacterized | -2.156888257 | 1.8022E-05 |
| A0A0H3ADZ7 | *hup* | Uncharacterized | -2.070583157 | 7.98445E-09 |
| A0A0H3PA50 | *CJJ81176_0126* | Uncharacterized | -1.992516907 | 5.76066E-05 |
| A0A0H3PH47 | *CJJ81176_1185* | Uncharacterized | -1.981804674 | 7.42004E-15 |
| A0A0H3PI41 | *CJJ81176_1600* | Uncharacterized | -1.869659825 | 2.24794E-16 |
| A0A0H3PEX3 | *CJJ81176_0544* | Uncharacterized | -1.735376814 | 0.002373957 |
| Q29VV2 | *CJB1432c* | Uncharacterized | -1.693639504 | 0.004517986 |
| A0A0H3PEG8 | *CJJ81176_0642* | Uncharacterized | -1.56083428 | 1.30015E-08 |
| A0A0H3PBX6 | *CJJ81176_0358* | Uncharacterized | -1.527453937 | 0.000317857 |
| A0A0H3PES2 | *CJJ81176_0377* | Uncharacterized | -1.503031759 | 9.88867E-06 |
| A0A0H3P9T5 | *CJJ81176_1649* | Uncharacterized | -1.493220011 | 0.009877019 |
| A0A0H3PA88 | *CJJ81176_0125* | Uncharacterized | -1.484601464 | 3.94867E-13 |
| A0A0H3P9B9 | *cjaA* | Uncharacterized | -1.326559777 | 2.40839E-08 |
| A0A0H3PGE8 | *CJJ81176_pTet0018* | Uncharacterized | -1.322435353 | 7.38298E-10 |
| A0A0H3PBJ5 | *dsbD* | Uncharacterized | -1.288045006 | 0.042164645 |
| A0A0H3PBE0 | *CJJ81176_0236* | Uncharacterized | -1.272034999 | 4.34741E-10 |
| A0A0H3PAV1 | *CJJ81176_0359* | Uncharacterized | -1.230962237 | 3.32231E-06 |
| Q8GJA7 | *Cjp48* | Uncharacterized | -1.198099905 | 1.73382E-08 |
| A0A0H3PA18 | *CJJ81176_0942* | Uncharacterized | -1.138138346 | 3.12462E-06 |
| A0A0H3PAP4 | *cjj81176_0439* | Uncharacterized | -1.137791038 | 8.25509E-11 |
| A0A0H3PGV9 | *CJJ81176_1198* | Uncharacterized | -1.11844178 | 0.03135851 |
| A0A0H3PBA4 | *CJJ81176_1508* | Uncharacterized | -1.08468241 | 1.02042E-12 |
| A0A0H3PI11 | *CJJ81176_1608* | Uncharacterized | -1.053392156 | 0.0008415 |
| A0A0H3PA44 | *CJJ81176_0124* | Uncharacterized | -1.053268697 | 6.41152E-09 |
| | | | | |
| **UniProt_Accession** | **Gene Name** | **Protein function** | **logFC** | **P.Value** |
| A0A0H3PDA2 | *ftsZ* | Cell cycle, cell division | 1.1408654 | 0.000346312 |
| A0A0H3P9L6 | *ftsA* | Cell cycle, cell division | 1.302100974 | 5.02596E-06 |
| A0A0H3PAM2 | *mreB* | Cell cycle, cell division | 1.333157894 | 1.36732E-06 |
| A1VXS1 | *tig* | Cell cycle, cell division | 1.428507617 | 1.42184E-14 |
| A1W043 | *murC* | Cell cycle, cell division | 1.45147549 | 7.80259E-16 |
| A0A0H3PD97 | *ffh* | Cell wall organization | 1.186977837 | 3.02194E-07 |
| A0A0H3PE69 | *murI* | Cell wall organization | 1.535901917 | 1.86606E-10 |
| A1VZK1 | *murA* | Cell wall organization | 1.923267778 | 1.29695E-13 |
| A0A0H3P9P7 | *cjj81176_1128* | Chemotaxis | 1.27930677 | 2.8277E-15 |

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|---|---|---|---|---|
| A0A0H3PA34 | *cheB* | Chemotaxis | 1.325162029 | 5.82475E-05 |
| A0A0H3P9C4 | *CJJ81176_1204* | Chemotaxis | 1.658325995 | 8.4036E-06 |
| A0A0H3PB06 | *TlpC* | Chemotaxis | 1.955613085 | 9.31169E-09 |
| A0A0H3PEP2 | *polA* | DNA Replication | 1.003642534 | 9.94153E-08 |
| A0A0H3PA46 | *topA* | DNA Replication | 1.004165788 | 1.1225E-06 |
| A0A0H3PH83 | *ssb* | DNA Replication | 1.012583599 | 2.0656E-09 |
| A0A0H3PH67 | *dnaX* | DNA Replication | 1.16746783 | 0.003410521 |
| A0A0H3PB11 | *CJJ81176_1474* | DNA Replication | 1.213569156 | 1.20275E-10 |
| A0A0H3PBJ8 | *CJJ81176_0612* | DNA Replication | 1.357694609 | 0.000696323 |
| A0A0H3PER2 | *dnaB* | DNA Replication | 1.473304034 | 9.51288E-05 |
| A0A0H3P989 | *recJ* | DNA Replication | 2.123647667 | 1.59352E-07 |
| A0A0H3P9R8 | *CJJ81176_1043* | DNA Transcription | 1.043986407 | 2.28658E-05 |
| A0A0H3PGQ1 | *fliA* | DNA Transcription | 1.237870878 | 0.025389376 |
| A0A0H3PER6 | *nusG* | DNA Transcription | 1.267910116 | 3.68886E-11 |
| A1VY10 | *greA* | DNA Transcription | 1.432257843 | 2.59249E-10 |
| A0A0H3PB61 | *nusA* | DNA Transcription | 1.439390303 | 6.65591E-11 |
| A0A0H3P9Q7 | *mfd* | DNA Transcription | 2.434489002 | 1.68169E-11 |
| A0A0H3PBB6 | *trpE* | Metabolism | 1.003189125 | 6.0061E-10 |
| A0A0H3PBY2 | *CJJ81176_0318* | Metabolism | 1.006310949 | 0.001865944 |
| A0A0H3PAJ4 | *hisI* | Metabolism | 1.014809361 | 1.6677E-09 |
| A0A0H3PA14 | *prsA* | Metabolism | 1.025508685 | 2.78553E-09 |
| A0A0H3PIL4 | *hisD* | Metabolism | 1.032790757 | 1.0399E-13 |
| Q1HG72 | *gltD* | Metabolism | 1.036335448 | 5.04443E-09 |
| A1W0U9 | *hisH1* | Metabolism | 1.050387738 | 0.049152845 |
| A0A0H3PIY4 | *aroE* | Metabolism | 1.074931759 | 2.52527E-07 |
| A0A0H3PEA7 | *oorB* | Metabolism | 1.076483625 | 7.4671E-15 |
| A0A0H3P9V8 | *CJJ81176_1527* | Metabolism | 1.078298625 | 0.043097272 |
| A0A0H3PBC8 | *CJJ81176_1415* | Metabolism | 1.082007016 | 0.091170526 |
| A0A0H3PB58 | *CJJ81176_0610* | Metabolism | 1.08590804 | 7.91844E-07 |
| A0A0H3PET1 | *trpD* | Metabolism | 1.130851725 | 1.37694E-10 |
| A0A0H3PA90 | *cj81176_0571* | Metabolism | 1.134427124 | 2.75768E-07 |
| A1VXU8 | *argB* | Metabolism | 1.146965546 | 1.85255E-05 |
| A0A0H3PHU2 | *CJJ81176_1517* | Metabolism | 1.147912263 | 5.35909E-06 |
| A1W116 | *pgk* | Metabolism | 1.148249164 | 8.38903E-10 |
| A1W1X5 | *queF* | Metabolism | 1.162193924 | 0.043057037 |
| A1VXU6 | *argC* | Metabolism | 1.162584654 | 2.15892E-05 |
| A0A0H3P9Q8 | *CJJ81176_1286* | Metabolism | 1.187518884 | 2.6617E-11 |
| A0A0H3PEE7 | *oorC* | Metabolism | 1.194525073 | 7.07002E-07 |
| A0A0H3P9A4 | *CJJ81176_0120* | Metabolism | 1.212228258 | 7.43778E-05 |
| A1W0R9 | *mqnA* | Metabolism | 1.214076693 | 0.001904392 |
| A1W1K3 | *hisA* | Metabolism | 1.215753381 | 0.040471373 |
| A0A0H3P9A3 | *CJJ81176_0013* | Metabolism | 1.218159617 | 0.009926103 |
| A1VZI4 | *fbp* | Metabolism | 1.222618407 | 4.3338E-14 |
| A0A0H3PE58 | *CJJ81176_1470* | Metabolism | 1.2290586 | 0.027978501 |
| A0A0H3PAJ2 | *CJJ81176_1039* | Metabolism | 1.237732666 | 6.28018E-08 |
| A0A0H3PHG1 | *coaBC* | Metabolism | 1.248329797 | 6.8283E-09 |
| A0A0H3P9P8 | *tkt* | Metabolism | 1.280218398 | 1.28551E-08 |
| A0A0H3PBG9 | *purN* | Metabolism | 1.28323879 | 3.07067E-11 |
| A0A0H3P9P9 | *guaB* | Metabolism | 1.289397339 | 1.78E-13 |
| A0A0H3P9Q2 | *nrfA* | Metabolism | 1.292618327 | 4.99923E-09 |
| A0A0H3PBV9 | *oorD* | Metabolism | 1.302291292 | 0.006953596 |
| A0A0H3PCK6 | *ansA* | Metabolism | 1.311331491 | 5.36278E-15 |
| A0A0H3PHE7 | *CJJ81176_0739* | Metabolism | 1.312959243 | 6.81963E-05 |
| A0A0H3PJF7 | *coaX* | Metabolism | 1.328366939 | 0.012221932 |
| A0A0H3PAU6 | *pyrC* | Metabolism | 1.341597719 | 0.104250195 |
| A0A0H3PAK3 | *hisH-2* | Metabolism | 1.342677341 | 0.003139101 |
| A1W091 | *ispE* | Metabolism | 1.343067248 | 0.005822463 |
| A0A0H3PA20 | *dcd* | Metabolism | 1.358508874 | 1.40789E-10 |
| A0A0H3P9G7 | *hemN* | Metabolism | 1.35948935 | 5.22666E-12 |

| | | | | |
|---|---|---|---|---|
| A0A0H3PC48 | *purQ* | Metabolism | 1.381069155 | 8.7794E-06 |
| A0A0H3PAP2 | *pgi* | Metabolism | 1.39339181 | 7.2788E-12 |
| A0A0H3PF06 | *CJJ81176_0186* | Metabolism | 1.404184091 | 4.54797E-13 |
| A1VZM8 | *ispH* | Metabolism | 1.415169414 | 0.004440061 |
| A0A0H3PAA8 | *CJJ81176_0533* | Metabolism | 1.41685347 | 4.07597E-10 |
| A0A0H3PBA0 | *carA* | Metabolism | 1.431771171 | 7.17243E-14 |
| A1W0I0 | *gpsA* | Metabolism | 1.440378192 | 1.30312E-10 |
| A0A0H3PAC7 | *nuoM* | Metabolism | 1.444783163 | 0.008752744 |
| A0A0H3PF31 | *CJJ81176_0427* | Metabolism | 1.44487348 | 0.022659681 |
| A0A0H3PH92 | *glcD* | Metabolism | 1.45464072 | 4.33352E-08 |
| A0A0H3PAD5 | *lpxD* | Metabolism | 1.457740599 | 2.87705E-14 |
| A1VXZ8 | *lpxA* | Metabolism | 1.462139579 | 2.77241E-12 |
| A0A0H3PB10 | *CJJ81176_0255* | Metabolism | 1.478183573 | 6.51609E-07 |
| A1VZF0 | *cysS* | Metabolism | 1.485927401 | 3.02098E-11 |
| A0A0H3PAG6 | *tpiA* | Metabolism | 1.489387813 | 2.56575E-06 |
| A0A0H3PBB5 | *purD* | Metabolism | 1.490061045 | 8.12768E-06 |
| A1VZJ6 | *hemL* | Metabolism | 1.500375258 | 5.34246E-12 |
| A1VYG9 | *thiC* | Metabolism | 1.505042952 | 1.42616E-05 |
| Q0ZSS3 | | Metabolism | 1.505140619 | 8.68437E-14 |
| A1VYF9 | *acpP* | Metabolism | 1.505819439 | 2.91892E-05 |
| Q1HG74 | *gltB* | Metabolism | 1.510185162 | 1.21899E-11 |
| Q29VW1 | *gmhA-2* | Metabolism | 1.517891003 | 2.44883E-09 |
| A0A0H3PBV0 | *ilvH* | Metabolism | 1.566697776 | 1.63556E-08 |
| Q29VH0 | *kpsF* | Metabolism | 1.57311925 | 1.29174E-09 |
| A0A0H3PHX0 | *cjj81176_1379* | Metabolism | 1.594505859 | 6.97557E-06 |
| A0A0H3PBH6 | *cj81176_1322* | Metabolism | 1.600996888 | 1.70325E-06 |
| A0A0H3P9B6 | *thiF* | Metabolism | 1.614767758 | 1.15048E-05 |
| A0A0H3PAH1 | *tyrA* | Metabolism | 1.622698689 | 6.15934E-05 |
| A0A0H3P9S3 | *hydD* | Metabolism | 1.650716691 | 0.009792 |
| A0A0H3PEI7 | *folP* | Metabolism | 1.65311143 | 5.16213E-05 |
| A1VZZ8 | *tgt* | Metabolism | 1.660724146 | 0.00484531 |
| A1W1W9 | *leuD* | Metabolism | 1.671375897 | 6.74317E-09 |
| A1VY40 | *dxs* | Metabolism | 1.682532191 | 8.17888E-05 |
| A0A0H3PCM5 | *metX* | Metabolism | 1.709023388 | 1.44925E-05 |
| A1VY69 | *trpB* | Metabolism | 1.720225813 | 2.56733E-11 |
| A1W068 | *thiE* | Metabolism | 1.725062063 | 3.01406E-06 |
| Q29VV6 | *fcl* | Metabolism | 1.73476975 | 7.41717E-18 |
| A0A0H3PH15 | *thiD* | Metabolism | 1.735761228 | 9.66361E-06 |
| A0A0H3PIZ2 | *CJJ81176_0601* | Metabolism | 1.768583118 | 0.000119642 |
| Q2M5Q4 | *accP* | Metabolism | 1.804263489 | 2.88463E-14 |
| A1VY70 | *trpA* | Metabolism | 1.821044646 | 3.7185E-09 |
| A0A0H3PEJ1 | *ilvB* | Metabolism | 1.835556939 | 1.46034E-10 |
| A1VXA6 | *pyrG* | Metabolism | 1.848186797 | 1.21919E-11 |
| Q0Q7I1 | *purM* | Metabolism | 1.860740172 | 8.53514E-10 |
| A1VXH9 | *obg* | Metabolism | 1.875463509 | 0.000564786 |
| A1W0W6 | *mobA* | Metabolism | 1.88532065 | 2.08662E-06 |
| A1W1X0 | *leuC* | Metabolism | 1.888401688 | 1.13276E-09 |
| A0A0H3PD29 | *cobB* | Metabolism | 1.908113094 | 0.000985836 |
| A1VZR0 | *apt* | Metabolism | 1.926925569 | 6.91372E-14 |
| A1VZ41 | *ispG* | Metabolism | 1.971925466 | 1.11281E-07 |
| A0A0H3PBF9 | *rpe* | Metabolism | 1.987926018 | 1.25005E-05 |
| A0A0H3PBD0 | *bioA* | Metabolism | 1.991192703 | 0.00330893 |
| A1W0I5 | *metE* | Metabolism | 2.028422557 | 4.88455E-11 |
| A0A0H3P982 | *rpiB* | Metabolism | 2.040743676 | 1.35659E-07 |
| A1VY44 | *xseA* | Metabolism | 2.076710686 | 4.07431E-07 |
| A1VZF8 | *nadE* | Metabolism | 2.084518532 | 1.70268E-06 |
| Q0Q7I7 | *mqnE* | Metabolism | 2.117168109 | 1.81018E-13 |
| A0A0H3PEZ1 | *frdB* | Metabolism | 2.138919185 | 1.53147E-07 |
| A1VYG1 | *accA* | Metabolism | 2.168172853 | 1.36934E-07 |

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|---|---|---|---|---|
| A0A0H3PJ93 | *lysA* | Metabolism | 2.171060285 | 1.52338E-15 |
| A0A0H3PBR9 | *ccpA-2* | Metabolism | 2.209146418 | 6.87342E-18 |
| A0A0H3PHM5 | *mobB* | Metabolism | 2.284964714 | 8.84675E-09 |
| A1VYU1 | *rppH* | Metabolism | 2.302246504 | 5.51762E-08 |
| A1VYL8 | *alaS* | Metabolism | 2.366478893 | 4.15855E-12 |
| A0A0H3P9B2 | *thiH* | Metabolism | 2.396720373 | 4.24782E-08 |
| A0A0H3PC31 | *hom* | Metabolism | 2.40525962 | 1.96533E-08 |
| A1W0N8 | *pnp* | Metabolism | 2.480065752 | 3.25744E-11 |
| A1VXF1 | *aroQ* | Metabolism | 2.553944619 | 0.00091255 |
| A0A0H3PJ06 | *mqnC* | Metabolism | 2.813863937 | 3.20786E-08 |
| A0A0H3PGM1 | *aspA* | Metabolism | 2.919307004 | 1.03812E-17 |
| A0A0H3PGR5 | *cjj81176_0063* | Metabolism | 3.12742143 | 2.22382E-10 |
| A0A0H3PAG3 | *sdhC* | Metabolism | 3.657099223 | 1.43621E-11 |
| A0A0H3PJB7 | *sdhB* | Metabolism | 4.05847421 | 5.04621E-19 |
| A0A0H3PAD9 | *pglD* | Metabolism | 1.516161068 | 0.009592022 |
| A0A0H3PBQ2 | *sdhA* | Metabolism | 4.557015291 | 1.0262E-21 |
| A0A0H3PEY5 | *fliS* | Motility | 1.133867454 | 0.002921781 |
| Q2M5R1 | *CJJ81176_1340* | Motility | 1.401756903 | 0.00721649 |
| A0A0H3P9L2 | *fliM* | Motility | 1.460937551 | 0.009794324 |
| A0A0H3PIU8 | *fliD* | Motility | 1.530766417 | 1.04017E-08 |
| A1W062 | *fliW* | Motility | 1.614319716 | 0.028209869 |
| A0A0H3PDD9 | *flaC* | Motility | 2.038788627 | 6.79442E-07 |
| A0A0H3PBK9 | *flaG* | Motility | 2.303062456 | 0.000993683 |
| A0A0H3PD33 | *sixA* | Protein modification | 3.50838383 | 2.40126E-08 |
| A1W165 | *truD* | Protein synthesis | 1.028157144 | 0.066861889 |
| A1VYA9 | *serS* | Protein synthesis | 1.034173751 | 3.63845E-14 |
| A1W1U6 | *rpsH* | Protein synthesis | 1.060099802 | 2.30622E-07 |
| A1VZH5 | *trmA* | Protein synthesis | 1.146227604 | 2.46356E-09 |
| A1VXT6 | *infC* | Protein synthesis | 1.14763899 | 5.68313E-07 |
| A0A0H3PAZ6 | *hypB* | Protein synthesis | 1.176579911 | 4.38416E-05 |
| A1W0I1 | *gatB* | Protein synthesis | 1.259726121 | 3.69034E-08 |
| A0A0H3PDU5 | *tyrS* | Protein synthesis | 1.288520058 | 2.86632E-13 |
| A1VZU7 | *mnmE* | Protein synthesis | 1.290261118 | 0.057844223 |
| A1VXL9 | *infB* | Protein synthesis | 1.323011744 | 3.19364E-08 |
| A1VYA6 | *der* | Protein synthesis | 1.324954354 | 5.2472E-06 |
| A1VYR0 | *efp* | Protein synthesis | 1.326666677 | 2.20448E-08 |
| A1W0H2 | *mnmG* | Protein synthesis | 1.35272772 | 6.25583E-08 |
| A0A0H3PAI4 | *ileS* | Protein synthesis | 1.383518332 | 3.19872E-15 |
| A1VXM1 | *rimP* | Protein synthesis | 1.394635285 | 0.000216305 |
| A0A0H3PB64 | *trpS* | Protein synthesis | 1.404087215 | 6.76275E-07 |
| A1W162 | *rimO* | Protein synthesis | 1.412443976 | 6.97299E-06 |
| A1W048 | *gatA* | Protein synthesis | 1.461525347 | 2.4725E-12 |
| A1VZW5 | *cmoB* | Protein synthesis | 1.465588084 | 0.000719815 |
| A1W163 | *prfB* | Protein synthesis | 1.535603691 | 2.2066E-11 |
| A1VYH4 | *miaB* | Protein synthesis | 1.583679774 | 7.31594E-06 |
| A1W1L4 | *prfA* | Protein synthesis | 1.59118897 | 2.06483E-09 |
| A0A0H3P9K7 | *metS* | Protein synthesis | 1.593951393 | 2.57393E-11 |
| A0A0H3PCJ0 | *CJJ81176_0101* | Protein synthesis | 1.726454881 | 1.9167E-07 |
| A0A0H3PBB3 | *rbfA* | Protein synthesis | 1.732167633 | 0.026284724 |
| A1VZB3 | *hisS* | Protein synthesis | 1.777135779 | 5.78592E-16 |
| A1W0R3 | *trmB* | Protein synthesis | 1.925358637 | 0.007751629 |
| A0A0H3P9L9 | *rpsA* | Protein synthesis | 1.937216852 | 5.43025E-14 |
| A1W1J4 | *rpsM* | Protein synthesis | 1.976800811 | 1.70131E-05 |
| A1VZ20 | *era* | Protein synthesis | 2.218847896 | 0.002357589 |
| A1VYB8 | *gatC* | Protein synthesis | 2.515538006 | 8.96293E-05 |
| A0A0H3PIS8 | *clpX* | Stress Response | 1.000868316 | 4.11912E-08 |
| A0A0H3P9Q3 | *csrA* | Stress Response | 1.010181468 | 0.023939028 |
| Q7X518 | *pseD* | Stress Response | 1.069026011 | 6.20301E-05 |
| A1VXS0 | *clpP* | Stress Response | 1.108983015 | 5.68675E-10 |

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|---|---|---|---|---|
| A1VZ21 | *hslU* | Stress Response | 1.126012373 | 2.70991E-11 |
| A0A0H3PBY8 | *CJJ81176_0298* | Stress Response | 1.139662765 | 4.65604E-10 |
| A0A0H3PAZ2 | *slyD* | Stress Response | 1.142440374 | 1.6743E-11 |
| A0A0H3PB76 | *dnaJ-1* | Stress Response | 1.179159393 | 0.092966683 |
| A0A0H3PHS4 | *CJJ81176_1536* | Stress Response | 1.186503532 | 0.013071774 |
| Q5QKR7 | *pseC* | Stress Response | 1.213674551 | 6.19784E-07 |
| A0A0H3PC09 | *hypC* | Stress Response | 1.253643969 | 0.000149657 |
| A0A0H3PAN1 | *CJJ81176_1158* | Stress Response | 1.27992193 | 5.64323E-08 |
| Q3I354 | *luxS* | Stress Response | 1.288212661 | 0.100860533 |
| A1VXQ2 | *sodB* | Stress Response | 1.342616101 | 1.81021E-10 |
| A0A0H3PAG5 | *radA* | Stress Response | 1.542327732 | 0.00799796 |
| A1W0P5 | *dnaJ* | Stress Response | 1.553230164 | 0.001104859 |
| A0A0H3PJI4 | *recN* | Stress Response | 1.734055854 | 9.4529E-06 |
| A0A0H3PEB4 | *nth* | Stress Response | 1.772489163 | 7.55877E-12 |
| A0A0H3PAC3 | *CJJ81176_1161* | Stress Response | 1.866184386 | 1.96754E-13 |
| A1VYU6 | *ligA* | Stress Response | 1.866330728 | 2.06032E-09 |
| A0A0H3P9V7 | *CJJ81176_1101* | Stress Response | 1.896127554 | 4.22233E-08 |
| A0A0H3P9M1 | *napD* | Stress Response | 1.969956486 | 0.000514118 |
| A0A0H3P9D2 | *CJJ81176_1077* | Stress Response | 2.128397855 | 4.8725E-06 |
| A0A0H3PGY0 | *ppiB* | Stress Response | 2.153548917 | 1.22657E-10 |
| A0A0H3PIA1 | *CJJ81176_1539* | Stress Response | 2.663445686 | 1.75308E-09 |
| A0A0H3PAU2 | *CJJ81176_1538* | Stress Response | 2.870142876 | 9.92744E-07 |
| A1W0U6 | *pseG* | Stress Response | 3.040628086 | 2.31316E-05 |
| A0A0H3PA51 | *napG* | Transport | 1.100156175 | 0.003588484 |
| A0A0H3PBL7 | *fur* | Transport | 1.253431932 | 0.090209041 |
| A1VZT4 | *secA* | Transport | 1.287004642 | 4.43645E-07 |
| A1VZC8 | *napA* | Transport | 1.325001806 | 4.45714E-12 |
| A1VXI7 | *atpH* | Transport | 1.464762002 | 0.004297885 |
| A0A0H3PAG9 | *CJJ81176_0672* | Transport | 1.46688738 | 2.4599E-09 |
| A0A0H3PDE7 | *CJJ81176_0897* | Transport | 1.630136757 | 0.018923871 |
| A0A0H3PA74 | *napB* | Transport | 1.770042288 | 4.1411E-13 |
| A0A0H3PEE2 | *secG* | Transport | 1.882106369 | 0.003158638 |
| A0A0H3PA60 | *dcuA* | Transport | 2.900436113 | 4.0325E-05 |
| Q0Q7H5 | *CJJ81176_1574* | Transport | 3.06743769 | 1.28427E-11 |
| A0A0H3PBF3 | *CJJ81176_1241* | Two component regulatory system | 1.186590423 | 4.42809E-07 |
| A0A0H3PJ41 | *cj81176_0671* | Two component regulatory system | 1.482600846 | 3.34561E-12 |
| A0A0H3P9R0 | *CJJ81176_1236* | Two component regulatory system | 1.916532986 | 3.62374E-09 |
| A0A0H3PA31 | *CJJ81176_0693* | Uncharacterized | 1.011450668 | 0.139232646 |
| A0A0H3P991 | *CJJ81176_0018* | Uncharacterized | 1.046898695 | 3.55284E-07 |
| A0A0H3PAM9 | *CJJ81176_1458* | Uncharacterized | 1.069399224 | 0.000125353 |
| A0A0H3PBR7 | *CJJ81176_0420* | Uncharacterized | 1.069838273 | 0.124429478 |
| A0A0H3P9U1 | *CJJ81176_1487* | Uncharacterized | 1.071468129 | 3.43809E-10 |
| A1VYL9 | *CJJ81176_0535* | Uncharacterized | 1.076431392 | 0.156886486 |
| A0A0H3PE88 | *CJJ81176_1417* | Uncharacterized | 1.088019062 | 1.52733E-11 |
| A0A0H3PCE6 | *CJJ81176_0935* | Uncharacterized | 1.102228856 | 0.09896491 |
| A1VY95 | *CJJ81176_0398* | Uncharacterized | 1.107976374 | 5.993E-09 |
| A0A0H3PEC2 | *CJJ81176_0189* | Uncharacterized | 1.110044274 | 0.105298429 |
| A0A0H3PAS3 | *CJJ81176_0705* | Uncharacterized | 1.129127816 | 0.108896822 |
| A0A0H3PAA2 | *CJJ81176_0288* | Uncharacterized | 1.141692559 | 8.73309E-07 |
| A0A0H3PIE6 | *CJJ81176_1488* | Uncharacterized | 1.15819802 | 5.56613E-06 |
| A0A0H3P9Y5 | *CJJ81176_pTet0016* | Uncharacterized | 1.178538124 | 0.006376335 |
| A0A0H3P9J4 | *CJJ81176* | Uncharacterized | 1.196272957 | 1.73634E-06 |
| A0A0H3PDS7 | *CJJ81176_1355* | Uncharacterized | 1.217815208 | 0.000896173 |
| A0A0H3PDT1 | *CJJ81176_1265* | Uncharacterized | 1.229975051 | 1.30307E-05 |
| A0A0H3PGL0 | *CJJ81176_1732* | Uncharacterized | 1.23711704 | 0.032023131 |
| A0A0H3PAI2 | *CJJ81176_1230* | Uncharacterized | 1.24558028 | 3.65559E-06 |

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|---|---|---|---|---|
| A0A0H3PJC9 | *CJJ81176_0518* | Uncharacterized | 1.248801296 | 0.007101476 |
| A0A0H3PHT8 | *CJJ81176_1541* | Uncharacterized | 1.265629473 | 0.006830475 |
| A0A0H3PJE6 | *CJJ81176_0622* | Uncharacterized | 1.284662984 | 0.000457811 |
| A0A0H3PJ75 | *CJJ81176_0306* | Uncharacterized | 1.288539279 | 0.003029216 |
| A0A0H3PDK8 | *CJJ81176_1475* | Uncharacterized | 1.292190688 | 3.15307E-05 |
| A0A0H3PA27 | *CJJ81176_0713* | Uncharacterized | 1.305923998 | 1.20103E-09 |
| A1W0U8 | *hisF2* | Uncharacterized | 1.316691038 | 0.011944518 |
| A0A0H3PDH2 | *CJJ81176_0856* | Uncharacterized | 1.323851921 | 0.074311693 |
| Q29VV5 | *CJB1429c* | Uncharacterized | 1.332425918 | 3.42607E-13 |
| A0A0H3PIQ2 | *CJJ81176_1657* | Uncharacterized | 1.408099612 | 0.022925976 |
| A0A0H3P9M3 | *CJJ81176_0048* | Uncharacterized | 1.411345637 | 0.000138788 |
| A0A0H3P9Z9 | *CJJ81176_0708* | Uncharacterized | 1.421875564 | 0.001365204 |
| A0A0H3P9P2 | *CJJ81176_1326* | Uncharacterized | 1.448402988 | 2.01788E-07 |
| A0A0H3PB39 | *CJJ81176_1673* | Uncharacterized | 1.448615032 | 0.009310839 |
| A0A0H3P9B0 | *CJJ81176_0135* | Uncharacterized | 1.45313159 | 1.12926E-06 |
| A0A0H3PAF1 | *CJJ81176_1363* | Uncharacterized | 1.459583714 | 0.002229546 |
| Q8GJE8 | *Cjp04* | Uncharacterized | 1.480258865 | 0.008283191 |
| A0A0H3PED7 | *CJJ81176_0477* | Uncharacterized | 1.480616811 | 0.000247027 |
| Q2M5Q7 | *CJJ81176_1317* | Uncharacterized | 1.488507364 | 0.000272481 |
| A0A0H3PAS5 | *CJJ81176_0840* | Uncharacterized | 1.499445897 | 0.003008268 |
| Q2M5Q6 | *CJJ81176_1318* | Uncharacterized | 1.50523359 | 2.58508E-07 |
| A0A0H3PAA3 | *CJJ81176_1453* | Uncharacterized | 1.548266497 | 0.055976986 |
| A0A0H3PA08 | *CJJ81176_0742* | Uncharacterized | 1.551026004 | 2.97742E-06 |
| A0A0H3PB55 | *CJJ81176_0474* | Uncharacterized | 1.559499087 | 2.45943E-12 |
| A0A0H3P9N1 | *CJJ81176_0889* | Uncharacterized | 1.564552176 | 9.694E-08 |
| A0A0H3P968 | *CJJ81176_pTet0026* | Uncharacterized | 1.604041968 | 1.66615E-05 |
| A0A0H3P9T3 | *CJJ81176_1422* | Uncharacterized | 1.604671486 | 2.52942E-05 |
| A0A0H3P9J3 | *CJJ81176_0988* | Uncharacterized | 1.605917139 | 0.00793143 |
| A0A0H3PCA8 | *CJJ81176_pTet0048* | Uncharacterized | 1.631468308 | 3.59433E-09 |
| A0A0H3PEH2 | *CJJ81176_0532* | Uncharacterized | 1.664236101 | 3.54751E-10 |
| Q0Q7K6 | *CJJ81176_0776* | Uncharacterized | 1.689324156 | 3.02022E-07 |
| A0A0H3P9W6 | *A0A0H3P9W6* | Uncharacterized | 1.690343647 | 1.70021E-05 |
| A0A0H3PCA0 | *CJJ81176_pTet0008* | Uncharacterized | 1.710466444 | 2.04289E-11 |
| A0A0H3PIU3 | *CJJ81176_0188* | Uncharacterized | 1.725670658 | 0.001227559 |
| A0A0H3PAV9 | *CJJ81176_1416* | Uncharacterized | 1.768729121 | 6.88555E-08 |
| A0A0H3PAB0 | *CJJ81176_0107* | Uncharacterized | 1.775596566 | 3.9107E-12 |
| A0A0H3P9M8 | *CJJ81176_0136* | Uncharacterized | 1.78274895 | 1.70867E-09 |
| Q0Q7K3 | *CJJ81176_0779* | Uncharacterized | 1.789819011 | 4.18066E-06 |
| Q29VV4 | *CJJ81176_1430* | Uncharacterized | 1.796746553 | 6.83196E-17 |
| A0A0H3PDT4 | *CJJ81176_1617* | Uncharacterized | 1.798570056 | 0.082903445 |
| A0A0H3PEN5 | *CJJ81176_0484* | Uncharacterized | 1.815247556 | 0.000511668 |
| Q2A947 | *CJJ81176_1444* | Uncharacterized | 1.81763975 | 9.43337E-15 |
| A0A0H3PAA1 | *CJJ81176_1497* | Uncharacterized | 1.834523941 | 1.03159E-11 |
| A0A0H3PDG2 | *CJJ81176_0891* | Uncharacterized | 1.869031325 | 0.004783325 |
| A0A0H3P9I1 | *CJJ81176_0782* | Uncharacterized | 1.872076498 | 2.76873E-06 |
| A0A0H3PJA2 | *CJJ81176_0520* | Uncharacterized | 1.889814326 | 2.51041E-05 |
| A0A0H3PED0 | *CJJ81176_0391* | Uncharacterized | 1.954712401 | 3.2561E-05 |
| A0A0H3PJ11 | *CJJ81176_0153* | Uncharacterized | 1.981378352 | 4.23823E-10 |
| A0A0H3PBF4 | *CJJ81176_0342* | Uncharacterized | 1.989755696 | 0.000631073 |
| A0A0H3PAI7 | *CJJ81176_1559* | Uncharacterized | 2.044239894 | 3.27283E-15 |
| A0A0H3PB85 | *CJJ81176_0254* | Uncharacterized | 2.063460325 | 0.018845967 |
| A0A0H3PA59 | *CJJ81176_1259* | Uncharacterized | 2.063765778 | 1.64128E-06 |
| A0A0H3P973 | *CJJ81176_pTet0021* | Uncharacterized | 2.07184484 | 1.89136E-07 |
| A0A0H3PBM5 | *CJJ81176_0187* | Uncharacterized | 2.103346614 | 2.16776E-05 |
| A0A0H3PBZ1 | *CJJ81176_0414* | Uncharacterized | 2.104108023 | 0.005175637 |
| A0A0H3PAE1 | *CJJ81176_0192* | Uncharacterized | 2.109777793 | 0.005282709 |
| A0A0H3PAT8 | *CJJ81176_1274* | Uncharacterized | 2.120615253 | 4.58826E-06 |
| A0A0H3P9L3 | *CJJ81176_0728* | Uncharacterized | 2.133251916 | 4.95086E-06 |
| A0A0H3PHH8 | *CJJ81176_0888* | Uncharacterized | 2.136170609 | 0.001343462 |

| | | | | |
|---|---|---|---|---|
| Q1HG73 | *CJJ81176_0034* | Uncharacterized | 2.144531973 | 7.09985E-14 |
| Q2M5Q9 | *CJJ81176_1315* | Uncharacterized | 2.191865091 | 3.76418E-08 |
| A0A0H3PJB0 | *CJJ81176_0403* | Uncharacterized | 2.285163654 | 4.47456E-07 |
| A0A0H3PGW3 | *CJJ81176_1177* | Uncharacterized | 2.288993451 | 0.000150261 |
| A0A0H3PJK4 | *CJJ81176_0436* | Uncharacterized | 2.294984015 | 2.3348E-08 |
| A0A0H3PAF3 | *CJJ81176_0231* | Uncharacterized | 2.337724249 | 2.36023E-10 |
| A0A0H3PAL1 | *CJJ81176_1102* | Uncharacterized | 2.373502542 | 4.55585E-10 |
| A0A0H3PB78 | *CJJ81176_1414* | Uncharacterized | 2.391545958 | 3.69242E-05 |
| A0A0H3PDW4 | *CJJ81176_1424* | Uncharacterized | 2.407901171 | 1.4773E-07 |
| A0A0H3PB91 | *CJJ81176_0355* | Uncharacterized | 2.451761394 | 3.19382E-17 |
| A0A0H3PBN8 | *CJJ81176_0437* | Uncharacterized | 2.454809835 | 4.91324E-11 |
| A0A0H3PAS8 | *CJJ81176_0740* | Uncharacterized | 2.542192699 | 8.43202E-05 |
| A0A0H3PEW6 | *CJJ81176_0447* | Uncharacterized | 2.751304085 | 9.69262E-13 |
| A0A0H3PB96 | *CJJ81176_0611* | Uncharacterized | 2.813846229 | 1.18082E-08 |
| A0A0H3PAR1 | *napL* | Uncharacterized | 2.855113696 | 1.3788E-05 |
| Q2M5R0 | *CJJ81176_1341* | Uncharacterized | 3.008373278 | 1.27588E-14 |
| A0A0H3P9D8 | *CJJ81176_1104* | Uncharacterized | 3.29864659 | 2.81688E-08 |

**Appendix 7**: Significantly differentiated proteins between 81-176 cultured in CDB at 37$^{o}$C for 24h and 42$^{o}$C for 24h

| | **Significantly downregulated** | | | |
|---|---|---|---|---|
| **UniProt_Accession** | **Gene Name** | **Protein function** | **logFC** | **P.Value** |
| A1VZM0 | *ftsK* | Cell cycle, cell division | -1.20474896 | 0.004230464 |
| A0A0H3P9C4 | *CJJ81176_1204* | Chemotaxis | -1.035823029 | 4.24941E-12 |
| A0A0H3P9Q7 | *mfd* | DNA Transcription | -1.22666595 | 2.40029E-14 |
| A1W068 | *thiE* | Metabolism | -2.009158025 | 2.23153E-05 |
| A0A0H3PGM1 | *aspA* | Metabolism | -1.890099225 | 5.31322E-14 |
| A1VY44 | *xseA* | Metabolism | -1.547614878 | 0.006384254 |
| A1VY70 | *trpA* | Metabolism | -1.509672276 | 9.71639E-08 |
| A1VY69 | *trpB* | Metabolism | -1.381791525 | 4.01416E-10 |
| A0A0H3PH15 | *thiD* | Metabolism | -1.321444213 | 8.94999E-13 |
| A0A0H3P9S3 | *hydD* | Metabolism | -1.308128592 | 0.001564111 |
| A0A0H3PCZ7 | *CJJ81176_1082* | Metabolism | -1.251112019 | 0.055654729 |
| A0A0H3PHM5 | *mobB* | Metabolism | -1.182982394 | 0.060239688 |
| A0A0H3PBQ2 | *sdhA* | Metabolism | -1.150476408 | 5.73816E-12 |
| A0A0H3PJB7 | *sdhB* | Metabolism | -1.118747887 | 2.82749E-14 |
| A0A0H3PBB6 | *trpE* | Metabolism | -1.101618852 | 8.21177E-07 |
| A1W062 | *fliW* | Motility | -1.171004942 | 0.110800187 |
| A1W1J3 | *rpmJ* | Protein synthesis | -4.445303331 | 0.0005841 |
| A0A0H3PBB3 | *rbfA* | Protein synthesis | -1.417005111 | 0.109312767 |
| A1VXH9 | *obg* | Protein synthesis | -1.381048955 | 0.008940218 |
| A0A0H3PDV7 | *selB* | Protein synthesis | -1.062590486 | 0.068775623 |
| A1VZ20 | *era* | Protein synthesis | -1.018442569 | 0.018310119 |
| A0A0H3PAU2 | *CJJ81176_1538* | Stress Response | -1.256788113 | 2.78386E-06 |
| Q3I354 | *luxS* | Stress Response | -1.25277018 | 0.088147969 |
| A0A0H3P9V7 | *CJJ81176_1101* | Stress Response | -1.099808664 | 6.07096E-06 |
| A0A0H3PE81 | *CiaC* | Stress Response | -1.090438163 | 0.019756288 |
| A0A0H3PA60 | *dcuA* | Transport | -1.3731478 | 1.48649E-08 |
| Q0Q7H5 | *CJJ81176_1574* | Transport | -1.123500175 | 4.66337E-08 |
| A0A0H3PBF3 | *CJJ81176_1241* | Two-component regulatory system | -1.1594126 | 2.09953E-09 |
| A0A0H3PH34 | *CJJ81176_1055* | Uncharacterized | -2.489932867 | 0.003187697 |
| A0A0H3PAF3 | *CJJ81176_0231* | Uncharacterized | -1.769249606 | 9.72991E-09 |
| Q8GJE8 | *Cjp04* | Uncharacterized | -1.733102229 | 0.019927421 |

| | | | | |
|---|---|---|---|---|
| A0A0H3PAL1 | *CJJ81176_1102* | Uncharacterized | -1.651613239 | 1.28592E-09 |
| A0A0H3PJA2 | *CJJ81176_0520* | Uncharacterized | -1.569143913 | 7.56635E-05 |
| A0A0H3PHG6 | *CJJ81176_0854* | Uncharacterized | -1.506690926 | 0.002479818 |
| A0A0H3PJ75 | *CJJ81176_0306* | Uncharacterized | -1.500720015 | 0.000493759 |
| Q2M5R0 | *CJJ81176_1341* | Uncharacterized | -1.256468072 | 6.02718E-10 |
| A0A0H3PIC7 | *CJJ81176_1509* | Uncharacterized | -1.256350259 | 0.009843407 |
| A0A0H3PIW6 | *CJJ81176_0547* | Uncharacterized | -1.159016383 | 0.001202373 |
| A0A0H3PJB0 | *CJJ81176_0403* | Uncharacterized | -1.144908063 | 1.64597E-06 |
| A0A0H3P9V0 | *CJJ81176_1433* | Uncharacterized | -1.135152327 | 0.059317961 |
| A0A0H3PGW3 | *CJJ81176_1177* | Uncharacterized | -1.132828646 | 0.014311253 |
| A0A0H3P9D8 | *CJJ81176_1104* | Uncharacterized | -1.074878404 | 5.55413E-05 |
| A0A0H3PEW6 | *CJJ81176_0447* | Uncharacterized | -1.069469976 | 6.49657E-12 |
| Q2M5Q9 | *CJJ81176_1315* | Uncharacterized | -1.014281314 | 5.72494E-07 |
| Q2M5Q0 | *CJJ81176_1314* | Uncharacterized | -1.013555098 | 0.172609895 |
| | | | | |
| | **Significantly upregulated** | | | |
| **UniProt_Accession** | **Gene Name** | **Protein function** | **logFC** | **P.Value** |
| A0A0H3PEV8 | *pbpA* | Cell cycle, cell division | 1.086928089 | 0.051214493 |
| A0A0H3P9T7 | *cj81176_1498* | Chemotaxis | 1.003934051 | 2.88928E-06 |
| A0A0H3PEL1 | *cjj81176_0289* | Chemotaxis | 1.864654961 | 2.43E-06 |
| A0A0H3P9E8 | *petC* | Metabolism | 1.003628931 | 1.73188E-05 |
| A0A0H3PD90 | *purE* | Metabolism | 1.003687748 | 0.00203151 |
| A0A0H3PDM3 | *sdaC* | Metabolism | 1.034201918 | 2.30004E-06 |
| A1W0X7 | *ppk* | Metabolism | 1.045897542 | 2.08733E-06 |
| A0A0H3PI37 | *nuoC* | Metabolism | 1.076929531 | 0.000801471 |
| A0A0H3PCI0 | *cjj81176* | Metabolism | 1.090934978 | 1.50056E-08 |
| A0A0H3PAC1 | *nuoG* | Metabolism | 1.109217101 | 2.27597E-11 |
| A0A0H3PB89 | *CJJ81176_1237* | Metabolism | 1.10969296 | 0.023202938 |
| A0A0H3PDD6 | *CJJ81176_0814* | Metabolism | 1.158767957 | 0.008647741 |
| A0A0H3P9R4 | *sdaA* | Metabolism | 1.200300402 | 5.49352E-08 |
| A0A0H3P9Q2 | *nrfA* | Metabolism | 1.22993766 | 2.56694E-10 |
| A0A0H3PEJ9 | *frdC* | Metabolism | 1.243172386 | 0.001825513 |
| A0A0H3PEX3 | *CJJ81176_0544* | Metabolism | 1.311352748 | 0.017042922 |
| A0A0H3PAQ1 | *CJJ81176_0849* | Metabolism | 1.319366315 | 0.022040424 |
| A0A0H3PHB9 | *petA* | Metabolism | 1.48190009 | 3.98065E-10 |
| A0A0H3PI21 | *nrfH* | Metabolism | 1.691280791 | 0.000420962 |
| A0A0H3PA38 | *cydA* | Metabolism | 1.729442395 | 0.004420028 |
| A0A0H3PIF6 | *fliL* | Motility | 1.050277067 | 6.1643E-07 |
| A1W1U3 | *rpsE* | Protein synthesis | 1.032367963 | 0.000292876 |
| A1VY90 | *rpsU* | Protein synthesis | 1.16168108 | 5.90784E-10 |
| A1VYI7 | *rpmG* | Protein synthesis | 1.239822446 | 0.002556072 |
| A1VXH8 | *rpmA* | Protein synthesis | 1.635338757 | 4.07831E-11 |
| A1W1L3 | *rpsT* | Protein synthesis | 1.756996902 | 0.001599569 |
| A1W1V8 | *rplW* | Protein synthesis | 1.859007364 | 0.000136365 |
| A0A0H3PBW9 | *clpB* | Stress Response | 1.009828881 | 1.23669E-11 |
| A0A0H3PB76 | *dnaJ-1* | Stress Response | 1.019217245 | 0.020208818 |
| A1W0K4 | *groL* | Stress Response | 1.029285959 | 2.63301E-15 |
| A1W0P5 | *dnaJ* | Stress Response | 1.094490901 | 2.39007E-09 |
| Q0Q7K7 | *dnaK* | Stress Response | 1.120778286 | 2.11638E-18 |
| A0A0H3P9Z0 | *CJJ81176_pTet0010* | Stress Response | 1.146456678 | 0.000167558 |
| A0A0H3PA35 | *dsbA* | Stress Response | 1.208240518 | 0.00243096 |
| Q0Q7K8 | *grpE* | Stress Response | 1.429643573 | 5.60992E-13 |
| A0A0H3PDE7 | *CJJ81176_0897* | Transport | 1.118483048 | 0.000949373 |
| A0A0H3PAQ2 | *CJJ81176_0494* | Transport | 1.184647717 | 0.02614528 |
| A0A0H3P9L8 | *atpF* | Transport | 1.299150728 | 0.034367151 |
| A0A0H3PAY0 | *tatB* | Transport | 1.369143595 | 0.001863966 |
| A0A0H3PB37 | *CJJ81176_1244* | Two-component | 1.327856279 | 0.026858687 |

| | | | | |
|---|---|---|---|---|
| | | regulatory system | | |
| A0A0H3PBU4 | *CJJ81176_0392* | Uncharacterized | 1.292671478 | 0.00123255 |
| A0A0H3PJB3 | *CJJ81176_0263* | Uncharacterized | 1.306677978 | 0.007967564 |
| A0A0H3PH47 | *CJJ81176_1185* | Uncharacterized | 1.332043969 | 1.10882E-12 |
| A0A0H3P9S8 | *CJJ81176_1184* | Uncharacterized | 1.430281836 | 5.58088E-10 |
| A0A0H3PEX7 | *CJJ81176_0438* | Uncharacterized | 1.502025847 | 0.002799808 |
| A0A0H3PEW9 | *CJJ81176_0659* | Uncharacterized | 1.605057927 | 0.000123031 |
| A0A0H3PCF8 | *CJJ81176_0975* | Uncharacterized | 1.660548853 | 0.006829101 |
| A0A0H3PAH4 | *CJJ81176_0565* | Uncharacterized | 1.743396073 | 0.011199139 |
| Q8GJC5 | *Cjp29* | Uncharacterized | 2.086928658 | 3.54031E-05 |
| A0A0H3PBB0 | *CJJ81176_1666* | Uncharacterized | 2.277171619 | 0.011192803 |

**Appendix 8:** Significantly differentiated communal adaptation proteins between 81-176 cultured in CDM supplemented with CA 0.1%, DCA 0.05%, LCA 0.5%, TCA 0.5%, CDCA 0.05%, UDCA 0.5% and GCA 0.4% cultured at 37$^{o}$C for 12h and 42$^{o}$C for 24h

| **Uniprot code** | **Gene name** | **Protein function** |
|---|---|---|
| A0A0H3PDA2 | ftsZ | Cell division |
| A1W043 | murC | Cell structure |
| A1W0A5 | cheY | Chemotaxis |
| A0A0H3P989 | recJ | DNA recombination |
| A0A0H3PED7 | CJJ81176_0477 | DNA replication |
| A0A0H3PAM5 | CJJ81176_0297 | Metabolism |
| A1W035 | thiG | Metabolism |
| A0A0H3PHF5 | CJJ81176_0907 | Metabolism |
| A1VZR0 | apt | Metabolism |
| A0A0H3P9J6 | pta | Metabolism |
| A0A0H3PB78 | CJJ81176_1414 | Metabolism |
| Q29VH0 | kpsF | Metabolism |
| Q2M5Q2 | pseF | Metabolism |
| A0A0H3P9K9 | CJJ81176_0850 | Metabolism |
| A0A0H3PC31 | hom | Metabolism |
| A1W0I0 | gpsA | Metabolism |
| A0A0H3PDU5 | tyrS | Metabolism |
| A0A0H3PAH1 | tyrA | Metabolism |
| A0A0H3PBK5 | purS | Metabolism |
| A0A0H3P9B2 | thiH | Metabolism |
| A0A0H3PA59 | CJJ81176_1259 | Metabolism |
| A1W0W6 | mobA | Metabolism |
| A1VY40 | dxs | Metabolism |
| A0A0H3P9K8 | CJJ81176_0111 | Metabolism |
| A0A0H3PB85 | CJJ81176_0254 | Metabolism |
| A0A0H3PI81 | gltA | Metabolism |
| A0A0H3PA64 | ggt | Metabolism |
| A0A0H3PGR5 | CJJ81176_0063 | Metabolism |
| A1VY31 | pth | Metabolism |
| Q0Q7I1 | purM | Metabolism |
| A1VYU1 | rppH | Metabolism |
| A0A0H3PD33 | sixA | Metabolism |
| A0A0H3PH94 | gmk | Metabolism |
| A1VYM4 | purC | Metabolism |
| A1VYQ4 | hemC | Metabolism |
| A1W1K3 | hisA | Metabolism |
| A0A0H3PAJ4 | hisI | Metabolism |
| A1W1W9 | leuD | Metabolism |
| A0A0H3P9R4 | sdaA | Metabolism |

| | | |
|---|---|---|
| A1VYA9 | serS | Metabolism |
| A0A0H3PA78 | fliY | Motility |
| A0A0H3PBL4 | hypE | Protein synthesis |
| A0A0H3PB64 | trpS | Protein synthesis |
| A0A0H3PAZ6 | hypB | Protein synthesis |
| A0A0H3PDX5 | rnc | Protein synthesis |
| A1W0R3 | trmB | Protein synthesis |
| Q0Q7K8 | grpE | Stress Response |
| Q0Q7K2 | CJJ81176_0780 | Stress Response |
| A0A0H3PBW9 | clpB | Stress Response |
| A1VXQ2 | sodB | Stress Response |
| A0A0H3PED0 | CJJ81176_0391 | Transcription, Transcription regulation |
| A0A0H3PDE7 | CJJ81176_0897 | Transport |
| A0A0H3PF18 | CJJ81176_0446 | Transport |
| A0A0H3P9J7 | CJJ81176_0137 | Transport |
| A0A0H3PEX7 | | Uncharacterized |
| A0A0H3PB55 | CJJ81176_0474 | Uncharacterized |
| A0A0H3P9T3 | CJJ81176_1422 | Uncharacterized |
| A0A0H3PAF1 | CJJ81176_1363 | Uncharacterized |
| A0A0H3PDG2 | CJJ81176_0891 | Uncharacterized |
| A0A0H3P991 | CJJ81176_0018 | Uncharacterized |
| A0A0H3PAW5 | CJJ81176_1624 | Uncharacterized |
| A0A0H3PC13 | CJJ81176_0374 | Uncharacterized |
| A0A0H3PEW9 | CJJ81176_0659 | Uncharacterized |
| A0A0H3PDS7 | CJJ81176_1355 | Uncharacterized |
| A0A0H3PEL5 | CJJ81176_0280 | Uncharacterized |
| Q8GJC5 | Cjp29 | Uncharacterized |
| A0A0H3P9M1 | napD | Uncharacterized |
| A0A0H3PDW4 | CJJ81176_1424 | Uncharacterized |
| A0A0H3PA08 | CJJ81176_0742 | Uncharacterized |
| A0A0H3PBF4 | CJJ81176_0342 | Uncharacterized |
| Q0Q7K3 | CJJ81176_0779 | Uncharacterized |
| A0A0H3PB96 | CJJ81176_0611 | Uncharacterized |
| A1W0U8 | hisF2 | Uncharacterized |
| A0A0H3PAA1 | CJJ81176_1497 | Uncharacterized |
| A0A0H3P9U1 | CJJ81176_1487 | Uncharacterized |
| A0A0H3P986 | | Uncharacterized |
| A0A0H3P9Z0 | CJJ81176_pTet0010 | Uncharacterized |
| A0A0H3P9L3 | CJJ81176_0728 | Uncharacterized |
| A0A0H3PAC3 | CJJ81176_1161 | Uncharacterized |
| A0A0H3P9G9 | Cpp35 | Uncharacterized |
| A0A0H3PD80 | CJJ81176_0830 | Uncharacterized |
| A0A0H3P9N6 | CJJ81176_0110 | Uncharacterized |
| A0A0H3P973 | CJJ81176_pTet0021 | Uncharacterized |
| A0A0H3P9B0 | CJJ81176_0135 | Uncharacterized |
| A0A0H3PIZ2 | CJJ81176_0601 | Uncharacterized |
| A0A0H3PJ41 | CJJ81176_0671 | Uncharacterized |
| A0A0H3PBB8 | CJJ81176_0472 | Uncharacterized |
| Q6QNL7 | CJJ81176_1356 | Uncharacterized |
| A0A0H3P9J3 | CJJ81176_0988 | Uncharacterized |
| A0A0H3PHU2 | CJJ81176_1517 | Uncharacterized |
| A0A0H3P9B6 | thiF | Uncharacterized |
| A0A0H3P9A5 | CJJ81176_0112 | Uncharacterized |