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MARMARA UNIVERSITY

INSTITUTE FOR GRADUATE STUDIES IN

PURE AND APPLIED SCIENCES

EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF BERBERINE

Kübra KARAOSMANOĞLU

THESIS

FOR THE DEGREE OF MASTER OF SCIENCE

IN

BIOENGINEERING

SUPERVISOR

Assoc. Prof. Dr. Berna SARIYAR AKBULUT

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BERBERİNİN ANTİMİKROBİYAL AKTİVİTESİNİN DEĞERLENDİRİLMESİ

Antimikrobiyal ilaçların hastanelerde ve tarımsal alanlarda yaygın olarak kullanımı, bakterilerin mevcut ilaçlara karşı direnç göstermelerine neden olmaktadır. Bu durum, sağlık sektöründe önemli bir problem teşkil etmektedir. Doğal kaynaklar olarak bitkiler ilaca dirençli olan mikroorganizmalara karşı savaşacak yeni ilaçlar için sayısız olanaklar sunmaktadırlar. Bitki kaynaklı berberin, suda çözünebilen birkaç alkoloidden biridir ve birçok bakteri ve mantara karşı antimikrobiyal etksini gösterdiği bilinmektedir. Bu çalışmada, berberinin *Escherichia coli* K12'ye olan etkisi transkriptomik ve proteomik düzeyde araştırılmıştır. Büyüme ortamındaki berberinin konsantrasyonu 750mg/L olarak seçilmiştir. Ilaç varlığında ve yokluğundaki büyüme profilleri, büyümedeki farklılığın 4. saatte başladığını göstermiş ve buna bağlı olarak RNA ve proteinler inokülasyondan 4,5 saat sonra ekstrakte edilmiştir. Transkriptom çalışmalarında, hücrelerin gen ekspresyon profilleri mikroarray teknolojisi kullanılarak belirlenmiştir. Mikroarray sonuçlarını berberin varlığında ekspresyonu azalan veya artan genlerin sayılarına göre değerlendirdiğimizde; katabolik proses, membran proteinleri ve taşınım proteinleri ile ilgili ekspresyonu artan genlerin sayısında önemli ölçüde artış, biyosentez mekanizması ve nükleik asit biyosentezi ile ilgili ekspresyonu azalan genlerin sayısında önemli ölçüde düşüş gözlemlenmiştir. Proteomik analizlerde, protein ekspresyon farklılıkları iki boyutlu jel elektroforez tekniği kullanılarak belirlenmiştir. 15 protein, LC-MS/MS ile analiz edilmek üzere seçilmiştir. Sonuçlar, berberinin metabolik yolaklarda, hücre bölünmesinde, taşınma sistemlerinde, glikolizde, ve hücre adhezyonunda yer alan proteinlerin ekspresyonlarını değiştirdiğini göstermiştir. Elektron taşınma sisteminde, protein biyosentezinde ve trikarboksilik asit döngüsündeki proteinlerin ekspresyonu artarken, nmpC, ompC ve MALE gibi membran proteinlerinin ekspresyonunda dikkate değer bir düşüş gözlemlenmiştir. Bu sonuçlar, ilaç adaylarının moleküler mekanizmalarının anlaşılması ve hedefe yönelik ilaç tasarımında gelecekteki çalışmalar için önemli birikim oluşturacaktır.

Haziran, 2012 **Kübra Karaosmanoğlu**

ABSTRACT

EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF BERBERINE

The extensive use of antimicrobial drugs in clinical and agricultural settings causes bacteria to acquire resistance against the commonly used antibiotics. This constitutes an important problem in health sector. Plants as natural products serve countless opportunities for new drugs to counter multi-drug resistance microorganisms. Plant-derived extract berberine is one of the few alkaloids soluble in water, and known to exhibit antimicrobial activity against several types of bacteria and fungi. In this work, the effect of berberine on *Escherichia coli* K12 was screened at both transcriptomic and proteomic level. The concentration of berberine in growth media was determined as 750mg/L. The analysis of growth profiles in the presence and absence of the drug candidate has shown that difference in growth started to be noticeable 4^{th} hour of growth, hence RNAs and proteins were extracted 4,5 hours after inoculation. In transcriptomic approach, gene expression profiles of cells were determined using microarray technology. As we evaluated microarray results according to number of genes up/down-regulated in the presence of berberine, we have seen a significant increase in the number of up-regulated genes related to catabolic processes, membrane proteins and transporter proteins, and a significant decrease in the number of down-regulated genes related to biosynthesis and nucleic acid metabolism. In proteomic analysis, 2-dimensional gel electrophoresis technique was used to find protein expression differences. 15 protein spots were selected to be analyzed with LC-MS/MS. The results have shown that berberine altered the expression of proteins take place in metabolic pathways, cell division, transport systems, glycolysis, and cell adhesion. There is a significant down- regulation in the expression of membrane proteins like nmpC, ompC and MALE, while the expression of proteins take place in electron tranport system, protein biosynthesis and tricarboxylic acid cycle were up-regulated. These results will provide valuable information to understand the molecular mechanism of the action of the drug candidates and to design new-target based drug candidates for future studies.

June,2012 **Kübra Karaosmanoğlu**

LIST OF SYMBOLS/ ABBREVIATIONS

1-D	**:** First Dimension
2-D	**:** Second Dimension
2-DE	**:** Two-Dimensional electrophoresis
APS	**:** Ammoniumpersulphate
aRNA	**:** antisenseRNA
BSA	**:** Bovine serum albumin
CBB-G250	**:** Coomassie brilitant blue G-250
CBB-R250	**:** Coomassie brilitant blue R- 250
CHAPS	**:** 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
Da	**:** Dalton
DMSO	**:** Dimethyl sulfoxide
DNA	**:** Deoxyribonucleic acid
DTT	**:** 1,4 dithiothreitiol
ESI-MS	**:** Electrospray ionization mass spectrometry
HPLC	**:** High performance liquid chromatography
IEF	**:** Isoelectric focusing
IPG	**:** Immobolized pH gradient
kDA	**:** Kilodalton
LC-MS/MS	**:** Liquid chromatography tandem mass spectrpmetry
mA	**:** miliAmpere
MALDI-MS	**:** Matrix-assisted laser desorption ionization mass spectrometry
MDR	**:** Multi-drug Resistance
mM	**:** Milimolar
mRNA	**:** Messenger ribonucleic acid
Mr	**:** Relative molecularmass
MS/MS	**:** Tandem Mass spectrometry
MW	**:** Molecular weight
m/z	**:**mass-to-charge ratio

NEpHGE	**:** Non-equilibrium pH gradient
nm	**:** Nanometer
OD	**:** Optical density
pI	**:** Isoelectric point
PMF	**:** Peptide mass finger printing
PMSF	**:** Phenylmethhylsulphonyl fluoride
Q-PCR	**:** Quantitative-Polimerase Chain Reaction
RMA	**:** Robust multi-array average
SDS	**:** Sodium dodecyl sulfate
SDS-PAGE	**:** Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TFA	**:** Trifluoroacetic acid
TOF	**:** Time of flight
UV	**:** Ultraviolet

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CHAPTER I

INTRODUCTION and AIM

Antimicrobial drugs used in health sector stand against various infectious diseases for centuries. However, the overuse and misuse of these drugs result in evolving bacterial resistance to these drugs which constitutes a significant menace to public health (Scholar et al., 2000). To fight with this major health problem, scientists focused on new sources with antimicrobial activities in addition to careful use of existing antimicrobials (Ozbalci et al., 2010). Unfortunetly, as scientists introduce new drug candidates to the market, bacteria develop new strategies to strengthen themselves. In this respect, the need to find novel sources that produce antimicrobial agents is getting increasingly important.

For decades, plants have been widely used to treat or prevent epidemic diseases by traditional healers mainly because plants synthesize new and natural bioactive molecules including tannins, terpenoids, flavanoids and alkaloids with *in-vitro* antimicrobial activities (Cowan 1999; Ngwendson et al., 2003; Klausmayer et al., 2004; Nita et al., 2002; Velickovich et al., 2003; Sakagami et al., 2001). This places plants as one of the main sources of new antimicrobial agents (McChesney et al., 2007). Plant-derived alkaloids are low molecular weight and nitrogen containing compounds that represent the largest group of bioactive molecules in the plant kingdom (Singla, 2010). Plants of the genus *Papaver* have known to produce a wide range of different alkaloids (Sarıyar 2002; Ünsal et al. 2007, 2009). The use of plant alkaloids for medicinal purposes has been practised for many years (Hanson, 2005; van Wyk and Wink, 2005). Morphine is the first discovered alkaloid used in medicine in treatment of both acute and chronic pain by myocardial infarction (Cowan, 1999). Both codeine and heroin are produced from morphine. Sanguinarine is another widespread alkaloid found in the family of *Papaveraceae*. It is reported to have antibacterial, cytotoxic and anti-inflammatory effect (Hoffman, 2003). Sanguinarine is a quaternary alkoloid compound as berberine. Berberine

demonstrates antimicrobial activity towards to various bacteria and fungi and its antimicrobial activity has been attributed to its specificity for the minor groove of AT-rich duplexes in DNA sequences (Saran et al. 1995; Choi et al. 2001; Sriwilaijareon et al. 2002; Mazzini et al. 2003;Chen et al. 2004; Qin et al. 2006). In addition, this alkaloid shows low toxicity to host cells in the *in-vitro* and *in-vivo* experiments (Burbaum and Tobal, 2002). Hence, berberine is a potent candidate for the development of new antimicrobial agents.

Upon exposure of bacteria to antimicrobial alkaloids, crucial changes takes place within cells can be anticipated depending on the mode of the action of these compounds. Therefore, clarification of the mechanism of the antimicrobial alkaloids is very important (Bandow et al., 2003). When a novel compound class stands out with its antibacterial activity, the molecular target of the compound has to be defined to estimate the unexpected side effects on the host organism (identification of the target). Moreover, for antimicrobials, which are structurally modified, or which are compounds derived from the target-based assays, it is important to confirm that the interaction with the cellular target is indeed the direct cause for bacterial cell death (validation of target) (Bandow, 2003). The analysis of all genes and proteins in a given genome and proteome by genomic and proteomic technologies opens up the possibility of providing details on the molecular mechanism (Wang et al., 2009). Transcriptional profiling enables the visualization of the whole genome under the stress of the antimicrobial agents (Tsiridis et al., 2006; González et al., 2008). Unfortunately, transcript levels detected in mRNA profiling clearly do not reflect all regulatory processes in the cell because post-transcriptional processing altering the amount of active-proteins are not considered (Silvestri et al., 2011). For this reason, proteomic tools should be complemented with transcriptomic tools to fully elucidate the regulation of biological functions (Freiberg et al., 2004; Kitchen et al., 2004). Only then would it be possible to develop or propose novel effective pro-drug compounds.

In the light of the information mentioned above, the aim of this thesis is to evaluate the molecular response of *Escherichia coli* K12 exposed to the berberine alkaloid at the gene, and protein levels. To this end, mRNAs and proteins were extracted from the cells grown in LB medium in the presence/absence of berberine at the predetermined times. With this study, the effect of berberine on *Escherichia coli*

K12 cells were evaluated integrating transcriptomic and proteomic profiles. In the evaluation of proteomic profiles of cells, 15 proteins were selected as either down/ up-regulated. Analysis of the gene expression profiles of the cells showed that, 330 genes were up-regulated and 193 genes were down-regulated when classified based on their biological processes. Integration of proteomic and transcriptomic results of this study has enabled us to elucidate the main targets of berberine and the blocked metabolic pathways. The results of this study will aid to propose new genes and proteins involved in bacterial resistance mechanism of *E. coli* K12.

CHAPTER II

GENERAL BACKGROUND

II.1. BACTERIAL RESISTANCE

Organisms have the ability to sustain their lives by their capability of sense and the response to environmental changes. Complex organisms can perceive sudden environmental changes as a danger and respond these stress conditions in a way regulated by numerous organ systems (Boor, 2006). For bacteria, stress can be defined as a deviation from optimal growth conditions that result in a reduced growth rate. Changes in temperature, pressure, osmolarity, pH can be perceived as stress conditions by microorganisms. Response to a stress condition can be in different levels of severity. Under stress conditions, microorganisms have developed signal transduction systems (movement of signals from outside the cell to inside) to sense environmental stresses and to control the coordinated expression of genes involved in cellular defense mechanisms (Kennelly and Potts, 1996). These evolved protective or adaptive networks assist microorganisms to modify their environments and/or to survive under the stress condition. If microorganisms are under little stress, cells can fully adapt to their new environment and keep on surviving in the same growth rate. Under severe stress, cells can endure the environmental changes but the growth rate of cells will decrease. Under extreme stress, cells limit their functions and use resources only to survive (Storz and Hengge, 2000).

II.1.1. Bacterial Resistance to Antimicrobial Agents

Antibiotic resistance can be defined as a case of decreased sensitivity to drugs that used to cause bacterial growth inhibition or cell death. Infectious bacteria are significantly resistant and have developed several ways to withstand antimicrobial drugs. As a result of widespread use of antibiotics in health sector, animal feeds and agriculture, the treatment of bacterial infections is getting increasingly difficult.

Evolution of bacteria against resistance to antimicrobial drugs, including multidrug resistance, is unavoidable since the general evolution of bacteria is

unstoppable. Therefore, to deal with this situation, emergence and subsequent dissemination of resistant bacteria or resistance genes have to be delayed. Resistance to antimicrobial drugs in bacteria can end up with mutations in structural or regulatory genes (vertical evolution). Alternatively, resistance can end up with the horizontal acquisition of foreign genetic information in same species or different species or genera (horizantal evolution). Both these events can be associated to the emergence and more effective spread of resistance (Courvalin, 2005).

Antimicrobial agents can perform their work in different ways according to their mechanism of action. These ways can be separated into 4 groups: (1) interference with cell wall synthesis, (2) inhibition of protein synthesis, (3) interference with nucleic acid synthesis, and (4) inhibition of a metabolic pathway (Neu, 1992). Therefore, bacteria develop different mechanisms to fight with antimicrobial agents before or after the antimicrobial agent enter the cell (McManus, 1997). Figure 1 depicts how bacteria resist antibiotics.

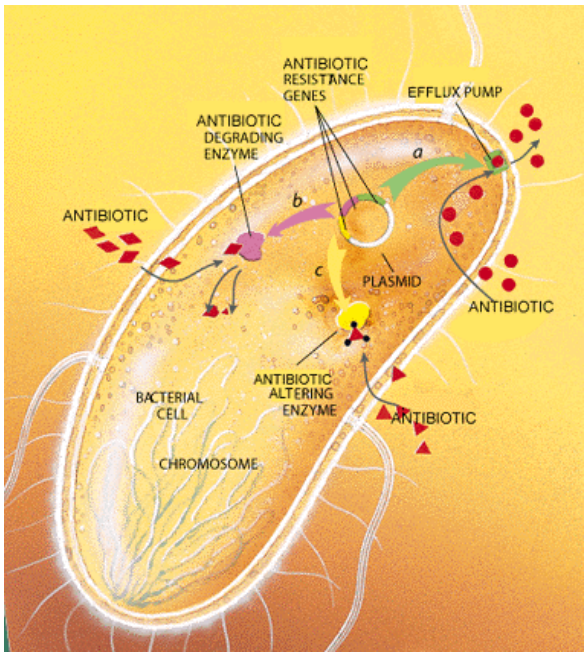


Figure 1: *Shematic Representation of Bacterial Resistance (Anne Cohen, 2011)*

Bacteria can upregulate the genes encoding enzymes, such as β-lactamases, to destroy the antibacterial agent before it reaches the bacterial target and have an effect on bacteria. By up-regulating outer membrane protein channels, efflux pumps that can throw out the antibiotic from the cell wall, bacteria can avoid from antibiotic

agent before it reaches its target site and perform its duty. There are also mutations that limit the access of antibiotics into the intracellular target by down-regulating porins on outer membrane protein channel. Bacteria can produce new genes to alter the target protein and preclude the binding of the antibiotic agent by modifying or eliminating the binding site on the cell wall (Tenover, 2006).

II.2. ESCHERICHIA COLI

Escherichia coli was first discovered in 1885 by Theodor Escherich, a German pediatrician, in the faeces of healthy individuals. Escherich called it *Bacterium coli* commune according to the fact that it is found in the colon and early classifications of Prokaryotes placed these in a handful of genera due to their shape and motility (Escherich, 1885). *E. coli* is a rod-shaped gram-negative, facultative anaerobic and non-sporulating bacteria belonging to Enterobacteriaciae family and commonly found in the lower intestine of endotherms. Most of the *E.coli* strains are non-pathogenic, but some serotypes are pathogenic and can cause food poisoning in humans. Cells are typically rod-shaped, and are about 2.0 microns (μm) long and 0.5 μm in diameter, with a cell volume of 0.6 – 0.7 $(\mu m)^3$ (Kubitschek, 1990).

Optimal growth of *E. coli* is at 37° C, but as an exception some laboratory strains can multiply at temperatures of up to 49° C (Fotadar et al., 2005). Growth can be sustained by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and the reduction of substrates such as oxygen, nitrate, fumarate, dimethyl sulfoxide and trimethylamine N-oxide (Ingledew and Poole, 1984).

E. coli encompasses an enormous population of bacteria that shows a very high degree of both genetic and phenotypic diversity. *E. coli* is one of the most diverse microorganism in bacterial species since it has only 20% of the genome is common to all strains (Lukjancenko et. al., 2010). *E. coli* is frequently used as a model organism in microbiology, as a host organism for the majority of work with recombinant DNA, biotechnology and genetics studies and also the most studied organism since it can live on a wide variety of substrates and has rapid growth rate. Cultivated strains (e.g. *E. coli* K12) are well-adapted to the laboratory environment, and, unlike wild type strains, have lost their ability to grow in the intestine. Many lab strains have lost their ability to form biofilms. In 1946, Joshua Lederberg and Edward Tatum first described the phenomenon known as bacterial

conjugation using E. coli as a model bacterium (Lederberg and Tatum, 1946), and it is still in use to study conjugation.

E. coli cells undergo a transition from a rapid growth phase to a stationary phase, which is accompanied by a variety of physiological changes that affect gene expression, the structure and composition of the cell wall, DNA organization, synthesis of storage compounds such as glycogen and polyphosphate, and other cellular processes (Bochkareva et al., 2002). As a result of these changes in different metabolic pathways, the cells become resistant to various toxical stresses such as heat shock (Jenkins et al.,1988), UV irradiation, acidic or basic conditions (Small et al., 1994), osmotic shock, and oxidation .

II.3. PLANT EXTRACTS

For decades people have taken the advantage of healing powers of plants to treat common infectious diseases. Some parts of these traditional medicines are stil used in the habitual treatment of various maladies (R´ıos et al.,2005). The use of plant extracts, as well as other derivatives, has gained great popularity in the late 1990s (Cowan, 1999).

Plants have limitless ability to synthesize aromatic substances, phenols or their oxygen-substituted derivatives (Geissman, 1963). Most of these substances serve as plant defense mechanisms against infection by microorganisms, insects, and herbivores. Plant extracts are rich in secondary metabolites which can be divided into several categories: phenolics and polyphenols, quinones, flavones, flavonoids, and flavonols, tannins, coumarins, terpenoids and essential oils, alkaloids, lectins and polypeptides (Cowan, 1999). Alkoloids are important plant-derived substances to cope with pathogenic microorganisms, therefore the investigation of their mechanism of action is an interesting research field to propose novel durg leads.

II.3.1. Plant Alkoloids, Berberine

An alkaloid is a cyclic compound containing nitrogen in a negative oxidation state which is of limited distribution in living organisms (Pelletier, 1999). Berberine is a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids and an important representative of the alkaloid group, found in *Coptidis rhizoma*, Berberis, some Papaver Species, *Coptis chinensis* (Burbaum and Tobal, 2002). Due to its quaternary structure, it can dissolve in water. The structure of berberine is given in Figure 2.

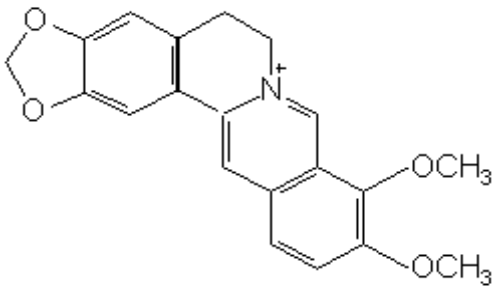


Figure2: *Structure of Berberine (PubChem)*

Extracts containing berberine have shown significant antimicrobial activity against several types of bacteria and fungi. This alkoloid is known to intercalate into DNA molecules however, if evaluated by a global analysis, the effect of berberine will be expected to be of different complexities (Burbaum and Tobal, 2002).

The antimicrobial activity of berberine alkaloid is depends on the type of the organism (Burbaum and Tobal, 2002). Bacteria such as *Staphylococcal*, *Streptococcal*, and *Enterococcal* species are vulnerable against berberine alkaloid. In addition, this alkaloid is used clinically to cure acute diarrhea caused by different pathogenic bacteria including *E. coli* (Wongbutdee, 2008). Ozbalci et al. (2010) have demonstrated that the minimum inhibitory concentration (MIC) of berberine containing *Papaver polychaetum* extract towards to *E. coli* was 1250 µg/ml. It is also reported that berberine alkaloid inhibits the growth of yeasts, such as *Candida* sp. (Seneviratne et al., 2007). The antimicrobial activity of this alkaloid has widely been imputed to its specificity for the DNA molecule (Burbaum and Tobal, 2002).

The toxicity and mutagenicity of berberine to host cells were relatively low in *in-vivo* and *in-vitro* experiments. The pharmacologic actions of this alkaloid was also investigated. The results indicated that berberine plays a major role on the metabolic inhibition of certain organisms (Kaneda et al., 1990, 1991), and inhibition of bacterial enterotoxin formation (Birdsall and Kelly, 1997). The more recently published report on the berberine alkaloid is focused on its antidiabetic properties. In that work, they have reported that berberine has an impact on the glucose metabolism (Cicero and Tartagani, 2012).

The reported studies so far have shown that berberine alkaloid has extreme importance on the health sector. Since the infections caused by the drug resistant bacteria comprise a global health threat, the investigation of the mechanism of action of antimicrobials such as berberine is gaining increasing importance. If evaluated by a global analysis, the effect of berberine will be anticipated to be of different complexities (Burbaum and Tobal, 2002). Integration of transcriptome and proteome analysis of bacteria exposed to berberine alkaloid will give important clues on the mode of action of this alkaloid.

II.4. PROTEOMICS

Proteomics is the large-scale study of structures and functions of proteins. The term "proteome" was coined to describe the complete set of proteins that an organism has produced under a defined set of conditions or life stages of a cell or organism (James, 1997). Since proteins are the main components of the physiological metabolic pathways of cells, they are vital parts of living organisms. One organism has basically different protein expression in different parts of its body, in different terms of its life and in different environmental conditions. Since the large increase in protein diversity is thought to be increasing due to alternative splicing and post-translational modification of proteins, protein diversity cannot be fully characterized by gene expression analysis alone. This makes proteomics a useful tool for characterizing cells of interest (Abhilash, 2009).

Proteomics facilitates a broad view on the cells' physiological state and, in combination with radioactive pulse-labeling, allows us to study the cellular response to any changes in growth conditions (VanBogelen, 1999). One of the areas where proteomic approaches find applications is the study of the responses of bacteria to antibacterial compounds.

II.4.1. Proteomics in Drug Discovery

Treating a bacterium with an antibiotic from an established class with well-understood mechanism of action can help us to get valuable insights about the physiological consequence of an impaired metabolic function or pathway. However, searching for novel antibacterial agents can lead the way to find more direct applications of proteomics in antibacterial drug discovery (Brötz, 2004).

Under antibiotic pressure, bacteria have developed various protective mechanisms such as additional barriers for antibiotic penetration, active pump

systems to extrude the drug from intracellular compartment, enzymatic modification of the drug to make it ineffective, and mutation of the molecular targets to inhibit the interaction between the drug and molecular targets (Walsh, 2003). Bacteria develop mechanisms to block these functions in order to survive.

Most of the few available studies of antibacterial discovery in which protemics was performed generally focus on either target validation or mode of action studies, including those studies that aim at a better molecular understanding of the mechanisms of action of existing drugs (Gray & Keck, 1999; Apfel et al., 2001; Evers et al., 2001; Gmuender et al., 2001; Singh, Jayaswal,&Wilkinson, 2001; Bandow et al., 2003; Ng et al., 2003).

II.4.2. Methods in Bacterial Proteome

Proteomics use various technologies like two-dimensional gel electrophoresis, mass spectrometry, protein arrays, isotope-encoding, two-hybrid systems, information technology and activity-based assays. These technologies, as part of the current proteomics techniques, are advancing the utility of proteomics in the drug-discovery process (Burbaum and Tobal, 2002).

II.4.2.1. Protein Extraction

Protein extraction methods can vary extensively in reproducibility and in representation of the total proteome. Since the methodical comparison of protein isolation methods is the first critical step for proteomic studies, it is important to choose the extraction method which might be used for bacterial studies (Cilia, 2009). In this situation, both the choice of the disruption method for cell lysis and the lysis buffer are also quite important (Dilgimen, 2008).

Some disruption methods for cell lysis are bead-vortexing (mechanical disruption), freeze/thaw, French pressing (liquid homogenization), and sonication. In freeze/thaw technique, repeated steps of freezing and thawing cause to disrupt bacterial membrane with forming intracellular which is generally fatal to cells and extracellular ice which can cause mechanical damage to the cell membrane due to crushing. This results periplasmic and intracellular products to release completely out of the cell. Freeze temperature, time period of each freeze-thaw cycle, and the thawing temperature arte the important parameters in this technique. In this study, liquid nitrogen is used as freezing solution and freeze/thaw step is repeated 5 times nearly in one hour.

Lysis buffers and extraction methods should be chosen according to the purpose of bacterial protein extraction. Different lysis buffers can be used for isolation of membrane protein, cytosolic protein, or whole cell protein fractions (Molloy et al., 1998; Blonder et al., 2002). There can be substances like nucleic acids, lipids, salts, proteases and polysaccharides that need to be removed from protein extracts, as they can interfere with proteins and cause problems in separation of proteins. DNA and RNA molecules can bind proteins and cause increase in sample viscosity and that lead to plugging gel pores. This effect can be overcome by nuclease, RNAse and DNAse, addition to the lysis buffer (Dilgimen, 2008). Effects of polysaccharides can be overcomed by centrifugation at high speeds. In addition to that, diffuculty in removing polysaccharides like musins can be overcome by addition of tributyl phosphine and thiourea (Herbert et al., 1997). Lipids in the protein extract can cause artificial migrations and horizantal streaking. Addition of non-ionic detergents like CHAPS to the lysis buffer can help to avoid this effect.

Bacterial growth media that have high amounts of salt can effect the electrophoresis systems in a way of inducing water accumulation and current by migrating through the pH gradient in the gel and accumulating in the ends of gels. Washing cells with buffers, widely used buffers like phosphate and tris buffers, can help in removing salt. Finally to avoid protein degradation, protease inhibitors like PMSF and EDTA against proteases like cycteine and metalloproteases can be added to lysis buffer (Dilgimen, 2008). DTT (dithiothreitol) is used in protein extraction for disrupting di-sulfide bonds and iodoacetamide for alklyating cysteine residues.

II.4.2.2. Protein Separation

Many proteomic technologies rely on the ability to separate complex proteins by one- or two-dimensional gel electrophoresis (Shevchenko, 2006). Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is the most commonly used technique for separating complex protein mixtures and gives us the ability to evaluate changes in protein expression levels by comparing spot intensities. The 2D-PAGE was developed by Patrick H. O'Farrell who successfully combined two known electrophoresis methods, isoeletric focusing (IEF) and sodium dodecyl sulfate electrophoresis (SDS-PAGE) (O'Farrell, 1975) with the aim of resolving large number of proteins in one single jel getting the 2D-PAGE technique and mass spectrometry together (Salvato, 2012) .

In 2D-PAGE technique, the first dimensional separation is performed according to isoelectric points (pI) of proteins, and the second dimensional separation is performed acoording to molecular weights of the proteins.

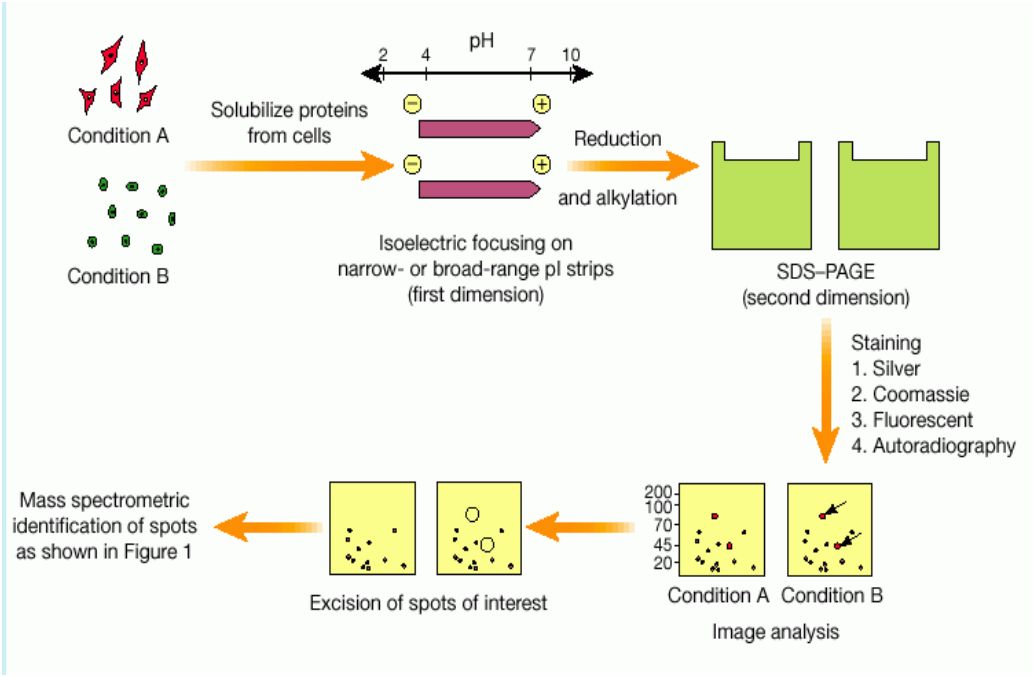


Figure3: *Shematic Representation of 2D- PAGE*

First dimensional electrophoresis can be carried out in different ways; using immobilized IPG strips with isoelectric focusing or using home-made gel in glass capillaries with the non-equilibrium pH gradient electrophoresis (NEpHGE) technique which allows pH scale built up with mixtures of soluble carrier ampholytes of different pH (O'Farrell, 1975). In NEpHGE technique, proteins move through the gel at different rates not according to their isoelectric points without the risk of precipitation at their isoelectric point. Therefore, accumulated volt-hours actually determine the pattern expand through the gel and this requires to adjust the volt hours consistently in order to get reproducible patterns (Lopez, 2002). The major difference between NEpHGE and IEF is that proteins migrate from anode to cathode and due to that basic proteins lead the separation. Another advantages of NEpHGE over IEF is that addition to basic proteins also acidic proteins are resolved at higher resolution. Following the first-dimension separation, the second-dimension separation is performed using SDS-PAGE technique.

II.4.2.3. Detection of Proteins

Visualization of proteins in gels is accomplished with different staining techniques. Staining techniques for common visualization of proteins separated by SDS-PAGE are Coomassie Brilliant Blue (CBB) and silver staining. In proteomic studies, protein stains must be compatible with mass spectrometry (MS). Coomassie Brilliant Blue R-250 and G-250 are organic dyes that constitues complexes stoichiometrically with basic amino acids, such as arginine, lysine, histidine, and tyrosine (Rabilloud, 2000). Coomassie R-250 requires on the order of 50-200 ng of protein per spot for detection. Absolute sensitivity and staining linearity depend on the proteins being stained.

Silver staining combines excellent sensitivity whilst using very simple and cheap equipment and chemicals. It is compatible with downstream processing such as mass spectrometry analysis after protein digestion (Chevallet et al., 2006). In staining principle, proteins bind silver ions, which can be reduced under appropriate conditions to develop a visible image made of finely divided silver metal. Silver staining methods are about 10-100 times more sensitive than various Coomassie Brilliant Blue staining techniques.

II.4.3. Protein Identification by Mass Spectrometry

In a 2-DE gel, thousands of protein spots can be separated at one single gel. The origin of the sample, methods used in extraction, amount of the protein, the technique used for separation, digestion methods, and the databases available for sample are the parameters that can effect the protein identification. There are several methods like protein partial hydrolysis coupled with chromatography, N-terminal sequence analysis by Edman degradation, ladder sequencing coupled with mass spectrometry, and mass spectrometry coupled with database analysis (Dilgimen, 2008).

II.4.3.1. Mass Spectrometry

Mass spectrometry (MS) is an analytical technique that forms ions from compounds by suitable methods, separates these ions according to their mass-to-charge ratio (m/z) and detects them qualitatively and quantitatively by their respective m/z and abundance (Jürgen, 2004). It is used in determining masses of charged particles, and elemental composition of an molecule. It is also used in elucidating the chemical structures of molecules like peptides.

A mass spectrometer has basically three main units; a source of ions, a mass analyzer and an ion detector. Adding to that main units, mass spectrometers can be equipped with an HPLC system and may have an ion trap unit. Basically in mass spectrometers, the ionization source converts the analyte into gas phase ions in vacuum, the ions are then accelerated in an electric field towards the analyzer, which separates them according to their m/z ratios on their way to the detector. The function of the detector is to record the impact of individual ions (Twyman, 2004).

Tandem mass spectrometry, known as MS/MS or MS^n, involves multiple steps of mass spectrometry selection, with some form of fragmentation occurring between the stages (Nic et al., 2006). MS/MS can be described as a series of events consisting of mass selection of a precursor ion in a first stage of analysis, an intermediate reaction event, followed by analysis of the product ions in a second stage of analysis (Busch et al.1988). Reaction event can be defined as an event that converts a precursor ion to a product ion having a different m/z value. The major strength of the tandem mass spectrometry method is that it provides extensive sequence information over the whole length of a protein chain in a single series of experiments that involve minimal effort directed toward separation and purification of oligopeptide fragments (Hunt, 1986). In proteomic studies, most commonly used mass spectrometers are MALDI-TOF MS, ESI-MS/MS, LC/ESI-MS/MS, ESI-Q-TOF MS.

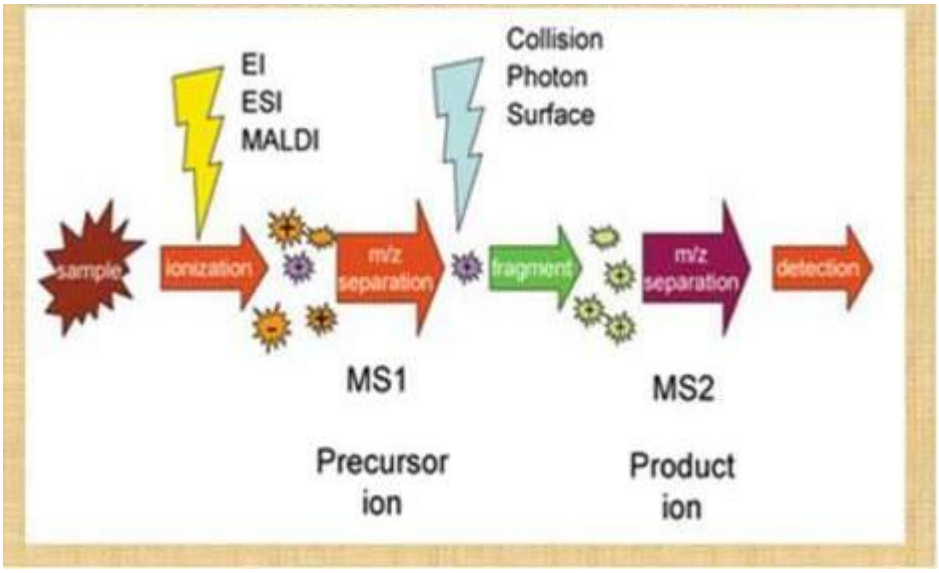


Figure4: *Shematic View of Tandem Mass Spectrometry*

Liquid chromatography–mass spectrometry (LC-MS,or alternatively HPLC-MS) technique is used by combining the physical separation capabilities of liquid chromatography and mass analysis capabilities of mass spectrometry. LC-MS/MS is a powerful technique used for many applications which has very high sensitivity and selectivity. LC-MS/MS can be applied to peptides digested from protein bands/spots separated by single-dimension (1D) and two-dimensional (2D) polyacrylamide gel electrophoresis (McDonald, 2002). The tandem mass spectrometry data can be related to the database sequences and peptides identified by their amino acid sequence (Eng et al, 1994; Yates et al, 1995).

II.4.3.2. Peptide mass fingerprinting

In peptide mass fingerprinting, interested proteins are first digested into smaller peptides, and their absolute masses accurately measured with a mass spectrometer such as MALDI-TOF or ESI-TOF (Clauser et al, 1999). Measured masses are then compared to a database containing known protein sequences. This is achieved by using computer programs that translate the known genome of the organism into proteins, then theoretically cut the proteins into peptides, and calculate the absolute masses of the peptides from each protein. Followed by this, the masses of the peptides of the unknown protein to the theoretical peptide masses of each protein encoded in the genome are compared. The results are statistically analyzed to find the best match. The advantage of this method is that only the masses of the peptides have to be known. A disadvantage is that the protein sequence has to be present in the database of interest.

II.5. TRANSCRIPTOMICS

The adaptive response of bacteria to different environmental conditions is related to its expressed gene products and their interactions with each other (Tsapakis et al., 2004). The analysis of the gene expression profiles is commonly performed at the total cellular RNA level (Friedel and Dölken, 2009). Transcriptome is a total set of all transcripts including coding RNAs (mRNAs), non-coding RNAs (tRNA, rRNA) and small RNAs in a cell. By studying transcriptome, it is possible to determine the transcriptional profiles of an organism that reflects the genes and pathways that are induced or repressed under different conditions (Adams, 2008). Transcriptomics is a term used to describe the methods that aid scientists to analyze the gene expression levels, gene regulation and regulatory sequences, to understand

the relation ship between the genome and functions of the cell, to demonstrate functional differences between cell types and to define the candidate genes for diseases (Wang et al., 2009).

Measuring the transcripts of messenger RNA, intermediate step between genes and proteins, fills the gap between the genetic code and the functional molecules that responsible for cell life. Variations in gene expression levels underline the wide range of physical, biochemical, and developmental differences seen between various cells and tissues (Adams et al, 2008).

II.5.1. Methods in Bacterial Transcriptome

Transcriptome analysis has an extreme importance to highlight altered expression of genes under the external environmental conditions. There has been several approaches to profile gene expression, including differential display and various types of microarrays and macroarrays, followed by validation methods, including real time quantitative polymerase chain reaction (Q-PCR) (Wilson et al, 2004). These technologies can be used independently or in paralel, where mRNA transcripts are investigated quantitatively by amplification of RNA from disease and control samples, with detection of specific complementary DNA (cDNA) or antisense RNA (aRNA) species (Tsapakis et al, 2004).

II.5.2. Microarray Technology

The DNA microarray technology has been developed and has become a powerful tool in studying the expressions of all genes in the genome concurrently. Microarrays can be applied to any organism of which the genome has been sequenced or a large collection of cDNA clones exists (Auesukaree, 2006).

Microarray techniques are based on the principle of spotting either short oligonucleotides or longer DNA fragments that are complementary to sequences of individual genes on a solid support such as coated glass surface or a nylon membrane (Tsapakis et al, 2004). Thus, DNA microarrays are categorized into cDNA microarrays and oligonucleotide arrays (Lipshutz et al, 1999).

cDNA microarrays have been the predominant method for the parallel analysis of gene expression in various biological processes. Arrays consist of DNA probes attached to the surface of a glass microscope slide at extremely high density. More than 20.000 genes can be arrayed on a surface of a glass slide (Auesukaree, 2006).

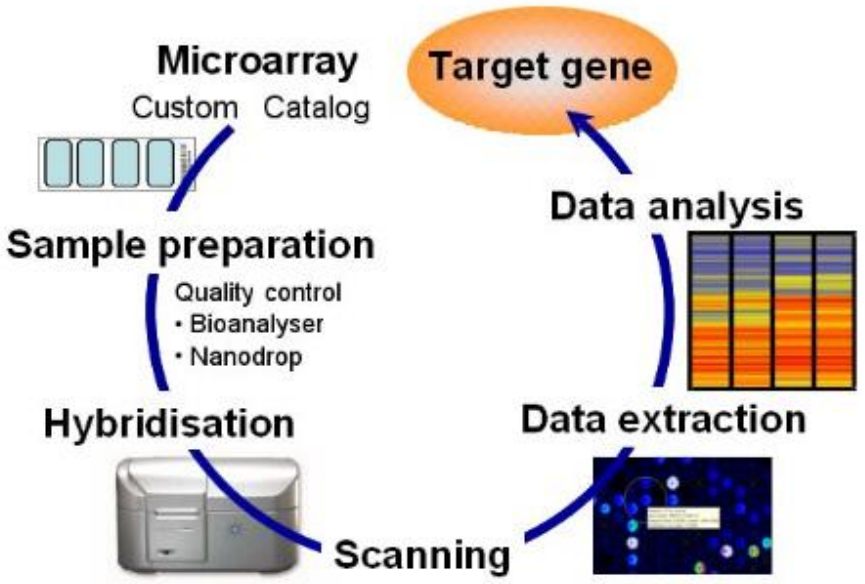


Figure5: *Workflow of Transcriptomic Study*

The expression levels of the genes are measured by hybridising cDNA, which is generally fluorescently labelled, with the spotted gene fragments. Analysis of microarray data help us to determine the expression levels of thousands of genes concurrently and to better understand the regulatory mechanisms, comprehensive bioactivity functions of genes, and simultaneous activity of cellular pathways. Therefore, cDNA microarrays have found wide applications in toxicological research, gene and drug discovery, and disease diagnosis (Lukac and Plataniotis, 2006).

II.6. INTEGRATING TRANSCRIPTOME AND PROTEOME PROFILES

Although genetic responses of bacteria to its environment are often regulated at transcriptional levels (Tsiridis and Giannoudis, 2006), transcript levels detected in mRNA profiling clearly do not reflect all regulatory processes in the cell since post-transcriptional processing altering the amount of active-protein are not considered (Silvestri et al., 2011). For this reason, in addition to transcriptomics, proteomics tools can be used to complete the knowledge on regulation of biological functions (Freiberg et al., 2004).

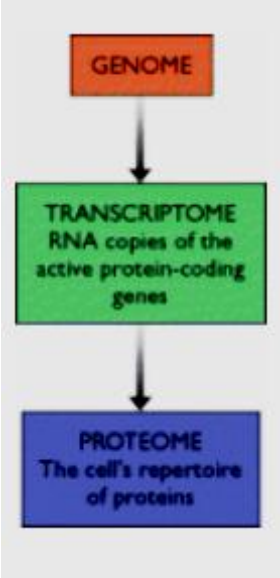


Figure6: *Relation between Transcriptome and Proteome* (Brown, T.A., 2002)

The proteome of a cell is the result of controlled biosynthesis and is regulated by gene expression (Kanapin et al, 2003). Nevertheless, the transcriptome can be regarded as a sensitive read-out of the proteome or the biochemical state of the cell. Therefore, transcriptome and proteome feed back to each other in a highly complex manner (Mijalski 2005).

CHAPTER III

THE STUDY

III.1. EXPERIMENTAL MATERIALS

III.1.1. List of Chemicals

Chemical	Supplier
Acetic acid	Sigma-Aldrich
Acetonitrile (HPLC Grade)	Sigma-Aldrich
Acrylamide	Sigma-Aldrich
Agarose	Sigma-Aldrich
Albumin bovine fraction5	Sigma-Aldrich
Ammonium persulfate (APS)	Amresco
Ammoniumsulfate	Merck
Ampholyte 4-6	Fluka
Ampholyte 3-10	Fluka
Ampholyte 5-8	Fluka
Berberinechloride	Sigma-Aldrich
Bromophenol blue	Sigma-Aldrich
Dithiothreitol	Amresco
CBB-G250	Sigma-Aldrich
Ethanol	Sigma-Aldrich
Ethylene diamine absolute	Sigma-Aldrich
Formaldehyde 37%	Sigma-Aldrich
Glycerol extrapure	OmniLife
Glycine	Merck
Hydrochloric acid 37%	Sigma-Aldrich
Isopropanol	Merck
Iodoacetamide	Merck
Methanol	Sigma-Aldrich

List of chemicals continuing

Mineral Oil	Bio-Rad
N,N-methylen bis-acrylamide	Sigma-Aldrich
PA12	Deconex
Potassiumchloride (KCl)	Sigma-Aldrich
Phosphoric acid (H_3PO_4)	Sigma-Aldrich
Sephadex G200	Sigma-Aldrich
Serdolit MB-1(Analytical Grade)	SERVA
Silvernitrate	Applichem
Sodium carbonate	Omni Life Science
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich
Sodium thiosulfate-pentahydrate	Merck
Sucrose (GC)	Sigma-Aldrich
TEMED	Merck
Tributhylphosphine	Sigma-Aldrich
Trifluoroacetic acid (TFA)	Merck
Tris-Base	Sigma-Aldrich
Tris-HCl	Sigma-Aldrich
Trypsin	Sigma-Aldrich
Urea (Ultra pure)	Merck
Urea (Pearl form)	Sigma-Aldrich
1,4 Bis(acryloyl) piperazine	Sigma-Aldrich

III.1.2. Bacterial Strains

Escherichia coli K12 strains were provided from The Scientific and Technological Research Council of Turkey (TUBITAK).

III.1.3. Growth Medium

LB Medium

Peptone	10g/L
Yeast Extract	5g/L
Sodium Chloride	10g/L

III.1.4. Buffers and Solutions

III.1.4.1. Buffers and Solutions Used in Determining Protein Concentration

Bradford Stock Solution

Ethanol (%95)	100ml
Phosphoric Acid (%88)	200ml
Coomasie Brilliant Blue-G250	350mg

Bradford Work Buffer

Deionized Water	425ml
Ethanol (%95)	15ml
Phosphoric Acid (%88)	30ml
Bradford Stock Solution	30ml

III.1.4.2. Buffers and Solutions Used in First Dimensional Electrophoresis

Separation Gel

Urea	9M
Glycerol	5% (w/v)
Acrylamide	4%
Ampholyte mix	2%
PDA	0,3%
TEMED	0,06% (v/v)

CAP Gel

Urea	9M
Glycerol	5% (w/v)
Acrylamide	10%
Ampholyte mix	2%
PDA	0,13%
TEMED	0,06% (v/v)

Ammoniumpersulphate (APS) Solution

APS	0,8%

Overlay Solution

Urea	5,2M
Glycerol	0,5M
Ampholyte	5%

Sephadex Solution

Sephadex (5%)	200µl
Urea	0,108g
Ampholyte (pH2-4)	10µl

Incubation Solution

Tris-Base	120mM
Glycerol	40% (w/v)
SDS	10mM

pH was adjusted to 6,8 with phosphoric acid

Ruuning Buffers:

Kathode Buffer

Urea	324g
Glycerol	30g (w/v)
Ethylendiamine	30ml

Completed to 600 ml with HPLC Grade dH_2O

Anode Buffer

Urea	72g
H_3PO_4 (85%)	20ml

Completed to 400 ml with HPLC Grade dH_2O

III.1.4.3. Buffers and Solutions Used in Second Dimensional Electrophoresis

SDS-PAGE Gel Solution

Tris-HCl (pH8.8)	375mM
SDS	0,1% (w/v)
Acrylamide	15% (w/v)
Bis-acrylamide	0,2% (w/v)
TEMED	0,03% (v/v)

Ammoniumpersulphate (APS) Solution

APS	1,28%

Overlay Agarose (0,5 %)

Tris	25mM
Glycine	192mM
SDS	0.1%
Bromophenol Blue	Trace

Completed to 50 ml with HPLC Grade dH_2O

SDS-PAGE Tank Buffer

Tris- Base	13,5g
Glycine	69,3g
SDS	4,5g

Completed to 4 liters with dH_2O

SDS-PAGE Running Buffer (10X)

Tris- Base	30g
Glycine	154g
SDS	10g

Completed to 1 liter with dH_2O

III.1.4.4. Solutions Used in Staining of 2-D Gels by Silver Staining

Fix Solution

Acetic acid (100%)	60ml
Ethanol (70%)	250ml
Formaldehyte (37%)	250µl

Completed to 500 ml with HPLC grade dH_2O

Washing Solution

Ethanol (70%)	100ml

Completed to 500 ml with HPLC grade dH_2O

Sensitizing Solution

Sodiumthiosulphate.$5H_2O$	157mg

Completed to 500 ml with HPLC grade dH_2O

Staining Solution

Silvernitrate	1g
Formaldehyte (37%)	380µl

Completed to 500 ml with HPLC grade dH_2O

Developing Solution

Sodiumthiosulphate.$5H_2O$	3,17mg
Sodiumcarbonate	30g
Formaldehyte (37%)	250µl

Completed to 500 ml with HPLC grade dH_2O

Stop Solution1

Acetic acid (100%)	30ml

Completed to 250 ml with HPLC grade dH_2O

Stop Solution2

Acetic acid (100%)	5ml

Completed to 250 ml with HPLC grade dH_2O

III.1.4.5. Solutions Used in Staining of 2-D Gels by CBB-G250 Staining

Fix Solution

Ethanol (70%)	1lt
H3PO4	28ml

Completed to 2 liters with dH_2O

Washing Solution

Methanol	680ml
Ammonium Sulphate	340g
H3PO4	28ml

Completed to 2 liters with dH_2O

Staining Solution

Methanol	680ml
Ammonium Sulphate	340g
H3PO4	28ml
CBBG-250	1.32g

Completed to 2 liters with dH_2O

III.1.4.6. Solutions Used in In-Gel Digestion Protocol

Dithiothreitol	10mM
Iodoacetamide	55mM
Ammoniumbicarbonate	100mM
Ammoniumbicarbonate	50mM
Trypsine	20ng/µl
Formic acid	1%
Acetonitrile	2%

III.1.4.7. Solutions Used in Zip-tip Protocol

Wetting & Elution Solution *(in LC grade water)*

Acetonitrile	70%
Trifluoroacetic acid	0.1%

Sample preparation solution *(in LC grade water)*

Trifluoroacetic acid	0.1%

III.1.5. Laboratory Equipment

Equipment	**Supplier**
Autoclave	NÜVE OT 032, Turkey
Water Purification Systems	ELGA PURELAB, United Kingdom
Spectrophotometer	PERKIN ELMER Lambda35 UV/VIS
Balance	Mettler Toledo AB204-S, USA
Etuves	NÜVE FN400/500
Refrigerators	ARÇELİK, Turkey
Deepfreezer (-20˚C)	LIEBHERR, England
Deepfreezer (-80˚ C)	HETO Mini Freeze, USA
Ice Machine	Arttex Bar Line, Italia
Centrifuge	Sigma 3-18k, Germany
Microsantrifuge	Sigma 1-15k, Germany
pHmeter	Mettler Toledo MP220, USA
Sonicator	Bandelin Sonorex, Germany
Rotational Vacuum Concentrator	Christ RVC2-25, Germany
Horizantal Laminar Flow Cabinet	ESCO ClassII type A2, USA
Orbital Shaker	Heidolph Unimax 2010, Germany
Incubator Shaker	Zhicheng ZHWY-211B, China
Magnetic Stirrers	Heidolph MR3001, Germany
Vortex	Labnet, USA
1st Dimensional Electrophoresi System	BioLab, Turkey
2nd Dimensional Electrophoresis System	Biorad Protean II xi Cell, USA Biolab, Turkey
Power Supply	Apelex PS1006 P, France
Gel Dryer	Thermo Scientific SG210D, USA
Gel Imaging System	GE Healthcare Image ScannerIII

III.2. EXPERIMENTAL METHODS

III.2.1. Sterilization

Sterilized equipments were used in all experiments. All the solutions and glasswares were sterilized for 15 minutes at 121˚C and 1.02atm pressure in an autoclave. Sterilized environment was provided under laminar flow.

III.2.2. Preparation and Maintenance of Bacterial Stocks

For storage, bacteria were grown in LB agar petri dishes, one colony was picked and used to inoculate LB medium. Bacteria in shake flasks were cultivated until their optical density reached 0.7 at 600nm. For long term storage, bacterial samples were kept at -80ºC in 50% (w/v) sterile glycerol with a ratio of 1:1. Bacteria also were stored in LB agar slant and LB agar plates for about a month.

III.2.3. Growth Experiments

III.2.3.1. Culture Conditions

E. coli K12 strains were grown in 100ml LB medium in retained 500ml flasks. Cultures were incubated at 37˚C, and 180rpm in an orbital incubator. 100ml LB medium as a control group and 100ml LB medium with the additon of berberine as a drug-treated group were inoculated with Escherichia coli K12 cells.

III.2.3.2. Determination of the Minimum Inhibitory Concentration of Berberine

Determination of minimum inhibitory concentration is a semi-quantitative test method that gives us the least concentration of an antimicrobial that inhibits bacterial growth. MIC is defined as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism (Turnidge, 2003).

Micro-dilution method was used to determine mic of the plant extract, berberine. For broth dilution method, sterile 96 well u-bottom micro-test plates were used. After addition of berberine to broth medium in wells, the plate was incubated at 37˚C for overnight.

III.2.3.3. Cell Growth and Cell Viability

5ml LB medium was inoculated from a frozen stock and incubated overnight at 37˚C with shaking (180rpm). 20ml LB medium was inoculated with 200µl overnight culture to prepare second preculture. 100µl culture was transfered to the 100ml LB medium and also 100ml LB medium supplemented with berberine in a 500ml of

flask when the second preculture's optical density at 600nm reached 0,7. Concentration of berberine in treated group was adjusted to 750mg/L.

Growth of control group of cells were determined spectrophotometrically. LB medium was used as a blank and to dilute samples to keep spectroscopic values within reliable limits. To monitor cell growth 0,1-0,5 ml samples were collected in every two hours for 18 hours.

Growth of both control and drug-treated group of cells were determined by viable cell count. To monitor cell viability, every two hours 0.5ml of culture was taken and transfered to 4,5ml of LB medium in a test tube. After mixing the test tube well, 0,5ml of this mixture was taken and this was repeated until reached desired dilution to make serial dilutions. 1ml of every dilution was taken and poured in the middle of a sterile plate. Approximately 25ml of LB-agar which was cooled to room temperature, and added to every plate. The plate was rotated immediately to spread cells homogeneosly in liquid agar. After solidification of agar medium, plates were incubated at 37˚C for overnight and then colonies were counted. Then, results were plotted on a graph.

III.2.4. Extraction of Whole Cell Proteins

Cells were harvested at the early-exponential and late-exponential phase. Before protein extraction, 50mM Tris buffer was added to centrifuge tube to suspend the pellet and centifuged at 15000g for 10 minutes. Cells were resuspended in tris buffer again and this was repeated for two times. Protein extraction was carried out according to ProteoPrep® Sample Extraction Kit's protocol (Sigma-Aldrich). For 10mg cells 2ml reagent type4 was added to cells pellet and incubated for 15 minutes. After incubation period, cells were freeze-thawed 5 times with liquid nitrogen. Solutions were centrifuged at 20000g for 10 minutes at 15˚C. Supernatants were transferred into new clean epperdorf tubes and non-soluble parts were discarded. Tributhylphospine was added to a final concentration of 5mM as a reducing agent and protein mixture was incubated at room tempereture for 1 hour. After incubation period, iodoacetamide was used to alkylate the protein samples to a final concentration of 15mM. Finally, reduced and alkylated samples were centrifuged at maximum speed for 5 minutes at room temperature to clarfy samples. Protein samples were aliquoted and stored at -20˚C for further applications.

III.2.5. Determination of Protein Concentration

Protein concentration was determined by using the Bradford (1976) assay. Bovine serum albumin (BSA) was used as a protein standard to prepare the calibration curve. BSA, ranged from 2µg to 30µg, was added to eppendorf tubes and with HPLC Grade water final volume was completed to 100µl. Different BSA concentrations used to prepare BSA calibration curve are given in the *Table 1*. 1ml of Bradford working solution was added to BSA and HPLC grade water mixture and vortexed. After mixing, samples were incubated at room temperature for 5 minutes and their optical density were measured at 595nm.

Table 1: *BSA Concentrations Used in BSA Calibration Curve*

BSA Concentrattion (µg)	Water (µl)	Bradford Reagent (µl)
0	100	1000
2	98	1000
4	96	1000
6	94	1000
8	92	1000
10	90	1000
12	88	1000
14	86	1000
16	84	1000
18	82	1000
20	80	1000
22	78	1000
24	76	1000
26	74	1000
28	72	1000
30	70	1000

The calibration curve was prepared by plotting BSA concentrations on the X axis, and their optical density measurements on the Y axis. The equation of the best fitting straight line was determined as y=0,0243x (See Appendix A for the graph).

The same assay was performed again for the protein samples of unknown concentrations.

III.2.6. Two-Dimensional Gel Electrophoresis

Two-dimensional separation of the proteins was performed using non – equilibrium pH gel electrophoresis (NEpHGE) in the first dimension and sodiumdodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension.

III.2.6.1. Separation of Proteins with First Dimensional Electrophoresis

First dimensional separation of proteins was carried out using NEpHGE technique. The advantage of this technique is that there is no risk of precipitation of proteins at their iso-electric points (Klose and Kobaltz, 1995).

In the first dimension protein are separated on glass capillary tubes filled with separation gels. For eight capillaries, 3600µl separation gel and approximately 200µl cap gel was prepared. For 3600µl separation gel, 400µl ampholyte mix was prepared. Ampholyte mixture was prepared mixing respectively two ampholytes, pH4-6 and 5-8 in a ratio of 3:1. After adding ampholyte mixture to the separation gel, mixture was degassed for ten minutes and then 100µl 0,8% APS solution was added to the solution. Gel solution was filled up to 15cm into the capillaries with a length of 19cm and 1,5mm inner diameter using syringes. At this stage, introduction of any air bubbles was avoided as oxygen molecules prevent polymerization of the gel. As polymerization stated in the capillaries, 5µl 0,8% APS and 20 µl pH2-4 ampholyte were added to the cap gel solution. Approximately one hour after the polymerization, cap gel was loaded to cathodic sides of the capillaries. The aim of adding cap solution to the capillaries is to prevent more dilute separation gels to go out of the capillaries during electrophoresis. After polymerization of the cap gel solution, cathodic sides of the capillaries were sealed with parafilm. Capillaries were stored in dark at room temperature for 2 nights. Water was produced as result of polymerization, and it was removed and capillaries were placed into the vertical electrophoresis system. Respectively, 10µl sephadex solution and 400µg protein sample were loaded each capillary. Finally to prevent mixing of sample with anode buffer, 10µl overlay solution was loaded each capillary. Then all capillaries were filled with anode buffer on the sample loading side and with cathode buffer on the opposite side. Proteins were allowed to run from anode to cathode for 20 hours by

respectively applying 100V for 60 minutes, 200V for 60 minutes, 400V for 990 minutes, 600V for 60 minutes and 1000V for 30 minutes. After the run was completed, gels were removed from capillaries. Prior to second dimensional separation, gels were transferred into incubation solution, and gently shaken for ten minutes at room temperature. Gels can be stored at -80ºC in petri dishes until proceeding second dimensional separation.

III.2.6.2. Separation of Proteins with Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis SDS-PAGE

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (Laemmli, 1970) was performed to separate proteins according to their molecular weights. SDS-PAGE gel solution containing 15% acrylamide and 0.2% bisacrylamide was prepared and degassed for ten minutes. Glass plates were cleaned with HPLC grade water, ethanol and isopropanol respectively. Two spacers were tucked between glass plates on both sides and glass plates were placed into the casting stand. 1.28% APS was gently added to SDS-PAGE gel solution and gel solution was poured into the electrophoresis chamber up to 10mM below the top of the glass plates. Surface of the gel was covered with isopropanol to obtain a flat surface and gel left at room temperature for about 30 minutes for polymerization. The gel sizes were 20x20x 1,5mm. During polymerization, gels were taken from -80˚C, and left at room temperature to thaw. Incubation buffer was prepared to equilibrate first dimensional gels. Gels were gently shaken for ten minutes in incubation buffer. Overlay agarose melted in a microwave oven prior to second dimensional electrophoresis. After polymerization of second dimensional gel, isopropanol was discarded and gel surface was washed with diluted running buffer. Incubation buffer was discarded and first dimensional gels were placed on top the second dimensonal gels. For about 2ml of overlay agarose was poured into the well of the SDS-PAGE gel without forming any bubbles to cover gels. When the overlay agarose solution was solidified, the gel sandwiches were placed into the electrophoresis tank filled with running buffer. Upper buffer well was filled with running buffer and the system was run for 15 minutes at 120mA and 134 minutes at 150mA. The gels were transferred to appropriate trays for staining.

III.2.6.3. Silver Staining of 2-D Gels

Gels were incubated in fix solution for overnight (least two hours). After discarding fix solution, gels were washed in washing solution for 20 minutes, and this was repeated two times more. Gels were incubated in sensitizing solution for two minutes and washed with HPLC grade water three times for 20 seconds. Gels were incubated in staining solution for 20 minutes. Staining solution was discarded and gels were washed with HPLC grade water three times for 20 seconds. After washing step, gels were incubated in developing solution approximately for 5 minutes until the spots were clearly seen. Developing solution was discarded and stop solution1 was added to trays for 5 minutes to stop the reaction. Gels were stored in stop solution2 at +4˚C.

III.2.6.4. Coomassie Blue G-250 Staining of 2-D Gels

Gels were incubated in fix solution for overnight. After discarding fix solution gels were incubated in wash solution for 2 hours. After discarding wash solution, staining solution was added to trays and gels were stained for two days.

III.2.6.5. Visualization of Stained Gels

Gels were visualized with GE HEALTCARE® Image Scanner II. Spots on control groups' gels were compared with treated groups' and interested protein spots were determined.

III.2.7. Mass Spectrometric Analysis

Each protein spot were analyzed by a liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) following digestion.

III.2.7.1. Sample Preparation for Mass Spectrometric Identification

Prior to excision of spots, gels were rinsed with HPLC grade water. Determined protein spots were excised with a clean scalpel under laminar flow and were transferred into labelled low-binding microcentrifuge eppendorf tubes. Spots in each eppendorf tube were cut into small pieces and spinned down on a bench-top microcentrifuge. 100µl of 100mM ammonium bicarbonate/acetonitrile (1:1, vol/vol) solution was added to each tube and incubated for 30 minutes with gentle shaking. 500µl acetonitrile was added to each tube and incubated at room temperature with gentle shaking until gel pieces became white and shrink. Gel pieces were spinned down to remove all the liquid. 30-50µl 10mM DTT solution was added to tubes and incubated at 80˚C for 30 minutes. 30-50µl 55mM iodoacetamide was added tubes

and incubated at room temperature for 20 minutes. Gel pieces were shrunk with acetonitrile and all the solution was discarded. Approximately 50µl trypsin buffer was added to gel pieces and tubes were left in an ice bucket for 30 minutes. After 30 minutes, more trypsin buffer was added to tubes since gel pieces absorb the buffer. Tubes were left for another 90 minutes to saturate the gel pieces with trypsin and 10-20µl of ammonium bicarbonate buffer added to keep the gel pieces wet during enzymatic reaction. Then, tubes were placed into an air circulation thermostat and incubated at 37˚C for overnight. After digestion period, tubes were chilled to room temperature and gel pieces were spinned down. Extraction buffer that contains 0.1 % formic acid and 2% acetonitrile was added each tube in a same volume of trypsin that used for digestion. 10µl of wetting solution was aspirated to the C^{18} coloumn and dispensed to waste to equilibrate the coloumn. This step was repeated two times more. 10µl of sample preparation solution was pippetted and dispensed to waste and repeated two times more. 10µl of sample mixture was aspirated and dispensed, up to 10 cycles, repeated again. LC grade water was aspirated and dispensed up to 3 cycles. 10µl of elution solution was transferred to a clean low-binding eppendorf tube with a standard tip and the eluant was aspirated and dispensed at least ten times without introducing air. Elution solution was removed under vacuum system. 10µl of 0,1% formic acid was added to each tube, vortexed for 20 seconds and spinned down for 30 seconds. Finally sample was transferred into LC vial. The samples were ready for analysis.

III.2.7.2. Obtaining Peptide Data with LC/MS-MS Mass Spectrometer

Mass spectra of digested peptides were obtained from LC- MS/MS (Waters Co., UK). Spectra obtained from each well were saved as text documents for further analysis.

III.2.7.3. Analysis of the Peptide Data in Database

Mascot is a search engine that uses mass spectrometryic datas to identify proteins using databases. Swissport is the most efficient database for protein identification. Enzyme was selected as trypsin, the taxonomy was selected as *E. coli*, and carboxymethyl and carboxamidomethyl were added as post translational modifications to increase the specificity of the results.

III.2.8. Transcriptome Analysis

III.2.8.1. RNA Isolation

One mililiters of culture from both control and treated group of cells were transferred into a DNAse&RNAse free eppendorf tubes. Total RNA isolation is carried out following the protocol provided by ROCHE®, High Pure RNA Isolation Kit. After RNA isolation, cDNA synthesis was carried out following the protocol provided by Invitrogen® SuperScript® VILO™ cDNA Synthesis Kit.

III.2.8.2. Microarray Technology

We applied microarray technology in bacterial gene expression experiments. After RNA isolation, isolates were sent to GENMAR Diagnostic Company laboratories (Izmir, Turkey), and all experiments including array fabrication, probe preparation and hybridization, data collection, and normalization carried out in GENMAR. Bacterial gene expression array was provided from NimbleGen, ROCHE®. We used 4X72K arrays with 72000 prober per sample and 45-60mer probe length. Microarrays for each duplicate group, control and treated cells, were constructed by arraying cDNA clones or genes on derivatized glass microscope slides. Arrays were scanned with MS200 Microarray Scanner (Roche, NimbleGen, U.S.A.) with 2µm resolution in 635nm. Collected data, RMA (Robust Multi-array Average) and annotation files, were loaded to Nexus Expr3.0 software (Biodiscovery, CA) and analyzed. Log-ratio threshold was adjusted to 1 to detect 2-fold change. Multiple gene expression profiles were compared alongside the gene annotations.

CHAPTER IV

RESULTS and DISCUSSION

In this study, the antimicrobial effect of berberine on *E. coli* K12 cells was investigated. For testing effectiveness of potential drugs, the universal solvent DMSO is commonly used to dissolve drug candidates. Unfortunately, such solvents have an inhibition effect on cell growth, especially when the minimum inhibitor concentration of the drug is high, as the case encountered in this study. Consequently for comparision of protein protein expression profiles, three independent gels should be compared simultaneously (one drug-treated gel, one DMSO-treated gel, and one control gel). This is a tedious job. Berberine is a quaternary alkoloid which is soluable in water. Hnece in the current study, this property of berberine was utilized. Using water as the solvent, protein expression in drug-treated gels were directly compared with the protein expression in control gels.

To understand the effectiveness of berberine as an antimicrobial drug, first the minimum inhibitory concentration of berberine for *E. coli* K12 cells was determined. Then taking 0,6xMIC as the final concentration of berberine in the growth culture, effect of berberine on actively growing cells were evaluated at both transcriptomic and proteomic levels. Results of gene and protein expression differences were determined and compared with each other.

This study provided important clues for understanding the action of mechanism of berberine and also gave reasons for bacterial survival under berberine treatment.

IV.1. MIC OF BERBERINE AGAINST *ESCHERICHIA COLI* K12 CELLS

Berberine is a potentially effective antimicrobial and has two stable targets to bind: the membrane and DNA (Amin et al., 1969). Although it is considered as an antimicrobic drug, based on its MIC value, berberine is reported to be not as effective for *E. coli* as it is to *Staphylococus aureus* strains. This outcome has been reasoned by its extrusion through the NorA Multi Drug Resistance (MDR) pump. It

has been reported that upon deletion of the NorA gene, the antibiotic action of berberine increased significantly (Stemitz et al., 2001).

In a previous study, the minimum inhibitory concentration (MIC) of berberine against *E. coli* K12 cells has been determined as 1250mg/L using micro-dilution method (Ozbalci et al., 2010). Using this result, the concentration of berberine in growth medium was adjusted to 750mg/L (0,6xMIC) to allow growth since the presence of 1250mg/L berberine fully stops cell growth.

IV.2. EVALUATION OF THE GROWTH OF E.COLI K12 CELLS

Cell growth was compared for cells in the presence and absence of berberine. Using a spectrophotometer, optical density at 600nm was measured every two hours. The growth profile of *E. coli* K12 control group cells is given in the *Figure 7.*

Unfortunately due to precipitaion of berberine, it was not possible to monitor growth of drug-treated cells. Precipitation of berberine has caused growth media to become blurry 3 hours following inoculation. Hence it was not possible to compared the difference in growth profiles using a spectrophotometric method.

Since measurement of optical density was not possible in order to investigate the effect of berberine on *E. coli* K12 cells, cell viability was measured every two hours by plating samples from control and berberine-treated cultures on solid growth media. Number of viable cells on both plates were counted and compared. The growth profiles of both control and drug-treated *E. coli* K12 cells are given in *Figure8* according to CFU results.

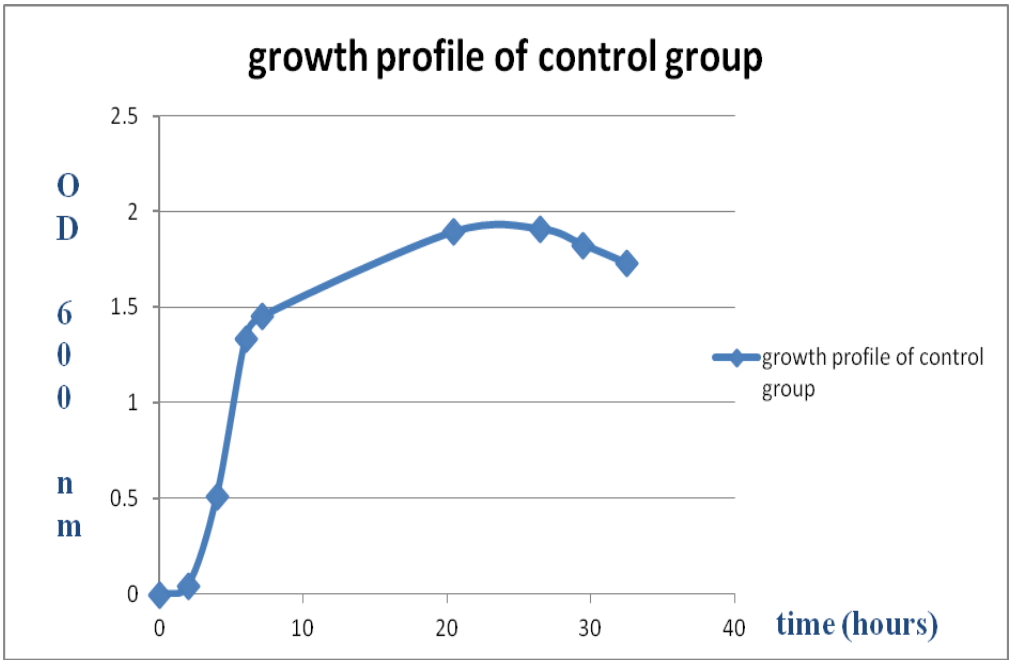


Figure 7: *Growth Profile of Escherichia coli K12 Cells*

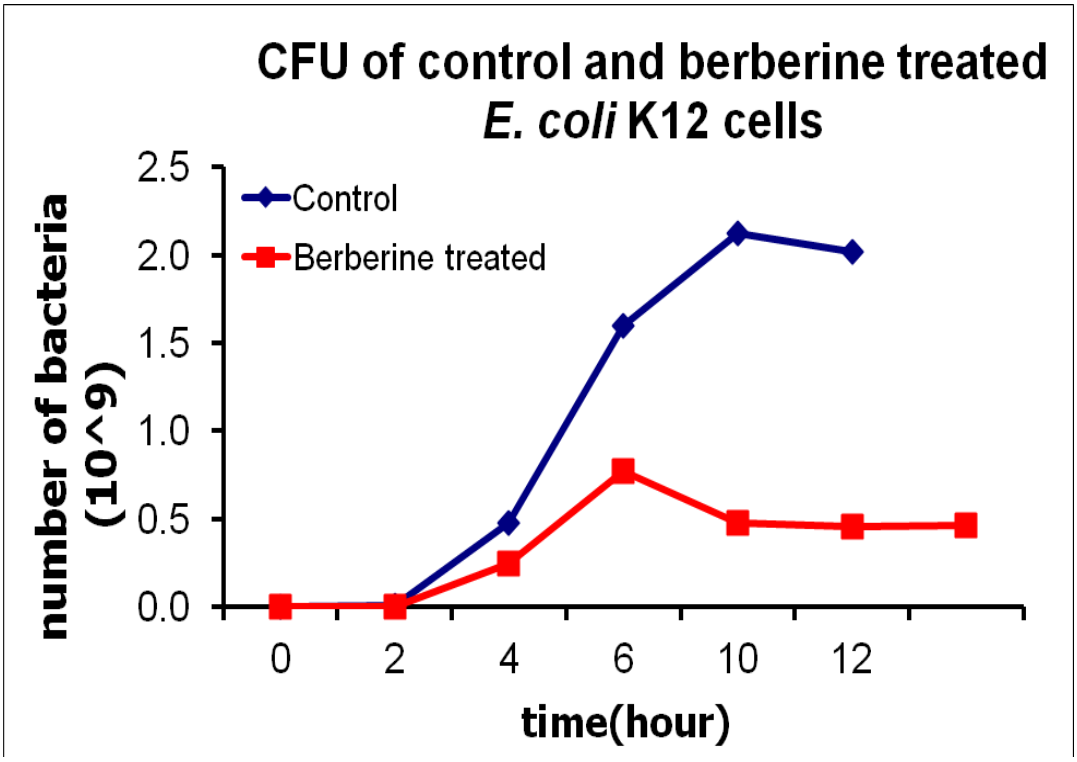


Figure 8: *Growth Profiles of Control and Berberine-treated Cells*

2 hours following the inoculation, growth profiles of control and berberine-treated *E. coli* K12 cells started to show a difference. The number of viable cells

in the berberine-treated cells was lower than the number of viable cells in the control culture. Viable cell number in the control culture continued to increase 10 hours following inoculation whereas viable cell number in the berberine-tretaed culture continued to increase only 6 hours. In this time interval, the growth rate of the control after 6 hours of growth, the increase in cell number ceased and cell death was monitored in berberine-treated cells. Using these results, within the scope of this work, proteomic and transcriptomic data from control and berberine-treated cells were compared after 4,5 hours of growth. This has allowed to evaluate the survival mechanism of *E. coli* K12 cells under berberine stress.

IV.3. ANALYSIS of PROTEOMIC DATA

In proteomic studies, separation of proteins was carried out using 2-DE techniques. In the first dimension, proteins were separated with NEpHGE, and in the second dimension with SDS-PAGE techniques. Selected protein spots were trypsinized and peptides obtained were analyzed by LC-MS/MS. Mass spectrometric data were searched on SWISS-PROT database to identify selected proteins.

IV.3.1. Analysis of Protein Expression Differences

Detecting protein bands on polyacrylamide gels is the first critical point in 2-DE experiments, since it can effect further mass spectrometric analysis. The staining method should be compatible with mass spectrometric analysis. Though, silver staining is a more sensitive technique to visualize proteins, CBB-G250 staining technique is more common in mass spectrometric analysis. Due to the diffuculty of removing silver stain from proteins bands during in-gel digestion protocol, CBB-G250 staining method was used in this study. 2-DE results have shown us that proteins were found to lie in the isoelectric point (p*I*) range from 4.5 to 7.5. Proteins spots were not separated distinctly on second dimensional gels with in the 3-10pH range and were mainly clustered in the neutral range to the acidic range. Thus, an optimization was required for the ampholyte mixture to be used in one-dimensional capillary gels to obtain every protein spot separate on second dimenional gels and aviod from cross-contamination risks of proteins during analysis. Based on the separation obtained using the 3-10pH range, first the pH range between 4.5-6.5 was enlarged. To achieve the required separation,

carrier ampholytes with pH ranges of 4-6, 5-8, and 3-10, were mixed in different ratios to obtain the best resolution. Best separation was obtained by mixing 4-6 and 5-8 pH–ranged carrier ampholytes in a ratio of 1:3. Experimental molecular masses of protein bands were determined by loading marker protein (SM0431, Fermentas) to the anode side prior to second dimensional run. According the 2-DE results, approximately 350 protein spots were detected on polyacrylamide gels with molecular weights (MW) between 120 and 10kDa. 2-DE images of control and berberine-treated group are given in the *Figure 10* and *Figure 11*.

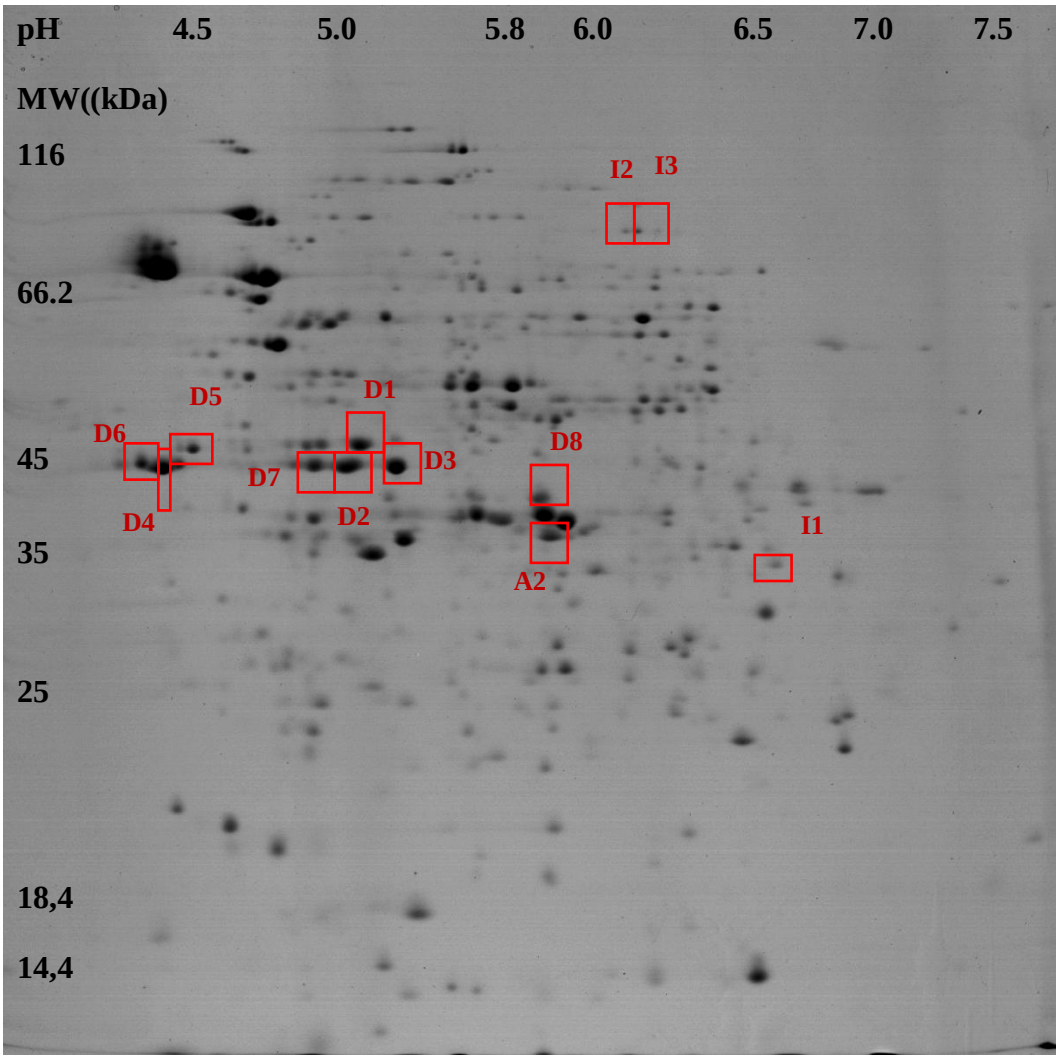


Figure 9: *2-DE Image of Control Group*

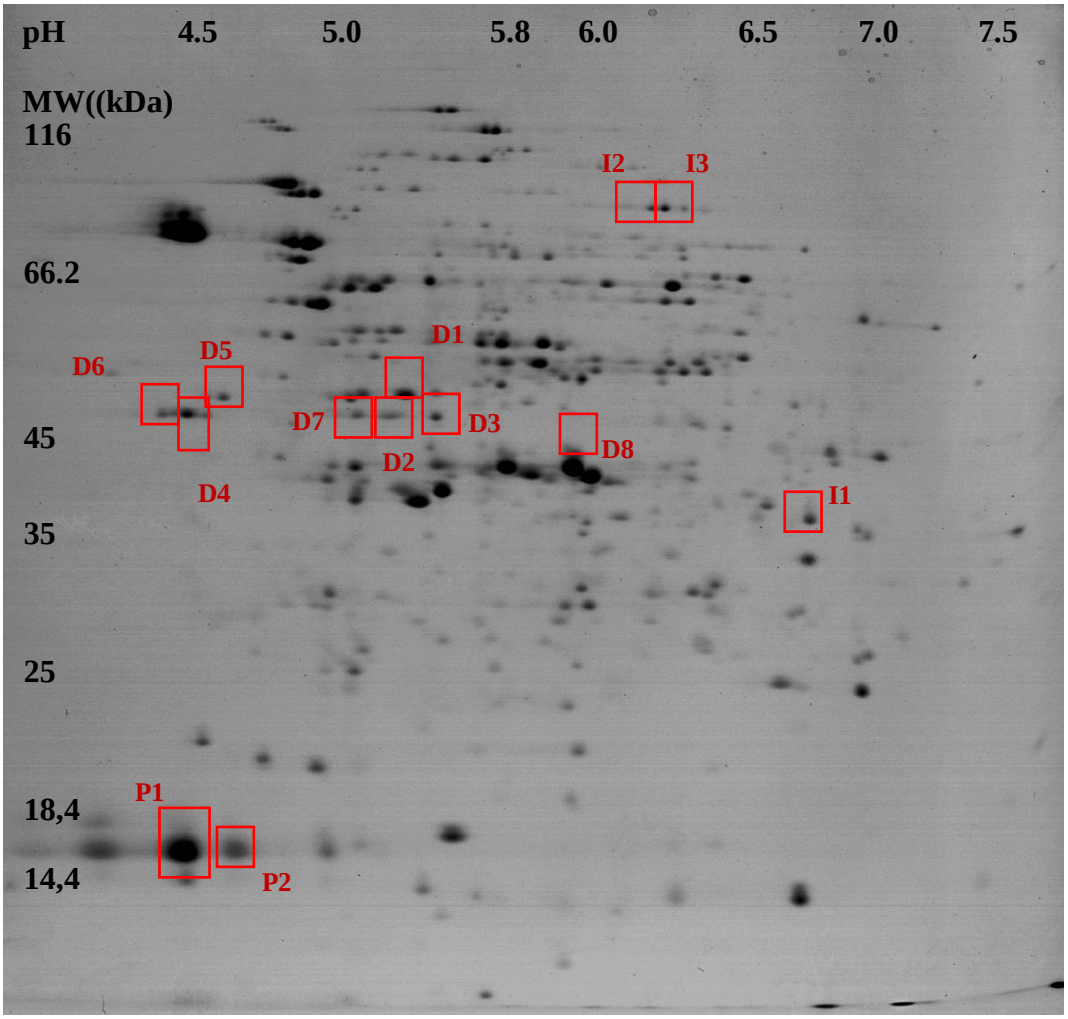


Figure 10: *2-DE Image of Berberine-treated group*

When the 2-DE gel of berberine-treated group was compared with control group, 14 protein spots were selected for further analysis. 13 protein spots were up-/down-regulated, and one protein was selected as control protein (D1). Of the selected proteins, 7 of them were down-regulated, 3 of them were up-regulated in the presence of berberine while 1 of them was expressed only in the presence of berberine and 2 proteins of them were expressed only in the absence of berberine.

The additon of berberine to the medium the expression of D2, D3, and D7 were down-regulated. These spots were found to lie in pH range of 4,8 to 5,4 and molecular weight range from 44kDa to 40kDa (*Figure 9*).

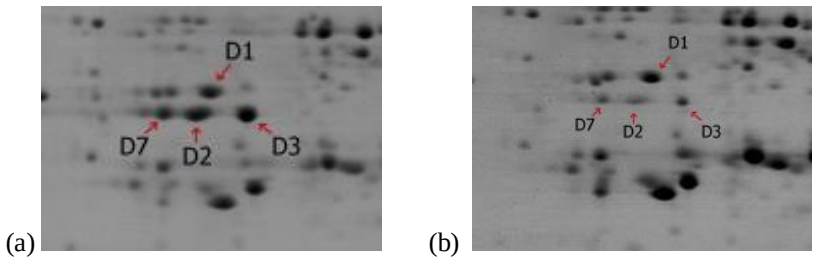


Figure 11: Zoomed views of spots numbered D1 (control protein), D2, D3, and D7 on 2-DE Gels (a) control group, (b) berberine-treated group.

The addition of berberine also down-regulated protein spots D4, D5, and D6. These spots were found to lie in pH range of 4,8 to 5,4 and molecular weight range from 44kDa to 40kDa (*Figure 10*).

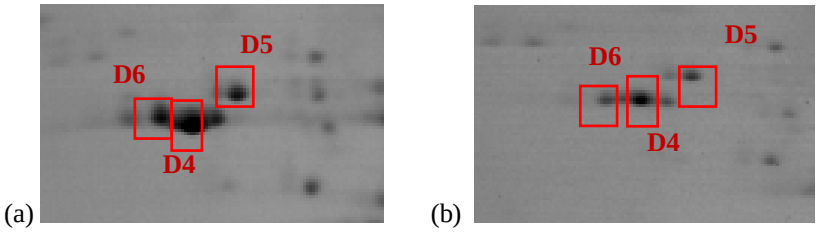


Figure 12: Zoomed views of spots numbered D4, D5, and D6 on 2-DE Gels (a) control group, (b) berberine-treated group.

Spots numbered D8 and A2 were found to be in the pH range of 5,4 to 5,9 and molecular weight range from 37 to 32kDa. While D8 was down-regulated in the presence of berberine, A2 was only expressed in the absence of berberine (*Figure 11).*

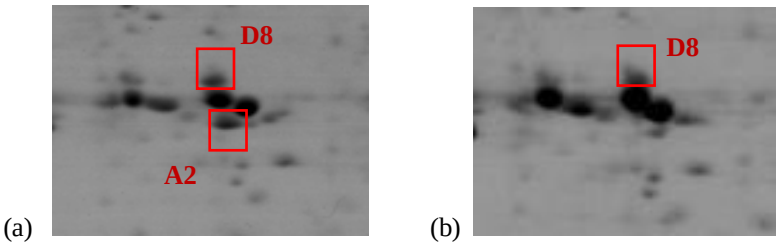
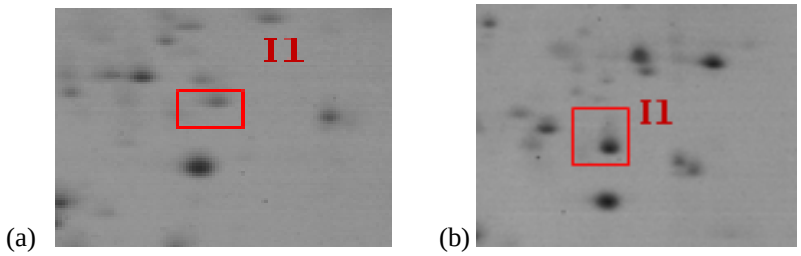


Figure 13: Zoomed views of spots numbered D8 and A2 on 2-DE Gels (a) control group, (b) berberine-treated group

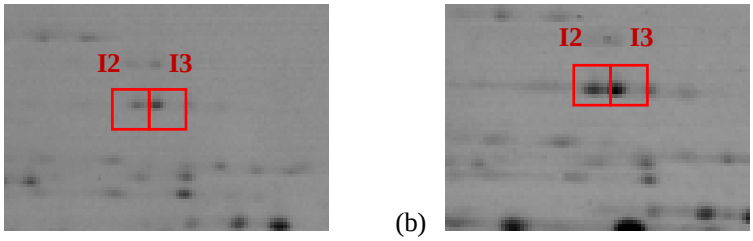
Protein spots numbered I1 was up-regulated in the presence of berberine and was found to lie in the pH range of 5,8 and molecular weight of 64kDa (*Figure 12).*



(a) (b)

Figure 14: Zoomed views of spot numbered I1 on 2-DE Gels (a) control group, (b) berberine-treated group.

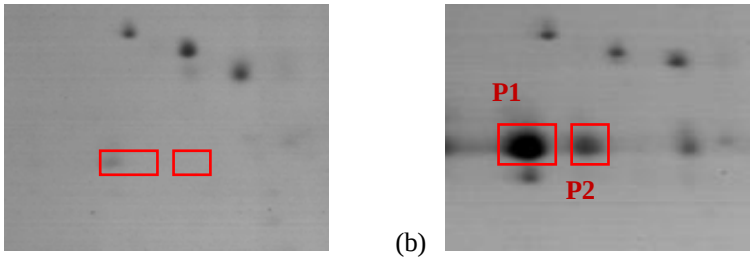
Protein spots numbered I2 and I3 were up-regulated in the presence of berberine and were found to lie in the pH range of 5,8 and molecular weight of 64kDa (*Figure 12).*



(a) (b)

Figure 15: *Zoomed views of spots numbered I2 and I3 on 2-DE Gels* (a) control group, (b) berberine-treated group.

Both P1 and P2 were found to be expressed only in the presence of berberine. They lied in the pH range of 4.92 to 4.98 and molecular weight range from 18 to 16kDa (*Figure 13).*



(a) (b)

Figure 16: *Zoomed views of spots numbered P1 and P2 on 2-DE Gels* (a) control group, (b) berberine-treated group**.**

IV.3.2. Identification of Protein Spots

Selected 14 proteins were analyzed by LC-MS/MS. Obtained mass data was searched in Mascot Database. Identification results are given in the *Table 2* (See Appendix B for the full list of identified proteins). Results of the differentially expressed proteins have shown that, down-regulated proteins were related to metabolism, sugar transport sistem, ion transport and cell division. Up-regulated proteins takes place in the mechanisms of electron transport, riboflavin biosynthesis, tricarboxylic acid cycle, and cell adhesion.

Table2: *Identification Results of Selected Protein Results*

Spot no.	*Protein name*		*Accession no.*	*Coverage (%)*	*PLGS score*	*Theoretical kDa/pI*	*Experimental kDa/ pI*
Glycolysis							
D1/	**PGK_ECO1**	C	A1AFB0	78	8433	41092/4,8924	41000/4,8
Metabolism							
D8/	**ASPG2_ECOLI**	X	P00805	70	9456	36827/5,9233	36000/5,4
Sugar transport system							
D2/	**MALE_ECOLI**	↓	P0AEX9	83	6391	43360/5,3782	40000/4,8
D3/	**MALE_ECOLI**	↓	P0AEX9	83	8894	43360/5,3782	40000/4,9
D7/	**MALE_ECOLI**	↓	P0AEX9	80	5603	43360/5,3782	40000/4,6
Ion transport							
D4/	**NMPC_ECOLI**	↓	P21420	67	4731	40277/4,4469	40000/4,3
D6/	**OMPC_ECOLI**	↓	P06996	46	2218	40343/4,3848	40000/4,2
Cell cycle/ Cell division							
D5/	**FTSZ_ECOLI**	↓	P0A9A6	98	13120	40298/4,4385	40000/4,4

Table 2 continuing

Electron transport							
I2/	**DHSA_ECOLI**	↑	P0AC41	21	159	64381/5,8114	66000/5,75
I3/	**DHSA_ECOLI**	↑	P0AC41	75	3848	64381/5,8114	66000/5,8
Cell adhesion							
P1/	**FIMA1_ECOLI**	↑↑	P04128	43	6365	18100/4,9266	17000/4,3
Riboflavin biosynthesis							
P2/	**RISB ECO24**	↑↑	A7ZIG8	60	953	16146/4,9753	17000/4,6
Tricarboxylic acid cycle							
I1/	**SUCD_ECOLI**	↑	P0AGE9	67	8938	29758/6,343	31000/6,3
A2/	**MDH ECO55**	X	B7LHU4	79	1032	32290/5,4611	34000/5,5

(**C**: control protein, ↑ : up-regulated in the presence of berberine, ↑↑ : up-regulated only in the presence of berberine, ↓ : down-regulated in the presence of berberine, **X** : no expression in the presence of berberine)

A control protein, D1, on gels of proteins from control and berberine-treated cells was selected to confirm that the matched protein spots were identical on both gels. This protein was identified as **phosphoglycerate kinase,** PGK ECOK1 for both gels. This has proved that protein matches were reliable to evaluate differential protein expression. PGK is an enzyme located in cytoplasm and functions as a transferase and kinase. It plays roles in glycolysis, ATP binding mechanism and phosphoglycerate metabolism and also takes place in the mechanism of carbonhydrate degradation pathways. .

Spot **A2** was only visualized in control group of gels and was identified as **malate dehydrogenase**, MDH ECO55. Malate dehydrogenase (MDH) is an enzyme that reversibly catalyzes the oxidation of malate to oxaloacetate. It is found in malate metabolic pathway, cellular carbohydrate metabolic process and tricarboxylic acid cycle. It also plays role in gluconeogenesis. Oxaloacetate is a citric acid cycle intermediate, and formed from pyruvate in mitocondria by pyruvate carboxylase enzyme. In order to get the oxaloacetate out of the mitochondria, malate dehydrogenase reduces it to malate, and it then transmitted the inner mitochondrial membrane. Once in the cytosol, the malate is oxidized back to oxaloacetate by cytosolic malate dehydrogenase. Finally, phosphoenolpyruvate carboxykinase (PEPCK) converts oxaloacetate to phosphoenolpyruvate (PEP) (Banazsak et al., 1975). High concentrations of oxaloacetate can inhibit MDH. In its active state, MDH undergoes a conformational change that encloses the substrate to minimize solvent exposure and to position key residues in closer proximity to the substrate (Goward and Nicholls, 1994). Its disappearence might indicate a general decline in metabolism (Fu et al., 2010) or a broken TCA cycle.

The three down-regulated **D2, D3** and **D7** protein spots were identified as **maltose-binding periplasmic protein** (MALE_ECOLI). MALE is located in periplasm. It plays role in transport, sugar transport systems and is initial receptor for the active transport of chemotaxis toward maltooligosaccharides. It is induced by high pH during aerobic or anaerobic growth and it increases in the physiological short-term adaptation to glucose limitation (Han et al., 2006). Transport of amino acid and other carbon sources inside of the cell via periplasmic binding proteins is essential for *E. coli* cells during energy chrisis.

The protein spot numbered **D4** was identified as **putative outer membrane porin protein**, nmpC. The outer membrane of gram-negative bacteria like other biological

membranes acts as a barrier to the permeation of solutes. There are two major porin-based mechanisms for antibiotic resistance that have been reported: 1) alterations of outer membrane profiles, including reduction of porins or replacement of one or two major porins by another; 2) altered function because of the specific mutations that reduce permeability (Delcour, 2009). *E. coli* K12 has the genetic ability to produce additional pore proteins (new membrane proteins, designated as Nmp proteins) which are not present on the surface of wild-type cells. These Nmp proteins are expressed as a consequence of mutations at genetic loci (nmpA, nmpB, and nmpC) distinct from the various omp loci (Pugsleyand Schnaitman, 1978). These nmp proteins, multiple pore proteins, are probably of strong survival value to the organism. In our study, nmpC expression is down-regulated by the addition of berberine.

The down- regulated protein, spot **D5** was identified as **cell division protein, FtsZ.** FtsZ is found in cytoplasm and forms a cytokinetic ring, designated the Z ring, that directs cytokinesis in prokaryotes (Mukherjee et al., 1998). FtsZ is an essential cell division protein that forms a cytokinetic ring that directs cell division in bacteria (Bi and Lutkenhaus, 1991). This ring, FtsZ or Z ring, has been studied most extensively in *E. coli* but appears to direct the formation of the septum in all prokaryotic organisms (Lutkenhaus and Addinall, 1997). Boberek et al (2010) has reported that FtsZ is inhibited in *E. coli* by berberine and proposed that the primary mechanism of action of berberine is not DNA binding but could possibly be inhibition of cell division.

Spot **D6** was identified as **outer membrane protein C,** OMPC_ECOLI. OmpC is a protein that is located in the outer membrane of *E. coli* K12 and plays role in ion transport, transport and porin activity. OmpC expression is down-regulated by the addition of berberine. In gram-negative bacteria, outer membrane proteins are the units that antibiotics penetrate first in the resistance of bacteria to an antibiotic. The number and activity of efflux pumps and outer membrane proteins that constitute porins play major roles in membrane permeability and hence in the definition of intrinsic resistance in Gram-negative bacteria that is altered under antibiotic exposure. In E. coli, outer membrane permeability is regulated by the balance of porin proteins, the diffusion channels that are the major route for passage of small hydrophilic compounds (Nikaido, 2001; Pages et al., 2005; Nikaido, 2003). OmpC, one of the major outer membrane proteins of *E. coli*, consists of three 16-stranded β-barrels defining a transmembrane pore in the outer membrane porin (Basle et al., 2006). The level of expression of ompC

controls the permeability of the outer membrane to glucose and nitrogen uptake under nutrient limitation (Liu and Ferenci, 2001; Ferenci, 2005), but may also be differentially regulated by the concentration of certain antibiotics in the environment (Davin-Regli and Pages, 2006; Randall and Woodward, 2002; Castillo-Keller et al., 2006). OmpC is known to be accumulated in response to salt stress (Weber et a., 2006).

Spot **D8** was identified **as L-asparaginase 2,** ASPG2_ECOLI. It plays role in asparagine metabolic process and catalyzes the hydrolysis of L-asparagine to L-aspartate and ammonia. *E. coli* produces two L-asparaginase isozymes: L-asparaginase I, a low-affinity enzyme located in the cytoplasm, and L-asparaginase II, a high-affinity secreted enzyme, located in the periplasmic region of the bacteria. L-asparaginase II is subject to regulation by cyclic AMP receptor protein (CRP) and is also induced by anaerobiosis (Cedar et al., 1967; Chesney et al., 1983; Russell et al., 1983). L-asparaginase2 has been in clinical use for many years, affecting complete disease remission in some cases, as well as proving effective in maintenance therapy (Swain et al., 1993)

Spot **I1** was identified as **succinyl-CoA ligase [ADP-forming] subunit alpha** (sucD). This is an enzyme found in cytoplasm and catalyses the reversible thioesterification of succinate in three steps involving the phosphoryl enzyme and enzyme bound succinyl phosphate intermediates (Buck and Guest, 1989). SucD also plays role in tricarboxylic acid cycle and ATP binding mechanism. The succinyl-CoA synthetase of *E. coli* is encoded by two genes, sucC (fi subunit) and sucD (a subunit), which are located in the sucABCD operon. They are expressed from the suc promoter. This promoter also expresses the dehydrogenase and dihydrolipoyl succinyl-transferase subunits of the 2-oxoglutarate dehydrogenase complex. SucD expression increased with berberine.

Proteins spots **I2** and **I3** were identified as **succinate dehydrogenase flavoprotein subunit (**DHSA_ECOLI). DHSA is one of the enzymes that takes place in pathways of carbohydrate metabolism, tricarboxylic acid cycle, and synthesis of fumarate from succinate. It is located in inner cell membrane. DHSA expression was increased by the addition of berberine.

Protein spot numbered **P1** was identified as **type-1 fimbrial protein, A chain**, FIMA1_ECOLI. It is located in fibrium of bacteria and it is important in cell adhesion mechanisms. Type-1A pilin expression increased with the addition of berberine. Type

1 pili are a class of appendages of *Escherichia coli* and other gram-negative bacteria that let bacteria to attach a variety of eucaryotic cells my mediating a mannose-sensitive attachment (Old, 1972). Type 1 pili is thought to explain the role of adhesion pathogensis of extraintestinal infectious disease caused by E. coli (Orndorff and Falkow, 1985). Sun et al (1988) reported that the presence of berberine blocked adhesion. Therefore, in the presence of berberine to overcome this problem the expression of type-1 fimbrial protein has increased.

Protein spot numbered **P2** was identified as **6,7-dimethyl-8-ribityllumazine synthase (**RISB ECO24**).** This protein is only visulized in the berberine-treated cells. Riboflavin synthase is a bifunctional enzyme complex in catalyzing the formation of riboflavin from 5-amino-6-(1'-D)-ribityl-amino-2,4(1H,3H)-pyrimidinedione and L-3,4-dihydrohy-2-butanone-4-phosphate via 6,7-dimethyl-8-lumazine. It is found in most cells as a component of the coenzymes flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN).

IV.4. ANALYSIS OF TRANSCRIPTOMIC DATA

Gene expression profiles of control and berberine-treated cells were also compared using microarray technology. As in the proteomic study, transcriptomic data from cells grown for 4.5 hours were analyzed. Since the turnover rate of *E. coli* K12 cells is around 20 minutes, it was assumed that the transcriptomic data would complemet each other. *Gene* expression profiles of the cell have shown that 342 genes were up -regulated and 193 genes were down -regulated based on their roles in biological processes. Results according to functions of the cells has shown that 266 genes were up-regulated and 381 genes were down-regulated. In addition, according to the molecular functions, 13 genes were up-regulated and 20 genes were down-regulated (See Appendix C for classified list of down-regulated genes and Appendix D for classified list of up-regulated genes).

Comparison of the genes based on their up/down-regulation has shown that, there was a significant difference related to the number of genes involve in amino acid biosynthesis mechanism, 44 genes were up-regulated whereas only 5 genes were down-regulated. When we looked genes related to amino acid biosynthesis in detail, histidine and tryptophan were found to be up-regulated significantly. A detailed analysis of the up-regulated genes in amino acid metabolism has shown that, genes related to the biosynthesis of histidine and tryptophan were found to be up-regulated significantly.

Trancriptomic results has shown that catabolic processes increased in berberine-treated cells, 114 genes specifically in carbonhydrate catabolic processes were up-regulated and only 30 genes were down-regulated. It has been expected that for drug response genes to be up-regulated, but based on the results only 4 genes were up-regulated but 10 genes were down-regulated. In addition to that 30 genes were up-regulated but 10 genes were down-regulated which were involved in other response mechanisms such as starvation, oxidative stress, temperature and pH. Genes related to transporter and membrane proteins were generally down-regulated. This may be a result of the cell defence mechanism that cell used to try to prevent berberine passage through the cell membrane. The distribution of the number of genes in control and berberine-treated cells classified according to their biological processes and functions in the cell is given in the *Figure16.*

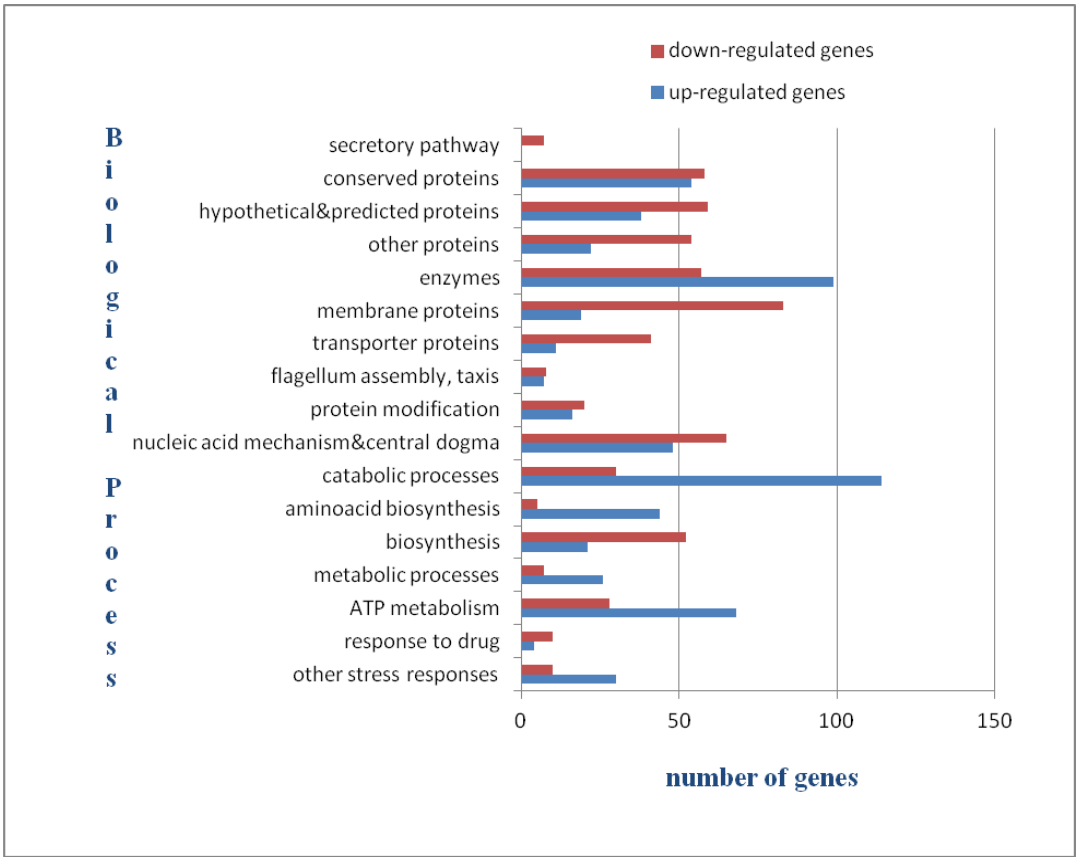


Figure 17: *Gen Expression Profiles of E.coli K12 control group vs berberine-treated group*

Great many of genes related to anaerobic respiration were found to be up-regulated. This can be explained by the fact that cells reduced their metabolic activity to cope with stress condition.

Genes related to transporter and membrane proteins were generally down-regulated. This may be a result of the cell defence mechanism which cells used to prevent berberine passage through the cell membrane (See Appendix E for the total numbers of genes according to their biological processes, functions and molecular functions).

IV.5. COMPARISION OF PROTEOMIC DATA WITH TRANSCRIPTOMIC DATA

In order to get a through ınderstanding of the effect of berberine on *E.coli* K12 cells, proteins identified as differentially expressed due to the drug treatment were compared with gene expression profiles. While some of the genes were correlated with proteomic results, some of them were not. Protein I1, SUCD, was found to be up-regulated in protein expression profiles, whereas in gene expression profiles SUCD was found to be down-regulated. This may occur due to the post-translational modifications of proteins. The outer membrane protein ompC was down-regulated, in the gene expression profile and there was a down-regulated enzyme related to ompC, induction of ompC, that is down-regulated. This protein may be down-regulated to minimize the passage of berberine through cell membrane via transporter proteins. Down-regulated protein FTSZ was up-regulated in gene expression profiles.

CHAPTER V

CONCLUDING REMARKS & RECOMMENDATIONS

V.1. CONCLUDING REMARKS

In this study, the aim was to understand the antimicrobial effect of berberine on *E. coli* K12 cells integrating transcriptomic and protemic approaches. *E. coli* has a great importance as a model organism in many research fields, such as molecular biology, genetics, and biochemistry due to its fully sequenced genome, rapid growth rate and simple nutritional requirements. The pathogenic strains of this microorganism are commonly encountered in hospitals and they constittute an important health problem.

In the presence of 750mg/L berberine the effect of the antimicrobial agent started to be measurable after 2 hours of growth. Based on this result, total mRNAs and proteins of *E. coli* K12 cells were extracted after 4.5 hours of growth and the extracts were analyzed. The transcriptomic results have shown that up-regulated genes related to catabolic processes, membrane proteins and transporter proteins, and repressed genes related to biosynthesis and nucleic acid metabolism. Proteomic profiles of cells have shown that in the presence of berberine, down-regulated proteins that were involved in glycolysis, sugar and ion transport system, cell division, and signalling pathways. On the other hand, proteins involved in electron transport system, protein and riboflavin biosynthesis, tricarboxylic acid cycle, and cell adhesion were up-regulated. Gene expression profiles of cells were determined using microarray technology. These results will provide valuable information to understand the molecular mechanism of the action of the drug candidates and to design new-target based drug candidates for future studies.

V.2. RECOMMENDATIONS

In this study, proteomic and transcriptomic profiles of E. coli K12 cells exposed to berberine for 4.5 hours were investigated. For further studies, cells can be harvested at different time points to see the effect of berberine as a fuction of time using both proteomic and transcriptomic approaches. In this study, a small number of proteins were identified, to see the effect of berberine deeply, and comment about the mechanism of the action of the alkoloid, proteomic profiles of cells can be investigated more comprehensively. In addition to that gene expression profiles of cells should be validated by quantitative real time PCR.

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APPENDIX A

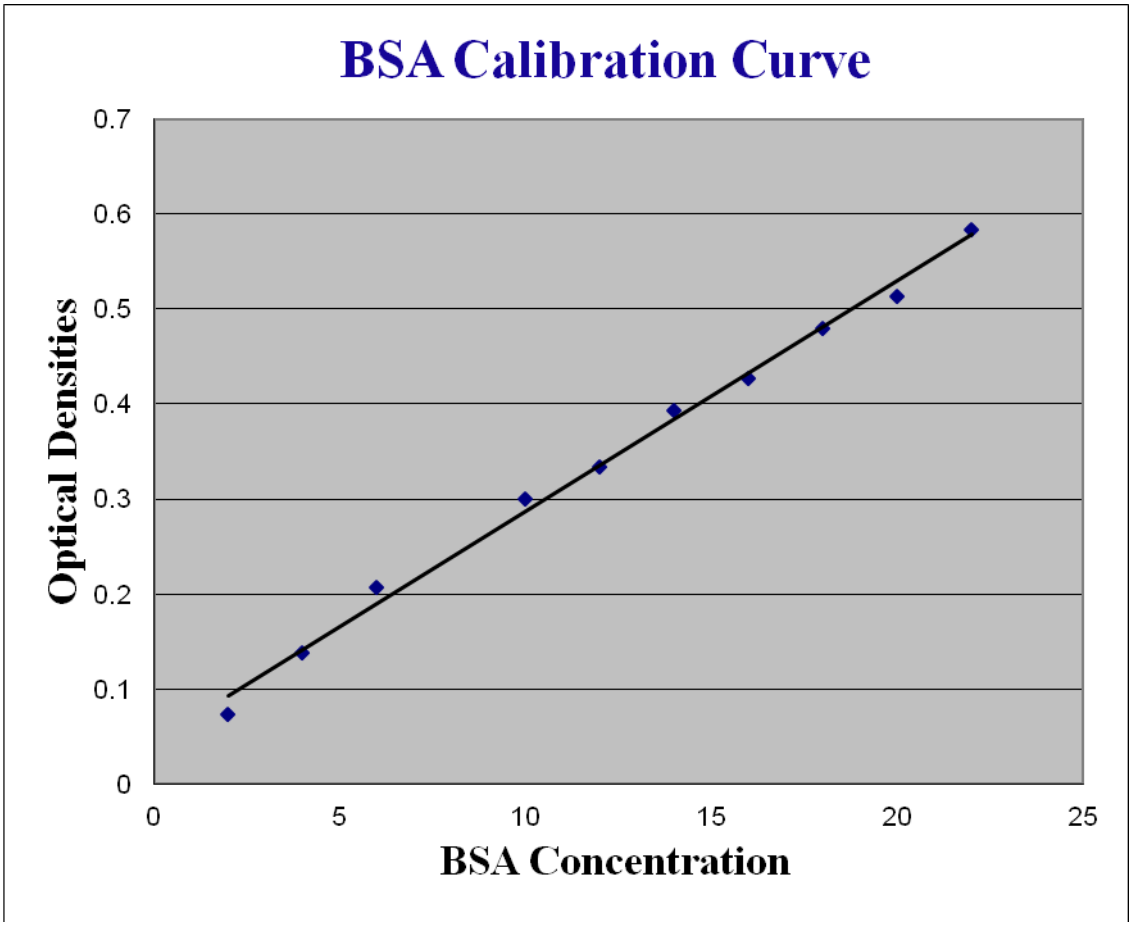

Figure 18: *BSA Calibration Curve*

APPENDIX B

Table 3: *Full List of Identified Proteins*

D1									
OK	**Accession**	**Entry**	**Description**	**mW (Da)**	**pI (pH)**	**PLGS Score**	**Peptides**	**Theoretical Peptides**	**Coverage (%)**
2	A1AFB0	A1AFB0	PGK ECOK1 Phosphoglycerate kinase OS Escherichia coli O1 K1 APEC GN pgk PE 3 SV 1	41092	4,8924	8433	112	29	78
2	P0AEX9	P0AEX9	MALE ECOLI Maltose binding periplasmic protein OS Escherichia coli strain K12 GN malE PE 1 SV 1	43360	5,3782	254	10	37	22
1	P09394	P09394	GLPQ ECOLI Glycerophosphoryl diester phosphodiesterase OS Escherichia coli strain K12 GN glpQ PE 1	40817	5,2297	169	12	25	34
0	RANDOM16652	RANDOM16652	Random Sequence 16652	30761	5,317	96	2	13	23
1	Q05523	Q05523	DACA BACST D alanyl D alanine carboxypeptidase dacA OS Bacillus stearothermophilus GN dacA PE 1 SV 1	50969	9,6839	78	8	41	13
1	A1AGI6	A1AGI6	RPOA ECOK1 DNA directed RNA polymerase subunit alpha OS Escherichia coli O1 K1 APEC GN rpoA PE 3 S	36489	4,7803	65	5	28	14
D2									
2	P0AEX9	P0AEX9	MALE ECOLI Maltose binding periplasmic protein OS Escherichia coli strain K12 GN malE PE 1 SV 1	43360	5,3782	6391	107	37	83
2	A1AFB0	A1AFB0	PGK ECOK1 Phosphoglycerate kinase OS Escherichia coli O1 K1 APEC GN pgk PE 3 SV 1	41092	4,8924	1334	7	29	25
D3									
2	P0AEX9	P0AEX9	MALE ECOLI Maltose binding periplasmic protein OS Escherichia coli strain K12 GN malE PE 1 SV 1	43360	5,3782	8894	151	37	83
D7									
2	P0AEX9	P0AEX9	MALE ECOLI Maltose binding periplasmic protein OS Escherichia coli strain K12 GN malE PE 1 SV 1	43360	5,3782	5603	60	37	80
1	P39377	P39377	IADA ECOLI Isoaspartyl dipeptidase OS Escherichia coli strain K12 GN iadA PE 1 SV 1	41058	4,9149	147	7	26	32

D4									
2	P21420	P21420	NMPC ECOLI Putative outer membrane porin protein nmpC OS Escherichia coli strain K12 GN nmpC PE 5	40277	4,4469	4731	94	22	67
D5									
2	P0A9A6	P0A9A6	FTSZ ECOLI Cell division protein ftsZ OS Escherichia coli strain K12 GN ftsZ PE 1 SV 1	40298	4,4385	13120	130	32	98
1	P0A9S5	P0A9S5	GLDA ECOLI Glycerol dehydrogenase OS Escherichia coli strain K12 GN gldA PE 1 SV 1	38687	4,604	161	5	19	19
D6									
2	P06996	P06996	OMPC ECOLI Outer membrane protein C OS Escherichia coli strain K12 GN ompC PE 1 SV 1	40343	4,3848	2218	41	26	46
I1									
2	P0AGE9	P0AGE9	SUCD ECOLI Succinyl CoA ligase ADP forming subunit alpha OS Escherichia coli strain K12 GN sucD	29758	6,343	8938	62	22	67
2	P0AFE0	P0AFE0	NUOJ ECOLI NADH quinone oxidoreductase subunit J OS Escherichia coli strain K12 GN nuoJ PE 1 SV 1	19861	5,4109	218	7	7	4
I2									
1	P0AGE9	P0AGE9	SUCD ECOLI Succinyl CoA ligase ADP forming subunit alpha OS Escherichia coli strain K12 GN sucD	29758	6,343	272	5	22	19
1	P0AC41	P0AC41	DHSA ECOLI Succinate dehydrogenase flavoprotein subunit OS Escherichia coli strain K12 GN sdhA PE	64381	5,8114	159	13	45	21
I3									
2	P0AC41	P0AC41	DHSA ECOLI Succinate dehydrogenase flavoprotein subunit OS Escherichia coli strain K12 GN sdhA PE	64381	5,8114	3848	63	45	75
A2									
2	P03841	P03841	MALM ECOLI Maltose operon periplasmic protein OS Escherichia coli strain K12 GN malM PE 4 SV 1	31923	8,4641	8867	33	15	40
2	P26646	P26646	YHDH ECOLI Putative quinone oxidoreductase yhdH OS Escherichia coli strain K12 GN yhdH PE 1 SV 1	34701	5,5325	1708	21	23	56
2	B7LHU4	B7LHU4	MDH ECO55 Malate dehydrogenase OS Escherichia coli strain 55989 EAEC GN mdh PE 3 SV 1	32290	5,4611	1032	21	22	79
2	A7ZHR0	A7ZHR0	EFTS ECO24 Elongation factor Ts OS Escherichia coli O139 H28 strain E24377A ETEC GN tsf PE 3 SV	30403	5,0349	678	14	31	36
2	P00805	P00805	ASPG2 ECOLI L asparaginase 2 OS Escherichia coli strain K12 GN ansB PE 1 SV 2	36827	5,9233	517	12	23	34

1	P0AFE0	P0AFE0	NUOJ ECOLI NADH quinone oxidoreductase subunit J OS Escherichia coli strain K12 GN nuoJ PE 1 SV 1	19861	5,4109	162	4	7	4
1	P0AEE5	P0AEE5	DGAL ECOLI D galactose binding periplasmic protein OS Escherichia coli strain K12 GN mglB PE 1 SV	35690	5,5833	136	6	31	33
1	B1XCN6	B1XCN6	NORR ECODH Anaerobic nitric oxide reductase transcription regulator norR OS Escherichia coli strain	55202	5,5393	76	8	40	15
0	RANDOM19209	RANDOM19209	Random Sequence 19209	22771	4,7919	61	1	13	16
0	P0AC41	P0AC41	DHSA ECOLI Succinate dehydrogenase flavoprotein subunit OS Escherichia coli strain K12 GN sdhA PE	64381	5,8114	44	2	45	5
0	RANDOM8017	RANDOM8017	Random Sequence 8017	38629	6,8721	40	2	28	10
0	P42592	P42592	YGJK ECOLI Uncharacterized protein ygjK OS Escherichia coli strain K12 GN ygjK PE 1 SV 1	88265	5,7468	36	4	64	10
0	P0AAI3	P0AAI3	FTSH ECOLI Cell division protease ftsH OS Escherichia coli strain K12 GN hflB PE 1 SV 1	70663	5,8341	22	4	54	7
D8									
2	P00805	P00805	ASPG2 ECOLI L asparaginase 2 OS Escherichia coli strain K12 GN ansB PE 1 SV 2	36827	5,9233	9456	82	23	70
2	A7ZUC9	A7ZUC9	K6PF ECO24 6 phosphofructokinase OS Escherichia coli O139 H28 strain E24377A ETEC GN pfkA PE 3 S	34819	5,3474	808	16	27	36
2	P0A910	P0A910	OMPA ECOLI Outer membrane protein A OS Escherichia coli strain K12 GN ompA PE 1 SV 1	37177	5,967	483	12	23	45
1	RANDOM1597	RANDOM1597	Random Sequence 1597	53849	5,4714	93	9	35	4
P1									
2	P04128	P04128	FIMA1 ECOLI Type 1 fimbrial protein A chain OS Escherichia coli strain K12 GN fimA PE 1 SV 2	18100	4,9266	6365	5	6	43
1	A1AIF7	A1AIF7	RL10 ECOK1 50S ribosomal protein L10 OS Escherichia coli O1 K1 APEC GN rplJ PE 3 SV 1	17700	9,4627	263	3	15	24
1	A1AJ55	A1AJ55	EFP ECOK1 Elongation factor P OS Escherichia coli O1 K1 APEC GN efp PE 3 SV 1	20578	4,7003	199	4	9	14
1	P00805	P00805	ASPG2 ECOLI L asparaginase 2 OS Escherichia coli strain K12 GN ansB PE 1 SV 2	36827	5,9233	132	4	23	20
1	P0AFE0	P0AFE0	NUOJ ECOLI NADH quinone oxidoreductase subunit J OS Escherichia coli strain K12 GN nuoJ PE 1 SV 1	19861	5,4109	113	6	7	6

0	P28590	P28590	ABRC ABRPR Abrin c OS Abrus precatorius PE 1 SV 1	62778	5,8775	35	4	35	4
0	A7ZPY2	A7ZPY2	HCAF ECO24 3 phenylpropionate cinnamic acid dioxygenase subunit beta OS Escherichia coli O139 H28 s	20566	6,2422	29	4	18	6
0	RANDOM27566	RANDOM27566	Random Sequence 27566	38538	5,051	18	2	25	4
0	RANDOM19460	RANDOM19460	Random Sequence 19460	79936	8,9698	16	7	57	8
P2									
2	P04128	P04128	FIMA1 ECOLI Type 1 fimbrial protein A chain OS Escherichia coli strain K12 GN fimA PE 1 SV 2	18100	4,9266	4087	4	6	62
2	A7ZIG8	A7ZIG8	RISB ECO24 6 7 dimethyl 8 ribityllumazine synthase OS Escherichia coli O139 H28 strain E24377A ET	16146	4,9753	953	6	10	60

APPENDIX C

Table 4: *Classified List of Down-regulated Genes*

	NAME	FUNCTION	DESCRIPTION	BIOLOGICAL PROCESS	CELLULAR COMPONENT	MOLECULAR FUNCTION
RESPONSE TO DRUG						
	predicted inner membrane protein	predicted inner membrane protein	regulates ampC	response to drug	organelle inner membrane, peptidoglycan-based cell wall	
	predicted transporter	predicted transporter	putative transport protein	response to drug	organelle inner membrane, peptidoglycan-based cell wall	
	transpeptidase involved in peptidoglycan synthesis (penicillin-binding protein 2)	transpeptidase involved in peptidoglycan synthesis (penicillin-binding protein 2)	cell elongation, e phase; peptidoglycan synthetase; penicillin-binding protein 2	peptidoglycan biosynthetic process, response to drug	organelle inner membrane, peptidoglycan-based cell wall	
	DNA-binding transcriptional dual activator of multiple antibiotic resistance	DNA-binding transcriptional dual activator of multiple antibiotic resistance	multiple antibiotic resistance; transcriptional activator of defense systems	response to drug, xenobiotic metabolic process	cytoplasm	transcription activator activity
	predicted protein	predicted protein	multiple antibiotic resistance protein	response to drug, xenobiotic metabolic process		
	inner membrane protein	predicted inner membrane protein	putative resistance protein	response to drug		
	inactive two-component system connector protein	polymyxin resistance protein B		response to drug		
	DNA-binding transcriptional regulator	DNA-binding transcriptional regulator	putative transcriptional regulator	response to drug	cytoplasm	transcription repressor activity

	multidrug efflux system protein	multidrug efflux system protein	integral transmembrane protein; acridine resistance	response to drug	organelle inner membrane, peptidoglycan-based cell wall	
	predicted chloramphenical resistance permease	predicted chloramphenical resistance permease		chloramphenicol transport, response to drug		
OTHER STRESS RESPONSES						
response to stress						
	RseC protein involved in reduction of the SoxR iron-sulfur cluster	RseC protein involved in reduction of the SoxR iron-sulfur cluster	sigma-E factor, negative regulatory protein	response to starvation	organelle inner membrane, peptidoglycan-based cell wall	
	stationary phase growth adaptation protein	heat shock protein	heat shock protein htrC	response to temperature stimulus		
	phosphoglycerol transferases I and II	phosphoglycerol transferase I		glucan biosynthetic process, phospholipid biosynthetic process, response to osmotic stress	organelle inner membrane, peptidoglycan-based cell wall	
SOS response						
	DNA translocase at septal ring sorting daughter chromsomes	DNA-binding membrane protein required for chromosome resolution and partitioning	cell division protein	SOS response	organelle inner membrane, peptidoglycan-based cell wall	
	conserved protein regulated by LexA	conserved protein regulated by LexA		DNA repair, SOS response	cytoplasm	
	DNA-damage-inducible SOS response protein	DNA-damage-inducible SOS response protein	DNA-damage-inducible protein F	DNA repair, SOS response, response to radiation	organelle inner membrane, peptidoglycan-based cell wall	
	toxic peptide	conserved protein		SOS response		

	regulated by antisense sRNA symR					
response to pH						
	sodium-proton antiporter	sodium-proton antiporter	Na+/H antiporter, pH dependent	response to pH	organelle inner membrane, peptidoglycan-based cell wall	
	DNA-binding transcriptional activator	DNA-binding transcriptional activator	transcriptional activator of nhaA	response to pH	cytoplasm	transcription activator activity
ENERGY METABOLISM						
ATP synthesis						
	flagellum-specific ATP synthase	flagellum-specific ATP synthase		ATP synthesis coupled proton transport	flagellum	
	ATP synthase, membrane-bound accessory factor	ATP synthase, membrane-bound accesory subunit	membrane-bound ATP synthase, dispensable protein, affects expression of atpB	ATP synthesis coupled proton transport	organelle inner membrane, peptidoglycan-based cell wall	
	magnesium transporter	magnesium transporter	Mg2+ transport ATPase, P-type 1	ATP synthesis coupled proton transport	organelle inner membrane, peptidoglycan-based cell wall	ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism
glycolysis						
	lysine decarboxylase 2, constitutive	lysine decarboxylase 2, constitutive		carbohydrate catabolic process, glycolysis		
	phosphatase	phosphoglyceromutase 2, co-factor independent	phosphoglyceromutase 2	gluconeogenesis, glycolysis	cytoplasm	
respiration						

	predicted membrane-bound lytic murein transglycosylase D	predicted membrane-bound lytic murein transglycosylase D		anaerobic respiration, cytochrome complex assembly	cytoplasm	transcription activator activity
	formate dehydrogenase formation protein	formate dehydrogenase formation protein	affects formate dehydrogenase-N	anaerobic respiration, protein folding		
	protoheme IX farnesyltransferase	protoheme IX farnesyltransferase	protoheme IX farnesyltransferase (haeme O biosynthesis)	aerobic respiration	organelle inner membrane, peptidoglycan-based cell wall	
	succinate dehydrogenase, FeS subunit	succinate dehydrogenase, FeS subunit	succinate dehydrogenase, iron sulfur protein	aerobic respiration, tricarboxylic acid cycle	cytoplasm	
	o-succinylbenzoate-CoA ligase	o-succinylbenzoate-CoA ligase		anaerobic respiration		
	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	3-octaprenyl-4-hydroxybenzoate carboxy-lyase		aerobic respiration		
	DNA-binding transcriptional activator, formate sensing	DNA-binding transcriptional activator, formate sensing	putative 2-component regulator, interaction with sigma 54	anaerobic respiration	cytoplasm	transcription activator activity
	flavodoxin 2	flavodoxin 2		aerobic respiration		electron carrier activity
	dual role activator/repressor for lldPRD operon	DNA-binding transcriptional repressor	transcriptional regulator	aerobic respiration	cytoplasm	transcription repressor activity
	formate dehydrogenase-O, cytochrome b556 subunit	formate dehydrogenase-O, cytochrome b556 subunit	formate dehydrogenase, cytochrome B556 (FDO) subunit	anaerobic respiration, cytochrome complex assembly	organelle inner membrane, peptidoglycan-based cell wall	
	1,4-dihydroxy-2-naphthoate octaprenyltransferase	1,4-dihydroxy-2-naphthoate octaprenyltransferase	1,4-dihydroxy-2-naphthoate --> dimethylmenaquinone	anaerobic respiration	organelle inner membrane, peptidoglycan-based cell wall	
	DNA-binding	DNA-binding		anaerobic	cytoplasm	

	response regulator in two-component regulatory system with DcuS	response regulator in two-component regulatory system with DcuS		respiration		
	hydrogenase 2 accessory protein	hydrogenase 2 accessory protein	hydrogenase-2 operon protein: may effect maturation of large subunit of hydrogenase-2	anaerobic respiration, protein folding		
CENTRAL DOGMA						
replication						
	DNA polymerase III alpha subunit	DNA polymerase III alpha subunit	DNA polymerase III, alpha subunit	DNA-dependent DNA replication	cytoplasm	
	ribonuclease HI, degrades RNA of DNA-RNA hybrids	ribonuclease HI, degrades RNA of DNA-RNA hybrids	RNase HI, degrades RNA of DNA-RNA hybrids, participates in DNA replication	DNA-dependent DNA replication, RNA catabolic process, proteolysis	cytoplasm	
	DNA polymerase III epsilon subunit	DNA polymerase III epsilon subunit	DNA polymerase III, epsilon subunit	DNA-dependent DNA replication, protein folding	cytoplasm	
	DNA polymerase III, theta subunit	DNA polymerase III, theta subunit		DNA-dependent DNA replication	cytoplasm	
	DNA primase	DNA primase	DNA biosynthesis; DNA primase	DNA-dependent DNA replication	cytoplasm	
	ATP-dependent DNA helicase	ATP-dependent DNA helicase	DNA helicase, resolution of Holliday junctions, branch migration	DNA-dependent DNA replication	cytoplasm	
	FMN-binding protein MioC	FMN-binding protein MioC	initiation of chromosome replication	DNA-dependent DNA replication		
	DNA polymerase IV	DNA polymerase IV	DNA polymerase IV DNA polymerase IV, devoid of proofreading, damage-inducible	DNA-dependent DNA replication, SOS response	cytoplasm	

			protein P			
	Primosome factor n' (replication factor Y)	Primosome factor n' (replication factor Y)	primosomal protein N'(= factor Y)(putative helicase)	DNA-dependent DNA replication	cytoplasm	
regulation of transcription						
	DNA-binding transcriptional dual regulator, fatty-acyl-binding	DNA-binding transcriptional dual regulator, fatty-acyl-binding	transcriptional regulator of succinylCoA synthetase operon	regulation of transcription, DNA-dependent, tricarboxylic acid cycle	cytoplasm	transcription repressor activity
	sigma 54-dependent transcriptional regulator of rtcBA expression	sigma 54-dependent transcriptional regulator of rtcBA expression	sigma54-dependent regulator of rtcBA expression	regulation of transcription, DNA-dependent	cytoplasm	
	predicted DNA-binding transcriptional regulator	predicted DNA-binding transcriptional regulator	putative DEOR-type transcriptional regulator	regulation of transcription, DNA-dependent		
	conserved protein, UPF0438 family	conserved protein		regulation of transcription, DNA-dependent		
	predicted DNA-binding transcriptional regulator; KpLE2 phage-like element	KpLE2 phage-like element; predicted DNA-binding transcriptional regulator		regulation of transcription, DNA-dependent		
	DNA-binding transcriptional activator for yjjMN; mutants fail to grow on L-galactonate	predicted DNA-binding transcriptional regulator		regulation of transcription, DNA-dependent		
translation						
	translation initiation factor IF-1	translation initiation factor IF-1	protein chain initiation factor IF-1	translation	cytoplasm	
	50S ribosomal subunit protein L19	50S ribosomal subunit protein L19		translation	cytoplasm	structural constituent of

						ribosome
	50S ribosomal subunit protein L17	50S ribosomal subunit protein L17		translation	cytoplasm	structural constituent of ribosome
	50S ribosomal subunit protein L36	50S ribosomal subunit protein L36		translation	cytoplasm	structural constituent of ribosome
	conserved protein, UPF0131 family	predicted mRNA endoribonuclease		translation		
	DNA-binding transcriptional activator for csgBA	DNA-binding transcriptional activator in two-component regulatory system	putative 2-component transcriptional regulator for 2nd curli operon			transcription activator activity
	quorum-sensing transcriptional activator	DNA-binding transcriptional activator	transcriptional regulator of ftsQAZ gene cluster		cytoplasm	transcription activator activity
	DNA-binding anti-FtsZ division inhibitor	division inhibitor	putative transcriptional regulator			transcription activator activity, transcription repressor activity
	DNA-binding transcriptional dual regulator	DNA-binding transcriptional dual regulator	positive regulator for ilvC		cytoplasm	transcription activator activity, transcription repressor activity
	DNA-binding transcriptional regulator	DNA-binding transcriptional regulator	transcriptional regulator of the flhDC operon		cytoplasm	transcription repressor activity
NUCLEIC ACID METABOLISM						
DNA recombination						
	ATP-dependent DNA	ATP-dependent DNA	Holliday junction	DNA	cytoplasm	

	helicase, component of RuvABC resolvasome	helicase, component of RuvABC resolvasome	helicase subunit A; branch migration; repair	recombination, DNA repair, SOS response		
	gap repair protein	gap repair protein	protein interacts with RecR and possibly RecF proteins	DNA recombination, DNA repair	cytoplasm	
	gap repair protein	gap repair protein	ssDNA and dsDNA binding, ATP binding	DNA recombination, DNA repair, DNA-dependent DNA replication	cytoplasm	
RNA modification						
	tRNA-specific adenosine deaminase	tRNA-specific adenosine deaminase		RNA modification		
	ribonuclease G	ribonuclease G		RNA modification	cytoplasm	
	tRNA mG18-2'-O-methyltransferase, SAM-dependen	tRNA (Guanosine-2'-O-)-methyltransferase		RNA modification		
	tRNA Leu mC34/mU34 2'-O-methyltransferase, SAM-dependent	predicted rRNA methylase		RNA modification		
nucleobase-containing small molecule interconversion						
	deoxyuridinetriphosphatase	deoxyuridinetriphosphatase		nucleobase-containing small molecule interconversion		
	diadenosine tetraphosphatase	diadenosine tetraphosphatase		nucleobase-containing small molecule interconversion	cytoplasm	
	uridylate kinase	uridylate kinase		nucleobase-containing small molecule	cytoplasm	

				interconversion		
	DNA-binding transcriptional repressor	DNA-binding transcriptional repressor	transcriptional repressor for deo operon, tsx, nupG	nucleobase-containing small molecule interconversion	cytoplasm	transcription repressor activity
DNA catabolic process						
	DNA glycosylase and apyrimidinic (AP) lyase (endonuclease III)	DNA glycosylase and apyrimidinic (AP) lyase (endonuclease III)	endonuclease III; specific for apurinic and/or apyrimidinic sites	DNA catabolic process, response to radiation	cytoplasm	
	DNA exonuclease X	DNA exonuclease X		DNA catabolic process, DNA repair	cytoplasm	
	DNA mismatch endonuclease of very short patch repair	DNA mismatch endonuclease of very short patch repair	DNA mismatch endonuclease, patch repair protein	DNA catabolic process, DNA repair		
	oxidative demethylase of N1-methyladenine or N3-methylcytosine DNA lesions	oxidative demethylase of N1-methyladenine or N3-methylcytosine DNA lesions	DNA repair system specific for alkylated DNA	DNA repair	cytoplasm	
	exonuclease VII, large subunit	exonuclease VII, large subunit		DNA catabolic process		
	Ssb-binding protein, misidentified as ExoIX	exonuclease IX (5'-3' exonuclease)	5'-3' exonuclease	DNA catabolic process		
RNA catabolic process						
	ribonuclease HII, degrades RNA of DNA-RNA hybrids	ribonuclease HII, degrades RNA of DNA-RNA hybrids	RNAse HII, degrades RNA of DNA-RNA hybrids	RNA catabolic process	cytoplasm	
nucleotide biosynthetic process						
	orotidine-5'-phosphate	orotidine-5'-phosphate		nucleobase-containing small		

	decarboxylase	decarboxylase		molecule interconversion, pyrimidine nucleotide biosynthetic process		
	orotate phosphoribosyltransferase	orotate phosphoribosyltransferase		pyrimidine nucleotide biosynthetic process	cytoplasm	
	pyrBI operon leader peptide	pyrBI operon leader peptide		pyrimidine nucleotide biosynthetic process	cytoplasm	
AMINOACID BIOSYNTHESIS						
aspartate biosynthetic process						
	glutamate, aspartate ABC transporter ATP-binding subunit	glutamate and aspartate transporter subunit	ATP-binding protein of glutamate/aspartate transport system	aspartate biosynthetic process, glutamate biosynthetic process	cytoplasm	
cysteine biosynthetic process						
	DNA-binding transcriptional activator for the ssuEADCB and tauABCD operons	DNA-binding transcriptional activator of cysteine biosynthesis	transcriptional regulator cys regulon; accessory regulatory circuit affecting cysM	cysteine biosynthetic process, sulfur compound metabolic process	cytoplasm	transcription activator activity, transcription repressor activity
methionine biosynthetic process						
	homoserine O-	homoserine O-		methionine	cytoplasm	

	transsuccinylase	transsuccinylase		biosynthetic process		
tyrosine biosynthetic process						
	tyrosine transporter	tyrosine transporter	tyrosine-specific transport system	tyrosine biosynthetic process	organelle inner membrane, peptidoglycan-based cell wall	
arginine biosynthetic process						
	histidine/lysine/arginine/ornithine transporter subunit	histidine/lysine/arginine/ornithine transporter subunit	histidine transport, membrane protein M; membrane component of ABC superfamily	arginine biosynthetic process, lysine biosynthetic process via diaminopimelate	organelle inner membrane, peptidoglycan-based cell wall	
LIPID AND PHOSPHOLIPID METABOLISM						
lipid biosynthetic process						
	tetraacyldisaccharide-1-P synthase	tetraacyldisaccharide-1-P synthase	tetraacyldisaccharide-1-P; lipid A biosynthesis, penultimate step	lipid A biosynthetic process	cytoplasm, peptidoglycan-based cell wall	cell surface antigen activity, host-interacting
	octanoyltransferase; octanoyl-[ACP]:protein N-octanoyltransferase	lipoyl-protein ligase	protein of lipoate biosynthesis	lipoate biosynthetic process		
phospholipid biosynthetic process						
	CDP-diglyceride synthase	CDP-diglyceride synthase	CDP-diglyceride synthetase	phospholipid biosynthetic process	organelle inner membrane, peptidoglycan-based	

					cell wall	
	phosphatidylserine synthase (CDP-diacylglycerol-serine O-phosphatidyltransferase)	phosphatidylserine synthase (CDP-diacylglycerol-serine O-phosphatidyltransferase)	phosphatidylserine synthase; phospholipid synthesis	phospholipid biosynthetic process	organelle inner membrane, peptidoglycan-based cell wall	
	phosphatidylglycerophosphatase A	phosphatidylglycerophosphatase A	phosphatidylglycerophosphatase	phospholipid biosynthetic process	organelle inner membrane, peptidoglycan-based cell wall	
CARBOHYDRATE CATABOLIC PROCESSES						
carbohydrate mechanism						
	2,5-diketo-D-gluconate reductase B	2,5-diketo-D-gluconate reductase B		carbohydrate catabolic process	cytoplasm	
	arabinose-inducible predicted transporter, MFS family	predicted transporter	involved in either transport or processing of arabinose polymers	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	maltose O-acetyltransferase	maltose O-acetyltransferase		carbohydrate catabolic process	cytoplasm	
	fused 2-O-a-mannosyl-D-glycerate specific PTS enzymes: IIA component/IIB component/IIC component	fused 2-O-a-mannosyl-D-glycerate specific PTS enzymes: IIA component/IIB component/IIC component	protein modification enzyme, induction of ompC	carbohydrate catabolic process, protein modification process, response to temperature stimulus	organelle inner membrane, peptidoglycan-based cell wall	
	alpha-mannosidase	alpha-mannosidase	putative sugar hydrolase	carbohydrate catabolic process, protein modification process, response to temperature	organelle inner membrane, peptidoglycan-based cell wall	

				stimulus		
	formate channel	formate transporter	probable formate transporter (formate channel 1)	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	xanthosine transporter	xanthosine transporter	xanthosine permease	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	alpha-ketoglutarate transporter	alpha-ketoglutarate transporter	alpha-ketoglutarate permease	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	L-fucose transporter	L-fucose transporter	fucose permease	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	DNA-binding transcriptional activator of maltose metabolism	DNA-binding transcriptional activator of maltose metabolism	regulatory factor of maltose metabolism; similar to Ner repressor protein of phage Mu	carbohydrate catabolic process	cytoplasm	transcription activator activity
	membrane protein regulates uhpT expression	membrane protein regulates uhpT expression	regulator of uhpT	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	sensory histidine kinase in two-component regulatory sytem with UhpA	sensory histidine kinase in two-component regulatory sytem with UhpA	sensor histidine protein kinase phosphorylates UhpA	carbohydrate catabolic process, protein modification process	organelle inner membrane, peptidoglycan-based cell wall	
	DNA-binding transcriptional repressor of ribose metabolism	DNA-binding transcriptional repressor of ribose metabolism	regulator for rbs operon	carbohydrate catabolic process	cytoplasm	transcription repressor activity
	melibiose:sodium symporter	melibiose:sodium symporter	melibiose permease II	carbohydrate catabolic process	organelle inner membrane,	

					peptidoglycan-based cell wall	
PROTEIN MODIFICATION and FOLDING						
protein modification process						
	sensory histidine kinase in two-component regulatory system with citB	sensory histidine kinase in two-component regulatory system with citB	putative sensor-type protein	protein modification process	organelle inner membrane, peptidoglycan-based cell wall	
	sensory histidine kinase in two-component regulatory system with PhoP	sensory histidine kinase in two-compoent regulatory system with PhoP	sensor protein PhoQ	protein modification process	organelle inner membrane, peptidoglycan-based cell wall	
	serine/threonine-specific protein phosphatase 1	serine/threonine-specific protein phosphatase 1		protein modification process, response to temperature stimulus	cytoplasm	
	serine/threonine-specific protein phosphatase 2	serine/threonine-specific protein phosphatase 2	protein phosphatase 2	protein modification process	cytoplasm	
	L-isoaspartate protein carboxylmethyltransferase type II	L-isoaspartate protein carboxylmethyltransferase type II		protein modification process		
	quorum sensing sensory histidine kinase in two-component regulatory system with QseB	sensory histidine kinase in two-component regulatory system with QseB	putative 2-component sensor protein	protein modification process		
	phosphohistidinoprotein-hexose phosphotransferase component of N-regulated PTS system	phosphohistidinoprotein-hexose phosphotransferase component of N-regulated PTS system	phosphocarrier protein HPr-like NPr, nitrogen related, exchanges phosphate with Enzyme I, Hpr	protein modification process	cytoplasm	

	(Npr)	(Npr)				
	isocitrate dehydrogenase kinase/phosphatase	isocitrate dehydrogenase kinase/phosphatase		glyoxylate cycle, protein modification process		
	anaerobic ribonucleotide reductase activating protein	anaerobic ribonucleotide reductase activating protein		nucleobase-containing small molecule interconversion, protein modification process		
	sensory histidine kinase in two-component regulatory system with CreB or PhoB, regulator of the CreBC regulon	sensory histidine kinase in two-component regulatory system with CreB or PhoB, regulator of the CreBC regulon	catabolite repression sensor kinase for PhoB; alternative sensor for pho regulon	protein modification process	organelle inner membrane, peptidoglycan-based cell wall	
protein folding						
	predicted periplasmic pilin chaperone	predicted periplasmic pilin chaperone	probable pilin chaperone similar to PapD	protein folding	organelle inner membrane	
	Hsp70 family chaperone Hsc62, binds to RpoD and inhibits transcription	Hsp70 family chaperone Hsc62, binds to RpoD and inhibits transcription		protein folding		
	heme lyase, CcmH subunit	heme lyase, CcmH subunit	possible subunit of heme lyase	cytochrome complex assembly, protein folding	organelle inner membrane, peptidoglycan-based cell wall	
	conserved protein	conserved protein		protein folding		
	DnaJ-like molecular chaperone specific for IscU	DnaJ-like molecular chaperone specific for IscU		protein folding		
	predicted periplasmic pilin chaperone	predicted periplasmic pilin chaperone	putative membrane protein	protein folding	organelle inner membrane, peptidoglycan-based	

					cell wall	
	peptidyl-prolyl cis-trans isomerase C (rotamase C)	peptidyl-prolyl cis-trans isomerase C (rotamase C)		protein folding		
FLAGELLUM ASSEMBLY, TAXIS						
	predicted flagellar export pore protein	predicted flagellar export pore protein	flagellar biosynthesis; possible export of flagellar proteins	flagellum assembly, taxis	flagellum, organelle inner membrane, peptidoglycan-based cell wall	
	protein that enables flagellar motor rotation	protein that enables flagellar motor rotation	enables flagellar motor rotation, linking torque machinery to cell wall	taxis	flagellum, organelle inner membrane, peptidoglycan-based cell wall	
	negative regulator of FliI ATPase activity	flagellar biosynthesis protein	flagellar biosynthesis; export of flagellar proteins?	flagellum assembly, taxis	flagellum	
	flagellar protein	flagellar protein	flagellar fliJ protein	flagellum assembly, taxis	flagellum	
	flagellar hook-length control protein	flagellar hook-length control protein		flagellum assembly, taxis	flagellum	
	flagellar biosynthesis protein	flagellar biosynthesis protein	flagellar biosynthesis	flagellum assembly, taxis	flagellum	
	flagellar biosynthesis protein	flagellar biosynthesis protein	flagellar biosynthesis	flagellum assembly, taxis	flagellum	
	flagellar protein	flagellar protein	flagellar fliJ protein	flagellum assembly, taxis	flagellum	
PEPTIDOGLYCAN BIOSYNTHETIC PROCESS						
	N-acetylglucosaminyl transferase	N-acetylglucosaminyl transferase		peptidoglycan biosynthetic process	organelle inner membrane, peptidoglycan-based cell wall	

	D-alanine:D-alanine ligase	D-alanine:D-alanine ligase	D-alanine-D-alanine ligase B, affects cell division	peptidoglycan biosynthetic process	cytoplasm, peptidoglycan-based cell wall	
	transpeptidase involved in septal peptidoglycan synthesis (penicillin-binding protein 3)	transpeptidase involved in septal peptidoglycan synthesis (penicillin-binding protein 3)	septum formation; penicillin-binding protein 3; peptidoglycan synthetase	peptidoglycan biosynthetic process, response to drug	organelle inner membrane, peptidoglycan-based cell wall	
	biosynthetic peptidoglycan transglycosylase	biosynthetic peptidoglycan transglycosylase	putative peptidoglycan enzyme	peptidoglycan biosynthetic process	peptidoglycan-based cell wall	
	cell wall structural complex MreBCD transmembrane component MreD	cell wall structural complex MreBCD transmembrane component MreD	rod shape-determining protein	peptidoglycan biosynthetic process	organelle inner membrane, peptidoglycan-based cell wall	
	cell wall structural complex MreBCD transmembrane component MreC	cell wall structural complex MreBCD transmembrane component MreC	rod shape-determining protein	peptidoglycan biosynthetic process	peptidoglycan-based cell wall	
	UDP-N-acetylenolpyruvoylglucosamine reductase, FAD-binding	UDP-N-acetylenolpyruvoylglucosamine reductase, FAD-binding	UDP-N-acetylenolpyruvoylglucosamine reductase	peptidoglycan biosynthetic process	cytoplasm, peptidoglycan-based cell wall	
	beta-lactamase/D-alanine carboxypeptidase	beta-lactamase/D-alanine carboxypeptidase	beta-lactamase; penicillin resistance	peptidoglycan biosynthetic process, response to drug	peptidoglycan-based cell wall, periplasmic space	
COFACTOR BIOSYNTHESIS MECHANISM						
cofactor biosynthetic process						
	molybdopterin-guanine dinucleotide biosynthesis protein B	molybdopterin-guanine dinucleotide biosynthesis protein B		Mo-molybdopterin cofactor biosynthetic process		

	molybdopterin-guanine dinucleotide synthase	molybdopterin-guanine dinucleotide synthase	molybdopterin ---> molybdopterin-guanine dinucleotide, protein Ar	Mo-molybdopterin cofactor biosynthetic process		
Mo-molybdopterin cofactor biosynthetic process						
	molybdopterin biosynthesis protein A	molybdopterin biosynthesis protein A	molybdopterin biosynthesis, protein A	Mo-molybdopterin cofactor biosynthetic process		
	molybdopterin biosynthesis protein B	molybdopterin biosynthesis protein B	molybdopterin biosynthesis, protein B	Mo-molybdopterin cofactor biosynthetic process		
ION/ELECTRON TRANSPORT MECHANISM						
	bacterioferritin, iron storage and detoxification protein	bacterioferritin, iron storage and detoxification protein	bacterioferrin, an iron storage homoprotein	iron ion transport	cytoplasm	
	bacterioferritin-associated ferredoxin	bacterioferritin-associated ferredoxin		iron ion transport	cytoplasm	
potassium:hydrogen antiporter activity						
	potassium:proton antiporter	potassium:proton antiporter	K+ efflux antiporter, glutathione-regulated		organelle inner membrane, peptidoglycan-based cell wall	potassium:hydrogen antiporter activity
electron carrier activity						
	conserved protein	conserved protein				electron carrier activity
	electron transport	predicted iron-sulfur				electron carrier

	complex protein, iron-sulfur protein, required for the reduction of SoxR	protein				activity
	predicted electron transfer flavoprotein, FAD-binding subunit	predicted electron transfer flavoprotein, FAD-binding	putative flavoprotein			electron carrier activity
	predicted oxidoreductase with FAD/NAD(P)-binding domain	predicted oxidoreductase with FAD/NAD(P)-binding domain	flavoprotein; probably electron transport			electron carrier activity
	predicted 3Fe-4S ferredoxin-type protein	predicted 4Fe-4S ferredoxin-type protein				electron carrier activity
	[2Fe-2S] ferredoxin	[2Fe-2S] ferredoxin	[2FE-2S] ferredoxin, electron carrer protein			electron carrier activity
	predicted 4Fe-4S cluster-containing protein	predicted 4Fe-4S cluster-containing protein				electron carrier activity
	NAD(P)H-flavin reductase	flavin reductase	ferrisiderophore reductase; flavin reductase (NADPH:flavin oxidoreductase)			electron carrier activity
cytochrome complex assembly						
	divalent-cation tolerance protein, copper sensitivity	copper binding protein, copper sensitivity	divalent cation tolerance protein; cytochrome c biogenesis	cytochrome complex assembly		
	O-antigen translocase	O-antigen translocase	O-antigen translocase in LPS biosynthesis	cytochrome complex assembly		
			pseudogene, truncated cytochrome b562 cytochrome b(562)	cytochrome complex assembly		electron carrier activity

EFFLUX PROTEINS						
	predicted drug efflux system	predicted drug efflux system	putative transport protein		organelle inner membrane, peptidoglycan-based cell wall	
	predicted multdrug exporter, MATE family	predicted multidrug efflux system			organelle inner membrane, peptidoglycan-based cell wall	
	cysteine and O-acetylserine exporter	neutral amino-acid efflux system				
	p-hydroxybenzoic acid efflux system component	p-hydroxybenzoic acid efflux system component			peptidoglycan-based cell wall	
	p-hydroxybenzoic acid efflux system component	p-hydroxybenzoic acid efflux system component			organelle inner membrane, peptidoglycan-based cell wall	
	membrane fusion protein (MFP) component of efflux pump, signal anchor	membrane fusion protein (MFP) component of efflux pump, signal anchor	putative membrane protein		organelle inner membrane, peptidoglycan-based cell wall	
	predicted multidrug or homocysteine efflux system	predicted multidrug or homocysteine efflux system	putative transport protein (MFS family) high copy suppressor of RspA: relieves RspA-mediated reduction of sigma(S) levels; overexpression also reduces MetE activity; effect proposed to be via an elevation in levels of homocysteine, causing a		organelle inner membrane, peptidoglycan-based cell wall	

			concomitant i			
	multidrug efflux system protein	multidrug efflux system protein	suppresses groEL, may be chaperone		organelle inner membrane, peptidoglycan-based cell wall	
ENZYMES						
transposase						
	IS186 transposase	IS186/IS421 transposase				
	IS1 transposase B	IS1 transposase InsAB'				
	predicted transposase	predicted transposase				
	IS5 transposase and trans-activator	IS5 transposase and trans-activator				
	IS1 transposase B	IS1 transposase InsAB'				
	IS1 transposase B	IS1 transposase InsAB'				
	predicted transposase	predicted transposase	putative receptor			
			pseudogene, transposase homolog			
			IS1 transposase InsAB';IS, phage, Tn; Transposon-related functions;8.3 extrachromosomal; transposon related			
transferase						
	predicted S-adenosyl-L-methionine-dependent methyltransferase	predicted S-adenosyl-L-methionine-dependent methyltransferase				
	predicted acyltransferase with acyl-CoA N-acyltransferase domain	predicted acyltransferase with acyl-CoA N-acyltransferase domain				

	phospholipid:lipid A palmitoyltransferase	palmitoyl transferase for Lipid A				
	predicted acyltransferase	predicted acyltransferase				
	tRNA (adenine-N(6)-)-methyltransferase	predicted S-adenosyl-L-methionine-dependent methyltransferase	putative enzyme			
	DNA adenine methyltransferase, SAM-dependent	predicted methyltransferase	putative methyltransferase			
	23S rRNA C2498 ribose 2'-O-methyltransferase, SAM-dependent	predicted methyltransferase				
	predicted glycosyl transferase	predicted glycosyl transferase	putative regulator			
	16S rRNA m(7)G527 methyltransferase, SAM-dependent; glucose-inhibited cell-division protein	methyltransferase, SAM-dependent methyltransferase, glucose-inhibited cell-division protein	glucose-inhibited division; chromosome replication?			
	predicted acetyltransferase	predicted acetyltransferase				
	TDP-Fuc4NAc:lipidIIFuc4NAc transferase	TDP-Fuc4NAc:lipidIIFuc4NAc transferase	TDP-Fuc4NAc:lipidII transferase			
ligase						
	short chain acyl-CoA synthetase, anaerobic	short chain acyl-CoA synthetase, anaerobic	putative ligase/synthetase			
	conserved protein, 5-formyltetrahydrofolate cyclo-ligase family	predicted ligase	putative ligase			
permease						
	predicted permease	predicted permease				
	inner membrane protein, predicted	predicted permease			peptidoglycan-based cell wall	

	permease					
cylase						
	diguanylate cyclase for cellulose, biofilm, motility regulation	predicted diguanylate cyclase				
	predicted diguanylate cyclase	predicted diguanylate cyclase				
	predicted diguanylate cyclase	predicted diguanylate cyclase				
	predicted diguanylate cyclase	predicted diguanylate cyclase				
	predicted diguanylate cyclase	predicted diguanylate cyclase				
	probable inner membrane diguanylate cyclase	predicted diguanylate cyclase				
isomerases						
	1-deoxy-D-xylulose 5-phosphate reductoisomerase	1-deoxy-D-xylulose 5-phosphate reductoisomerase				
	isopentenyl diphosphate isomerase	isopentenyl diphosphate isomerase			cytoplasm	
peptidase						
	lipoprotein	predicted aminopeptidase	putative aminopeptidase			
	DLP12 prophage; predicted murein endopeptidase	DLP12 prophage; predicted murein endopeptidase	bacteriophage lambda endopeptidase homolog			
	predicted peptidase	predicted peptidase	putative aminopeptidase			
	S9 peptidase family protein, function unknown	predicted peptidase	putative enzyme (3.4.-)			
	bifunctional prepilin leader peptidase/ methylase	bifunctional prepilin leader peptidase/ methylase			organelle inner membrane, peptidoglycan-based	

					cell wall	
	conserved protein, DUF830 family	predicted peptidoglycan peptidase				
phosphatases						
	phosphodiesterase with model substrate bis-pNPP	predicted phosphatase				
	predicted alpha-ribazole-5'-P phosphatase	predicted alpha-ribazole-5'-P phosphatase	homolog of Salmonella cobC, a phosphohistidine protein			
	predicted membrane fusion protein (MFP) of YdhJK efflux pump	undecaprenyl pyrophosphate phosphatase	putative membrane protein		organelle inner membrane, peptidoglycan-based cell wall	
	undecaprenyl pyrophosphate phosphatase	undecaprenyl pyrophosphate phosphatase			peptidoglycan-based cell wall	
lyase						
	predicted lyase	predicted lyase				
	predicted aminodeoxychorismate lyase	predicted aminodeoxychorismate lyase	putative thymidylate kinase			
	membrane-bound lytic transglycosylase F, murein hydrolase	predicted transglycosylase	putative periplasmic binding transport protein		periplasmic space	
hydrolases						
	nucleoside triphosphate pyrophosphohydrolase, marked preference for dGTP	nucleoside triphosphate pyrophosphohydrolase, marked preference for dGTP	7,8-dihydro-8-oxoguanine-triphosphatase, prefers dGTP, causes AT-GC transversions		cytoplasm	
	conserved protein with nucleoside triphosphate hydrolase domain	conserved protein with nucleoside triphosphate hydrolase domain				

	CTP pyrophosphohydrolase; also hydrolyzes 2-hydroxy-dATP, 8-hydroxy-dGTP, CTP, dCTP and 5-methyl-dCTP	pyrimidine (deoxy)nucleoside triphosphate pyrophosphohydrolase	5-hydroxy-CTP pyrophosphohydrolase			
	predicted hydrolase	predicted hydrolase				
	elongator methionine tRNA (ac4C34) acetyltransferase	predicted hydrolase				
	predicted ATP-binding protein	predicted protein with nucleoside triphosphate hydrolase domain				
	predicted ATP-binding protein	predicted protein with nucleoside triphosphate hydrolase domain				
	predicted ATP-binding protein	predicted protein with nucleoside triphosphate hydrolase domain				
	CDP-diacylglycerol phosphotidylhydrolase	CDP-diacylglycerol phosphotidylhydrolase				
	predicted hydrolase, inner membrane	predicted hydrolase, inner membrane	putative alkaline phosphatase I		peptidoglycan-based cell wall	
kinases						
	dephospho-CoA kinase	dephospho-CoA kinase	putative DNA repair protein			
	Thr/Ser kinase implicated in Cpx stress response	Thr/Ser kinase implicated in Cpx stress response				
PROPHAGE						
			pseudogene, Rac prophage			

	Rac prophage; predicted tail fiber assembly protein	Rac prophage; predicted tail fiber assembly protein	tail fiber assembly protein homolog from lambdoid prophage Rac			
	CP4-6 prophage; predicted protein	CP4-6 prophage; predicted protein				
	CP4-6 prophage; predicted protein	CP4-6 prophage; predicted protein				
	DLP12 prophage; predicted antitermination protein	DLP12 prophage; predicted antitermination protein				
	DLP12 prophage; predicted phage lysis protein	DLP12 prophage; predicted phage lysis protein				
	DLP12 prophage; predicted lysozyme	DLP12 prophage; predicted lysozyme	bacteriophage lambda lysozyme homolog			
			pseudogene, DLP12 prophage; predicted tail fiber assembly protein			
	conserved protein, Qin prophage		Qin prophage; predicted protein			
	Qin prophage; predicted protein	Qin prophage; predicted protein				
			Qin prophage; predicted protein			
			pseudogene, Qin prophage; predicted defective integrase			
	expressed protein, DLP12 prophage	DLP12 prophage; predicted protein			cytoplasm	
	DLP12 prophage; predicted lipoprotein	DLP12 prophage; predicted lipoprotein			cell outer membrane	
	IS1 repressor TnpA	KpLE2 phage-like element; IS1 repressor protein				

		InsA				
	Rac prophage; predicted lipoprotein	Rac prophage; predicted lipoprotein			cell outer membrane	
			DLP12 prophage; predicted tail fiber assembly protein (pseudogene)			
PSEUDOGENE						
			pseudogene			
			pseudogene, Rhs family			
			pseudogene, rhsA-linked			
			pseudogene, H repeat-associated protein			
			pseudogene, P4-like integrase remnant			
			pseudogene conserved hypothetical protein			
			pseudogene, H repeat-associated protein		cytoplasm	
			pseudogene			
CELL DIVISION						
	cell division modulator	cell division modulator				
	5-methylaminomethyl-2-thiouridine modification at tRNA U34	glucose-inhibited cell-division protein	glucose-inhibited division; chromosome replication?			
SECRETORY PATHWAY						
	pseudopilin, cryptic, general secretion	pseudopilin, cryptic, general secretion				

	pathway	pathway				
	predicted general secretory pathway component, cryptic	predicted general secretory pathway component, cryptic				
	general secretory pathway component, cryptic	general secretory pathway component, cryptic				
	predicted general secretory pathway component, cryptic	predicted general secretory pathway component, cryptic				
	general secretory pathway component, cryptic	general secretory pathway component, cryptic				
	general secretory pathway component, cryptic	general secretory pathway component, cryptic				
	general secretory pathway component, cryptic	general secretory pathway component, cryptic				
FIMBRIAL-LIKE ADHESIN PROTEINS						
	predicted fimbrial-like adhesin protein	predicted fimbrial-like adhesin protein	putative fimbrial-like protein		pilus	
	predicted fimbrial-like adhesin protein	predicted fimbrial-like adhesin protein	putative fimbrial-like protein		pilus	
	predicted fimbrial-like adhesin protein	predicted fimbrial-like adhesin protein	putative fimbrial-like protein		pilus	
	predicted fimbrial-like adhesin protein	predicted fimbrial-like adhesin protein	putative fimbrial-like protein		pilus	
	predicted fimbrial-like adhesin protein	predicted fimbrial-like adhesin protein	putative fimbrial-like protein		pilus	
BIOSYNTHETIC PROCESS						
biosynthetic process						
	DNA-binding	DNA-binding	positive regulator for	K antigen	cytoplasm	cell surface

	transcriptional activator, co-regulator with RcsB	transcriptional activator, co-regulator with RcsB	ctr capsule biosynthesis, positive transcription factor	biosynthetic process, colanic acid biosynthetic process		antigen activity, host-interacting, transcription activator activity
	lipoprotein required for capsular polysaccharide translocation through the outer membrane	lipoprotein required for capsular polysaccharide translocation through the outer membrane	putative polysaccharide export protein	colanic acid biosynthetic process		
	dehydroshikimate reductase, NAD(P)-binding	dehydroshikimate reductase, NAD(P)-binding	dehydroshikimate reductase	chorismate biosynthetic process		
	leucine/isoleucine/valine transporter subunit	leucine/isoleucine/valine transporter subunit	high-affinity branched-chain amino acid transport protein (ABC superfamily, peri_bind); periplasmic-binding component of ABC superfamily	leucine biosynthetic process	periplasmic space	
	glutamate transporter	glutamate transporter	glutamate transport	aspartate biosynthetic process, glutamate biosynthetic process	organelle inner membrane, peptidoglycan-based cell wall	
	DNA-binding transcriptional dual regulator	DNA-binding transcriptional dual regulator	regulator for asnA, asnC and gidA	asparagine biosynthetic process	cytoplasm	transcription activator activity, transcription repressor activity
	fused chorismate mutase T/prephenate dehydrogenase	fused chorismate mutase T/prephenate dehydrogenase	chorismate mutase-T and prephenate dehydrogenase	L-phenylalanine biosynthetic process, tyrosine biosynthetic process	cytoplasm	

	3-deoxy-D-arabino-heptulosonate-7-phosphate synthase, tyrosine-repressible	3-deoxy-D-arabino-heptulosonate-7-phosphate synthase, tyrosine-repressible	3-deoxy-D-arabinoheptulosonate-7-phosphate synthase (DAHP synthetase, tyrosine repressible)	chorismate biosynthetic process		
	threonine efflux pump	threonine efflux system	amino acid exporter (threonine) threonine efflux protein (RhtB family)	threonine biosynthetic process	organelle inner membrane	
	pantothenate kinase	pantothenate kinase		coenzyme A biosynthetic process		
	predicted Wzy protein involved in ECA polysaccharide chain elongation	predicted Wzy protein involved in ECA polysaccharide chain elongation	putative ECA polymerization protein	enterobacterial common antigen biosynthetic process	organelle inner membrane	cell surface antigen activity, host-interacting
lipopolysaccharide biosynthetic process						
	conserved protein, pfam09612 family	predicted protein	involved in lipopolysaccharide biosynthesis	lipopolysaccharide biosynthetic process		cell surface antigen activity, host-interacting
	O-antigen ligase	O-antigen ligase	O-antigen ligase; lipopolysaccharide core biosynthesis	lipopolysaccharide core region biosynthetic process	organelle inner membrane, peptidoglycan-based cell wall	cell surface antigen activity, host-interacting
	lipopolysaccharide core biosynthesis	lipopolysaccharide core biosynthesis	probably hexose transferase; lipopolysaccharide core biosynthesis	lipopolysaccharide core region biosynthetic process	organelle inner membrane	cell surface antigen activity, host-interacting
	DNA-binding transcriptional antiterminator	DNA-binding transcriptional antiterminator	transcriptional activator affecting biosynthesis of lipopolysaccharide core, F pilin, and haemolysin	lipopolysaccharide core region biosynthetic process, pilus assembly	cytoplasm, pilus	cell surface antigen activity, host-interacting, transcription activator activity

lipoprotein biosynthetic process						
	apolipoprotein N-acyltransferase	apolipoprotein N-acyltransferase	apolipoprotein N-acyltransferase, copper homeostasis protein, inner membrane	lipoprotein biosynthetic process	organelle inner membrane, peptidoglycan-based cell wall	
glycoprotein biosynthetic process						
	flagellar biosynthesis protein	flagellar biosynthesis protein	flagellar biosynthesis	flagellum assembly, glycoprotein biosynthetic process	flagellum, peptidoglycan-based cell wall	
	predicted protein	predicted protein	prepilin peptidase dependent protein C	glycoprotein biosynthetic process	pilus	
	minor component of type 1 fimbriae	minor component of type 1 fimbriae	minor fimbrial subunit, D-mannose specific adhesin	glycoprotein biosynthetic process	pilus	
biotin biosynthetic process						
	predicted dethiobiotin synthetase	predicted dethiobiotin synthetase		biotin biosynthetic process		
	conserved protein, DUF1407 family	predicted protein		biotin carboxyl carrier protein biosynthetic process		
	bifunctional biotin-[acetylCoA carboxylase] holoenzyme synthetase/ DNA-binding transcriptional repressor, bio-5'-	bifunctional biotin-[acetylCoA carboxylase] holoenzyme synthetase/ DNA-binding transcriptional repressor, bio-5'-	biotin-[acetylCoA carboxylase] holoenzyme synthetase and biotin operon repressor	biotin biosynthetic process	cytoplasm	transcription repressor activity

	AMP-binding	AMP-binding				
	predicted S-adenosyl-L-methionine-dependent methyltransferase	predicted S-adenosyl-L-methionine-dependent methyltransferase	putative biotin synthesis protein	biotin biosynthetic process		
folic acid biosynthetic process						
	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase	7,8-dihydro-6-hydroxymethylpterin-pyrophosphokinase	folic acid biosynthetic process		
	4-amino-4-deoxychorismate lyase component of para-aminobenzoate synthase multienzyme complex	4-amino-4-deoxychorismate lyase component of para-aminobenzoate synthase multienzyme complex	4-amino-4-deoxychorismate lyase	folic acid biosynthetic process		
thiamine biosynthetic process						
	thiamin transporter subunit	thiamin transporter subunit	putative ATP-binding component of a transport system	thiamine biosynthetic process	cytoplasm, peptidoglycan-based cell wall	
	fused thiamin transporter subunits of ABC superfamily: membrane components	fused thiamin transporter subunits of ABC superfamily: membrane components		thiamine biosynthetic process	organelle inner membrane, peptidoglycan-based cell wall	
	thiamin-monophosphate kinase	thiamin-monophosphate kinase		thiamine biosynthetic process		
	predicted thiamine biosynthesis lipoprotein	predicted thiamine biosynthesis lipoprotein		thiamine biosynthetic process		

	tyrosine lyase, involved in thiamin-thiazole moiety synthesis	thiamin biosynthesis ThiGH complex subunit		thiamine biosynthetic process		
NAD biosynthetic process						
	nicotinic acid mononucleotide adenylyltransferase, NAD(P)-dependent	nicotinic acid mononucleotide adenylyltransferase, NAD(P)-dependent		NAD biosynthetic process		
	quinolinate synthase, L-aspartate oxidase (B protein) subunit	quinolinate synthase, L-aspartate oxidase (B protein) subunit	quinolinate synthetase, B protein	NAD biosynthetic process		
METABOLIC PROSESS						
metabolic process						
	hydroxyacylglutathione hydrolase	predicted hydroxyacylglutathione hydrolase	probable hydroxyacylglutathione hydrolase	methylglyoxal metabolic process		
	rhodanase-like enzyme, sulfur transfer from thiosulfate	rhodanase-like enzyme, sulfur transfer from thiosulfate	enhanced serine sensitivity	sulfur compound metabolic process		
	phosphate transporter	phosphate transporter	low-affinity phosphate transport	phosphorus metabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	DNA-binding transcriptional activator in response to Zn(II)	DNA-binding transcriptional activator in response to Zn(II)		xenobiotic metabolic process	cytoplasm	transcription activator activity
	conserved inner membrane protein	conserved inner membrane protein		metabolic process	cytoplasm	
	arsenite/antimonite transporter	arsenite/antimonite transporter	arsenical pump membrane protein	xenobiotic metabolic process	organelle inner membrane, peptidoglycan-based cell wall	

nitrogen compound metabolic process						
	DNA-binding transcriptional dual regulator of nitrogen assimilation	DNA-binding transcriptional dual regulator of nitrogen assimilation	nitrogen assimilation control protein	nitrogen compound metabolic process	cytoplasm	transcription activator activity, transcription repressor activity
CATABOLIC PROCESS						
macromolecule catabolic process						
	periplasmic endochitinase	periplasmic endochitinase		macromolecule catabolic process	periplasmic space	
aminoacid catabolic process						
	predicted transporter	predicted transporter	putative amino acid/amine transport protein	cellular amino acid catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	DNA-binding transcriptional repressor, hypoxanthine-binding	DNA-binding transcriptional repressor, hypoxanthine-binding	transcriptional repressor for pur regulon, glyA, glnB, prsA, speA	cellular amino acid catabolic process, glycine biosynthetic process, histidine biosynthetic process, nitrogen compound metabolic process, nucleobase-containing small molecule interconversion, polyamine biosynthetic process, purine nucleotide	cytoplasm	transcription repressor activity

				biosynthetic process, pyrimidine nucleotide biosynthetic process		
	L-serine deaminase II	L-serine deaminase II	L-serine dehydratase (deaminase), L-SD2	cellular amino acid catabolic process, fatty acid oxidation		
			predicted amino-acid transporter subunit;putative transport; Not classified putative periplasmic binding transport protein; periplasmic-binding component of ABC superfamily	cellular amino acid catabolic process	periplasmic space	
amine catabolic process						
	taurine dioxygenase, 2-oxoglutarate-dependent	taurine dioxygenase, 2-oxoglutarate-dependent		amine catabolic process, sulfur compound metabolic process		
	putrescine/proton symporter: putrescine/ornithine antiporter	putrescine/proton symporter: putrescine/ornithine antiporter	putrescine transport protein	amine catabolic process, polyamine biosynthetic process, response to osmotic stress	organelle inner membrane, peptidoglycan-based cell wall	putrescine:ornithine antiporter activity
	polyamine transporter subunit	polyamine transporter subunit	spermidine/putrescine transport system permease; membrane component of ABC superfamily	amine catabolic process, polyamine biosynthetic process	organelle inner membrane, peptidoglycan-based cell wall	
	polyamine transporter subunit	polyamine transporter subunit	spermidine/putrescine transport system permease; membrane	amine catabolic process, polyamine biosynthetic	organelle inner membrane, peptidoglycan-based	

			component of ABC superfamily	process	cell wall	

APPENDIX D

Table 5: *Classified List of Up-regulated Genes*

	NAME	FUNCTION	DESCRIPTION	BIOLOGICAL PROCESS	CELLULAR COMPONENT	MOLECULAR FUNCTION
RESPONSE TO DRUG						
	anaerobic multidrug efflux transporter, ArcA-regulated	multidrug resistance efflux transporter	putative membrane protein		organelle inner membrane, peptidoglycan-based cell wall	
	anaerobic multidrug efflux transporter, ArcA-regulated	multidrug transporter, RpoS-dependent	putative transport system permease protein		cell outer membrane, peptidoglycan-based cell wall	
	dihydropteridine reductase, NAD(P)H-dependent, oxygen-insensitive	dihydropteridine reductase, NAD(P)H-dependent, oxygen-insensitive	oxygen-insensitive NAD(P)H nitroreductase	response to drug		
	tellurite resistance protein, SAM-dependent; predicted S-adenosyl-L-methionine-dependent methyltransferase	predicted S-adenosyl-L-methionine-dependent methyltransferase		response to drug		
OTHER STRESS RESPONSES						
	serine endoprotease (protease Do), membrane-associated	serine endoprotease (protease Do), membrane-associated	periplasmic serine protease Do; heat shock protein HtrA	response to temperature stimulus	periplasmic space	
	stationary-phase morphogene, transcriptional repressor for mreB; also regulator for dacA, dacC, and ampC	regulator of penicillin binding proteins and beta lactamase transcription (morphogene)	possible regulator of murein genes	response to osmotic stress	cytoplasm, peptidoglycan-based cell wall	
	proteolytic subunit of ClpA-	proteolytic subunit of	ATP-dependent	response to temperature	cytoplasm	peptidase activity

	ClpP and ClpX-ClpP ATP-dependent serine proteases	ClpA-ClpP and ClpX-ClpP ATP-dependent serine proteases	proteolytic subunit of clpA-clpP serine protease, heat shock protein F21.5	stimulus		
	DNA-binding transcriptional repressor	DNA-binding transcriptional repressor	cold shock protein	response to temperature stimulus	cytoplasm	transcription activator activity
	Fe-binding and storage protein	Fe-binding and storage protein	global regulator, starvation conditions	response to starvation	cytoplasm	
	phosphoanhydride phosphorylase	phosphoanhydride phosphorylase	phosphoanhydride phosphorylase; pH 2.5 acid phosphatase; periplasmic	phosphorus metabolic process, response to starvation	periplasmic space	
	DNA-binding transcriptional activator	DNA-binding transcriptional activator	activator of ntrL gene	response to osmotic stress	cytoplasm	transcription activator activity
	exonuclease III	exonuclease III		DNA catabolic process, response to radiation		
	stress protein, member of the CspA-family	stress protein, member of the CspA-family	cold shock protein	response to temperature stimulus		transcription activator activity
	predicted endopeptidase	predicted endopeptidase	heat shock protein, integral membrane protein	response to temperature stimulus	organelle inner membrane, peptidoglycan-based cell wall	
	palmitoleoyl-acyl carrier protein (ACP)-dependent acyltransferase	palmitoleoyl-acyl carrier protein (ACP)-dependent acyltransferase	putative heat shock protein	lipid A biosynthetic process, response to temperature stimulus	organelle inner membrane, peptidoglycan-based cell wall	cell surface antigen activity, host-interacting
	anti-sigma factor	anti-sigma factor	sigma-E factor, negative regulatory protein	response to temperature stimulus	cytoplasm	
	RNA polymerase, sigma 24	RNA polymerase,	RNA polymerase,	response to temperature	cytoplasm	

	(sigma E) factor	sigma 24 (sigma E) factor	sigma-E factor; heat shock and oxidative stress	stimulus		
	universal stress (ethanol tolerance) protein B	predicted universal stress (ethanol tolerance) protein B		response to stress, xenobiotic metabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	universal stress global response regulator	universal stress global response regulator	universal stress protein; broad regulatory function?	response to stress	cytoplasm	
	outer membrane lipoprotein	outer membrane lipoprotein	outer membrane protein induced after carbon starvation	response to starvation	cell outer membrane, peptidoglycan-based cell wall	
	RNA chaperone and anti-terminator, cold-inducible	major cold shock protein	cold shock protein 7.4, transcriptional activator of hns	response to temperature stimulus	cytoplasm	transcription activator activity
	stress-induced protein	stress-induced protein	putative regulator	response to stress		
	molecular chaperone and ATPase component of HslUV protease	molecular chaperone and ATPase component of HslUV protease	heat shock protein hslVU, ATPase subunit, homologous to chaperones	protein folding, proteolysis, response to temperature stimulus	cytoplasm	peptidase activity
	predicted inner membrane protein	predicted inner membrane protein	putative carbon starvation protein	response to starvation		
STRESS PROTEINS						
	universal stress protein UP12	universal stress protein UP12				
	stress-induced protein	stress-induced protein				
	stress-induced protein, ATP-binding protein	stress-induced protein, ATP-binding protein	putative filament protein			
	periplasmic ATP-independent protein	envelope stress induced periplasmic	periplasmic protein related to		periplasmic space	

	refolding chaperone, stress-induced	protein	spheroblast formation			
	stress response protein acid-resistance protein	stress response protein acid-resistance protein				
	peripheral inner membrane phage-shock protein	peripheral inner membrane phage-shock protein	phage shock protein			
	Qin prophage; cold shock protein	Qin prophage; cold shock protein				
	inhibitor of DNA replication, cold shock protein homolog	cold shock protein homolog	stress induced DNA replication inhibitor.		cytoplasm	
acid-resistance protein						
	acid-resistance protein	acid-resistance protein				
	acid-resistance membrane protein	acid-resistance membrane protein				
ENERGY METABOLISM						
gluconeogenesis, glycolysis						
	phosphoglyceromutase 1	phosphoglyceromutase 1		gluconeogenesis, glycolysis		
	glucose-1-phosphatase/inositol phosphatase	glucose-1-phosphatase/inositol phosphatase	periplasmic glucose-1-phosphatase	glucose metabolic process	periplasmic space	
	periplasmic trehalase	periplasmic trehalase	trehalase, periplasmic	glucose metabolic process, response to osmotic stress	periplasmic space	
	DNA-binding transcriptional repressor-activator for carbon metabolism	DNA-binding transcriptional dual regulator	transcriptional repressor of fru operon and others	glycolysis	cytoplasm	transcription activator activity, transcription repressor activity

	malate dehydrogenase, (decarboxylating, NAD-requiring) (malic enzyme)	malate dehydrogenase, (decarboxylating, NAD-requiring) (malic enzyme)	NAD-linked malate dehydrogenase (malic enzyme)	gluconeogenesis		
	glyceraldehyde-3-phosphate dehydrogenase A	glyceraldehyde-3-phosphate dehydrogenase A		gluconeogenesis, glycolysis, pyridoxine biosynthetic process	cytoplasm	
	trehalose-6-phosphate synthase	trehalose-6-phosphate synthase		glucose metabolic process, response to osmotic stress		
	trehalose-6-phosphate phosphatase, biosynthetic	trehalose-6-phosphate phosphatase, biosynthetic	trehalose-6-phosphate phophatase, biosynthetic	glucose metabolic process, response to osmotic stress	cytoplasm	
	fructose-bisphosphate aldolase class I	fructose-bisphosphate aldolase class I		glycolysis		
	fructose-1-phosphate kinase	fructose-1-phosphate kinase		glycolysis		
	glucosephosphate isomerase	glucosephosphate isomerase		gluconeogenesis, glycolysis	cytoplasm	
	fused trehalose(maltose)-specific PTS enzyme: IIB component/IIC component	fused trehalose(maltose)-specific PTS enzyme: IIB component/IIC component	PTS system enzyme II, trehalose specific	glucose metabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	DNA-binding transcriptional repressor	DNA-binding transcriptional repressor	repressor of treA,B,C	glucose metabolic process, response to osmotic stress	cytoplasm	transcription repressor activity
	copper transporter	copper transporter			organelle inner membrane, peptidoglycan-based cell wall	ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism
ATP synthesis						

	zinc, cobalt and lead efflux system	zinc, cobalt and lead efflux system	zinc-transporting ATPase	ATP synthesis coupled proton transport, xenobiotic metabolic process	organelle inner membrane, peptidoglycan-based cell wall	ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism
	ATPase and specificity subunit of ClpA-ClpP ATP-dependent serine protease, chaperone activity	ATPase and specificity subunit of ClpA-ClpP ATP-dependent serine protease, chaperone activity	ATP-binding component of serine protease		cytoplasm	
	membrane ATPase of the MinC-MinD-MinE system	membrane ATPase of the MinC-MinD-MinE system	cell division inhibitor, a membrane ATPase, activates minC		organelle inner membrane, peptidoglycan-based cell wall	
respiration						
	dimethyl sulfoxide reductase, anaerobic, subunit A	dimethyl sulfoxide reductase, anaerobic, subunit A	anaerobic dimethyl sulfoxide reductase subunit A	anaerobic respiration	organelle inner membrane, peptidoglycan-based cell wall	
	dimethyl sulfoxide reductase, anaerobic, subunit B	dimethyl sulfoxide reductase, anaerobic, subunit B	anaerobic dimethyl sulfoxide reductase subunit B	anaerobic respiration	organelle inner membrane, peptidoglycan-based cell wall	
	dimethyl sulfoxide reductase, anaerobic, subunit C	dimethyl sulfoxide reductase, anaerobic, subunit C	anaerobic dimethyl sulfoxide reductase subunit C	anaerobic respiration	organelle inner membrane, peptidoglycan-based cell wall	
	hydrogenase 1, small subunit	hydrogenase 1, small subunit	hydrogenase-1 small subunit	aerobic respiration, anaerobic respiration	organelle inner membrane	
	hydrogenase 1, large subunit	hydrogenase 1, large subunit	hydrogenase-1 large subunit	aerobic respiration, anaerobic respiration	organelle inner membrane	

	hydrogenase 1, b-type cytochrome subunit	hydrogenase 1, b-type cytochrome subunit	probable Ni/Fe-hydrogenase 1 b-type cytochrome subunit	aerobic respiration, anaerobic respiration, cytochrome complex assembly	organelle inner membrane, peptidoglycan-based cell wall	
	hydrogenase 1 maturation protease	protein involved in processing of HyaA and HyaB proteins	processing of HyaA and HyaB proteins	aerobic respiration, anaerobic respiration, protein folding		
	nitrate reductase 1, alpha subunit	nitrate reductase 1, alpha subunit		anaerobic respiration	organelle inner membrane, peptidoglycan-based cell wall	
	nitrate reductase 1, beta (Fe-S) subunit	nitrate reductase 1, beta (Fe-S) subunit	nitrate reductase 1, beta subunit	anaerobic respiration	organelle inner membrane, peptidoglycan-based cell wall	
	molybdenum-cofactor-assembly chaperone subunit (delta subunit) of nitrate reductase 1	molybdenum-cofactor-assembly chaperone subunit (delta subunit) of nitrate reductase 1	nitrate reductase 1, delta subunit, assembly function	anaerobic respiration, protein folding		
	nitrate reductase 2 (NRZ), beta subunit	nitrate reductase 2 (NRZ), beta subunit		anaerobic respiration	organelle inner membrane, peptidoglycan-based cell wall	
	nitrate reductase 2 (NRZ), alpha subunit	nitrate reductase 2 (NRZ), alpha subunit		anaerobic respiration	organelle inner membrane, peptidoglycan-based cell wall	
	pyruvate kinase I	pyruvate kinase I	pyruvate kinase I (formerly F), fructose stimulated	anaerobic respiration, fermentation, glycolysis		
	pyruvate kinase II	pyruvate kinase II	pyruvate kinase II, glucose stimulated	anaerobic respiration, fermentation, glycolysis		
	D-lactate dehydrogenase, FAD-binding, NADH	D-lactate dehydrogenase, FAD-	D-lactate dehydrogenase,	aerobic respiration	cytoplasm	

	independent	binding, NADH independent	FAD protein, NADH independent			
	nitrate reductase, periplasmic, large subunit	nitrate reductase, periplasmic, large subunit	probable nitrate reductase 3	anaerobic respiration	periplasmic space	
	sn-glycerol-3-phosphate dehydrogenase (anaerobic), large subunit, FAD/NAD(P)-binding	sn-glycerol-3-phosphate dehydrogenase (anaerobic), large subunit, FAD/NAD(P)-binding	sn-glycerol-3-phosphate dehydrogenase (anaerobic), large subunit	anaerobic respiration, glycerol metabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	sn-glycerol-3-phosphate dehydrogenase (anaerobic), membrane anchor subunit	sn-glycerol-3-phosphate dehydrogenase (anaerobic), membrane anchor subunit		anaerobic respiration, glycerol metabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	anaerobic sn-glycerol-3-phosphate dehydrogenase, C subunit, 4Fe-4S iron-sulfur cluster	sn-glycerol-3-phosphate dehydrogenase (anaerobic), small subunit	sn-glycerol-3-phosphate dehydrogenase (anaerobic), K-small subunit	anaerobic respiration, glycerol metabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	autonomous glycyl radical cofactor	pyruvate formate lyase subunit	putative formate acetyltransferase	anaerobic respiration		
	protein required for maturation of hydrogenases 1 and 3	protein required for maturation of hydrogenases 1 and 3	pleiotrophic effects on 3 hydrogenase isozymes	anaerobic respiration, protein modification process		
	protein required for maturation of hydrogenases	protein required for maturation of hydrogenases	pleiotrophic effects on 3 hydrogenase isozymes	anaerobic respiration, protein modification process		
	carbamoyl dehydratase, hydrogenases 1,2,3 maturation protein	carbamoyl phosphate phosphatase, hydrogenase 3	plays structural role in maturation of all 3	anaerobic respiration, protein folding		

		maturation protein	hydrogenases			
	hydrogenase 2, large subunit	hydrogenase 2, large subunit	probable large subunit, hydrogenase-2	anaerobic respiration	organelle inner membrane	
	pyruvate formate-lyase 4/2-ketobutyrate formate-lyase	pyruvate formate-lyase 4/2-ketobutyrate formate-lyase	probable formate acetyltransferase 3	anaerobic respiration, cellular amino acid catabolic process, fermentation	cytoplasm	
	nitrite reductase, large subunit, NAD(P)H-binding	nitrite reductase, large subunit, NAD(P)H-binding	nitrite reductase (NAD(P)H) subunit	anaerobic respiration	cytoplasm	
	nitrite reductase, NAD(P)H-binding, small subunit	nitrite reductase, NAD(P)H-binding, small subunit	nitrite reductase (NAD(P)H) subunit	anaerobic respiration	cytoplasm	
	glycerol-3-phosphate transporter subunit	glycerol-3-phosphate transporter subunit	sn-glycerol 3-phosphate transport system; periplasmic binding protein; periplasmic-binding component of ABC superfamily	aerobic respiration, anaerobic respiration, glycerol metabolic process, phospholipid biosynthetic process	periplasmic space	
	formate dehydrogenase formation protein	formate dehydrogenase formation protein	affects formate dehydrogenase-N	anaerobic respiration, protein folding	cytoplasm	
	nitrite reductase, formate-dependent, cytochrome	nitrite reductase, formate-dependent, cytochrome	periplasmic cytochrome c(552): plays a role in nitrite reduction	anaerobic respiration, cytochrome complex assembly	periplasmic space	
	nitrite reductase, formate-dependent, penta-heme cytochrome c	nitrite reductase, formate-dependent, penta-heme cytochrome c	formate-dependent nitrite reductase; a penta-haeme cytochrome c	anaerobic respiration, cytochrome complex assembly	periplasmic space	
	formate-dependent nitrite	formate-dependent	formate-	anaerobic respiration	organelle inner	

	reductase, 4Fe4S subunit	nitrite reductase, 4Fe4S subunit	dependent nitrite reductase; Fe-S centers		membrane, peptidoglycan-based cell wall	
	formate-dependent nitrite reductase, membrane subunit	formate-dependent nitrite reductase, membrane subunit	formate-dependent nitrate reductase complex; transmembrane protein	anaerobic respiration	organelle inner membrane, peptidoglycan-based cell wall	
	fumarate reductase (anaerobic), membrane anchor subunit	fumarate reductase (anaerobic), membrane anchor subunit	fumarate reductase, anaerobic, membrane anchor polypeptide	anaerobic respiration, fermentation	organelle inner membrane, peptidoglycan-based cell wall	
	fumarate reductase (anaerobic), Fe-S subunit	fumarate reductase (anaerobic), Fe-S subunit	fumarate reductase, anaerobic, iron-sulfur protein subunit	anaerobic respiration, fermentation	cytoplasm	
	fumarate reductase (anaerobic) catalytic and NAD/flavoprotein subunit	fumarate reductase (anaerobic) catalytic and NAD/flavoprotein subunit	fumarate reductase, anaerobic, flavoprotein subunit	anaerobic respiration, fermentation	cytoplasm	
pentose-phosphate pathway						
	transaldolase A	transaldolase A		pentose-phosphate shunt, non-oxidative branch		
	transaldolase B	transaldolase B		pentose-phosphate shunt, non-oxidative branch	cytoplasm	
fermentation						
	predicted pyruvate formate lyase	predicted pyruvate formate lyase		fermentation		
	fused acetaldehyde-CoA dehydrogenase/iron-dependent alcohol	fused acetaldehyde-CoA dehydrogenase/iron-	CoA-linked acetaldehyde dehydrogenase	fermentation		

	dehydrogenase/pyruvate-formate lyase deactivase	dependent alcohol dehydrogenase/pyruvate-formate lyase deactivase	and iron-dependent alcohol dehydrogenase; pyruvate-formate-lyase deactivase			
	fermentative D-lactate dehydrogenase, NAD-dependent	fermentative D-lactate dehydrogenase, NAD-dependent		fermentation		
	D-malate oxidase, NAD-dependent; probable tartrate dehydrogenase	predicted dehydrogenase	putative tartrate dehydrogenase	fermentation		
	GTP hydrolase involved in nickel liganding into hydrogenases	GTP hydrolase involved in nickel liganding into hydrogenases	guanine-nucleotide binding protein, functions as nickel donor for large subunit of hydrogenase 3	fermentation, protein folding		
	phosphoenolpyruvate carboxylase	phosphoenolpyruvate carboxylase		fermentation, tricarboxylic acid cycle	cytoplasm	
	formate dehydrogenase-H, selenopolypeptide subunit	formate dehydrogenase-H, selenopolypeptide subunit	selenopolypeptide subunit of formate dehydrogenase H	fermentation		
oxidation						
	DNA-binding transcriptional dual regulator of fatty acid metabolism	DNA-binding transcriptional dual regulator of fatty acid metabolism	negative regulator for fad regulon, and positive activator of fabA	fatty acid oxidation	cytoplasm	transcription activator activity, transcription repressor activity
	fused response regulator of ato operon, in two-component system with AtoS: response regulator/sigma54 interaction protein	fused response regulator of ato operon, in two-component system with AtoS: response regulator/sigma54 interaction protein	response regulator of ato, ornithine decarboxylase antizyme (sensor ATOS)	fatty acid oxidation, polyamine biosynthetic process	cytoplasm	transcription activator activity

	conserved protein	conserved protein	putative lipase	fatty acid oxidation		
METABOLIC PROCESS						
metabolic process						
	glyoxalase I, Ni-dependent	glyoxalase I, Ni-dependent	lactoylglutathione lyase	methylglyoxal metabolic process		
sulfur compound metabolic process						
	conserved protein	conserved protein	putative sulfatase	sulfur compound metabolic process		
	predicted thiosulfate sulfur transferase	predicted thiosulfate sulfur transferase	putative thiosulfate sulfur transferase	sulfur compound metabolic process		
	3-mercaptopyruvate sulfurtransferase	3-mercaptopyruvate sulfurtransferase	putative thiosulfate sulfurtransferase	sulfur compound metabolic process		
	PAPS (adenosine 3'-phosphate 5'-phosphosulfate) 3'(2'),5'-bisphosphate nucleotidase	PAPS (adenosine 3'-phosphate 5'-phosphosulfate) 3'(2'),5'-bisphosphate nucleotidase	affects pool of 3'-phosphoadenosine -5'-phosphosulfate in pathway of sulfite synthesis	sulfur compound metabolic process		
peptidoglycan metabolic process						
	beta N-acetyl-glucosaminidase	beta N-acetyl-glucosaminidase		peptidoglycan metabolic process		
	oligopeptide transporter subunit	oligopeptide transporter subunit	oligopeptide transport; periplasmic binding protein; periplasmic-binding component of ABC superfamily	peptidoglycan metabolic process, protein folding	peptidoglycan-based cell wall, periplasmic space	

	oligopeptide transporter subunit	oligopeptide transporter subunit	homolog of Salmonella ATP-binding protein of oligopeptide ABC transport system	peptidoglycan metabolic process	cytoplasm, peptidoglycan-based cell wall	
glycerol metabolic process						
	CP4-6 prophage; S-methylmethionine:homocysteine methyltransferase	CP4-6 prophage; S-methylmethionine:homocysteine methyltransferase		glycerol metabolic process, methionine biosynthetic process		
	glycerol kinase	glycerol kinase		glycerol metabolic process		
	glycerol facilitator	glycerol facilitator	facilitated diffusion of glycerol	glycerol metabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	glycerol dehydrogenase, NAD	glycerol dehydrogenase, NAD	glycerol dehydrogenase, (NAD)	glycerol metabolic process		
nitrogen compound metabolic process						
	allantoate amidohydrolase	allantoate amidohydrolase		allantoin assimilation pathway, nitrogen compound metabolic process		
	ureidoglycolate dehydrogenase	ureidoglycolate dehydrogenase		allantoin assimilation pathway, nitrogen compound metabolic process		
	nitrate/nitrite transporter	nitrate/nitrite transporter	nitrite extrusion protein	nitrogen compound metabolic process	organelle inner membrane, peptidoglycan-based cell wall	

phosphorus metabolic process						
	DNA-binding response regulator in two-component regulatory system with PhoR (or CreC)	DNA-binding response regulator in two-component regulatory system with PhoR (or CreC)	positive response regulator for pho regulon, sensor is PhoR (or CreC)	phosphorus metabolic process	cytoplasm	transcription activator activity
	conserved protein with nucleoside triphosphate hydrolase domain	conserved protein with nucleoside triphosphate hydrolase domain	PhoB-dependent, ATP-binding pho regulon component; may be helicase; induced by P starvation	phosphorus metabolic process, response to starvation	cytoplasm	
	negative regulator of PhoR/PhoB two-component regulator	negative regulator of PhoR/PhoB two-component regulator	negative regulator for pho regulon and putative enzyme in phosphate metabolism	phosphorus metabolic process		
	phosphate transporter subunit	phosphate transporter subunit	ATP-binding component of high-affinity phosphate-specific transport system; ATP-binding component of ABC superfamily	phosphorus metabolic process	cytoplasm	
xenobiotic metabolic process						
	alkyl hydroperoxide reductase, F52a subunit, FAD/NAD(P)-binding	alkyl hydroperoxide reductase, F52a subunit, FAD/NAD(P)-	alkyl hydroperoxide reductase, F52a subunit;	xenobiotic metabolic process		

		binding	detoxification of hydroperoxides			
	lipid hydroperoxide peroxidase	lipid hydroperoxide peroxidase	thiol peroxidase	xenobiotic metabolic process	periplasmic space	
	N-ethylmaleimide reductase, FMN-linked	N-ethylmaleimide reductase, FMN-linked	N-ethylmaleimide reductase	xenobiotic metabolic process		
	thiol peroxidase, thioredoxin-dependent	thiol peroxidase, thioredoxin-dependent	bacterioferritin comigratory protein	xenobiotic metabolic process	cytoplasm	
	DNA-binding response regulator in two-component regulatory system with CpxA	DNA-binding response regulator in two-component regulatory system with CpxA	transcriptional regulator in 2-component system	xenobiotic metabolic process	cytoplasm	transcription activator activity
	catalase-peroxidase HPI, heme b-containing	catalase/hydroperoxidase HPI(I)	catalase; hydroperoxidase HPI(I)	xenobiotic metabolic process		
	inhibitor of the cpx response; periplasmic adaptor protein	periplasmic protein combats stress	periplasmic repressor of cpx regulon by interaction with CpxA	xenobiotic metabolic process	cytoplasm, periplasmic space	
AMINOACID BIOSYNTHESIS						
arginine biosynthetic process						
	acetylornithine deacetylase	acetylornithine deacetylase		arginine biosynthetic process	cytoplasm	
	arginine binding protein, periplasmic	arginine transporter subunit	arginine 3rd transport system periplasmic binding protein	arginine biosynthetic process	periplasmic space	
	argininosuccinate synthetase	argininosuccinate		arginine biosynthetic	cytoplasm	

		synthetase		process		
proline biosynthetic process						
	gamma-glutamate kinase	gamma-glutamate kinase		proline biosynthetic process	cytoplasm	
	pyrroline-5-carboxylate reductase, NAD(P)-binding	pyrroline-5-carboxylate reductase, NAD(P)-binding	pyrroline-5-carboxylate reductase	proline biosynthetic process	cytoplasm	
L-phenylalanine biosynthetic process						
	3-deoxy-D-arabino-heptulosonate-7-phosphate synthase, phenylalanine repressible	3-deoxy-D-arabino-heptulosonate-7-phosphate synthase, phenylalanine repressible	3-deoxy-D-arabinoheptulosonate-7-phosphate synthase (DAHP synthetase, phenylalanine repressible)	L-phenylalanine biosynthetic process		
	tyrosine aminotransferase, tyrosine-repressible, PLP-dependent	tyrosine aminotransferase, tyrosine-repressible, PLP-dependent	tyrosine aminotransferase, tyrosine repressible	L-phenylalanine biosynthetic process, leucine biosynthetic process, tyrosine biosynthetic process	cytoplasm	
glycine biosynthetic process						
	L-allo-threonine aldolase, PLP-dependent	L-allo-threonine aldolase, PLP-dependent		glycine biosynthetic process	cytoplasm	
homoserine biosynthetic process						
	aspartokinase III	aspartokinase III	aspartokinase III,	homoserine biosynthetic	cytoplasm	

			lysine sensitive	process, lysine biosynthetic process via diaminopimelate		
	aspartate-semialdehyde dehydrogenase, NAD(P)-binding	aspartate-semialdehyde dehydrogenase, NAD(P)-binding	aspartate-semialdehyde dehydrogenase	homoserine biosynthetic process, lysine biosynthetic process via diaminopimelate, methionine biosynthetic process, threonine biosynthetic process		
asparagine biosynthetic process						
	asparagine synthetase B	asparagine synthetase B		asparagine biosynthetic process, cellular amino acid catabolic process	cytoplasm	
	asparagine synthetase A	asparagine synthetase A		asparagine biosynthetic process, cellular amino acid catabolic process	cytoplasm	
lysine biosynthetic process						
	2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase	2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase		lysine biosynthetic process via diaminopimelate	cytoplasm	
	diaminopimelate decarboxylase, PLP-binding	diaminopimelate decarboxylase, PLP-binding	diaminopimelate decarboxylase	lysine biosynthetic process via diaminopimelate		
	predicted lysine/cadaverine transporter	predicted lysine/cadaverine transporter	transport of lysine/cadaverine	lysine biosynthetic process via diaminopimelate	organelle inner membrane, peptidoglycan-based cell wall	
leucine biosynthetic process						
	3-isopropylmalate	3-isopropylmalate	3-isopropylmalate	leucine biosynthetic	cytoplasm	

	dehydratase large subunit	isomerase subunit, dehydratase component	isomerase (dehydratase) subunit	process		
	3-isopropylmalate dehydrogenase, NAD(+)-dependent	3-isopropylmalate dehydrogenase		leucine biosynthetic process	cytoplasm	
	2-isopropylmalate synthase	2-isopropylmalate synthase		leucine biosynthetic process	cytoplasm	
	DNA-binding transcriptional dual regulator, leucine-binding	DNA-binding transcriptional dual regulator, leucine-binding	regulator for leucine (or lrp) regulon and high-affinity branched-chain amino acid transport system	leucine biosynthetic process	cytoplasm	transcription activator activity, transcription repressor activity
threonine biosynthetic process						
	thr operon leader peptide	thr operon leader peptide		threonine biosynthetic process		
	fused aspartokinase I and homoserine dehydrogenase I	fused aspartokinase I and homoserine dehydrogenase I	bifunctional: aspartokinase I (N-terminal); homoserine dehydrogenase I (C-terminal)	homoserine biosynthetic process, methionine biosynthetic process, threonine biosynthetic process	cytoplasm	
	homoserine kinase	homoserine kinase		threonine biosynthetic process	cytoplasm	
	threonine synthase	threonine synthase		threonine biosynthetic process	cytoplasm	
histidine biosynthetic process						
	his operon leader peptide	his operon leader peptide		histidine biosynthetic process		
	ATP phosphoribosyltransferase	ATP phosphoribosyltransferase		histidine biosynthetic process	cytoplasm	

	bifunctional histidinal dehydrogenase/ histidinol dehydrogenase	bifunctional histidinal dehydrogenase/ histidinol dehydrogenase	L-histidinal:NAD+ oxidoreductase; L-histidinol:NAD+ oxidoreductase	histidine biosynthetic process		
	histidinol-phosphate aminotransferase	histidinol-phosphate aminotransferase		histidine biosynthetic process		
	fused histidinol-phosphatase/imidazoleglycerol-phosphate dehydratase	fused histidinol-phosphatase/imidazoleglycerol-phosphate dehydratase	imidazoleglycerol phosphate dehydratase and histidinol-phosphate phosphatase	histidine biosynthetic process		
	imidazole glycerol phosphate synthase, glutamine amidotransferase subunit with HisF	imidazole glycerol phosphate synthase, glutamine amidotransferase subunit with HisF	glutamine amidotransferase subunit of heterodimer with HisF = imidazole glycerol phosphate synthase holoenzyme	histidine biosynthetic process		
	N-(5'-phospho-L-ribosyl-formimino)-5-amino-1-(5'-phosphoribosyl)-4-imidazolecarboxamide isomerase	N-(5'-phospho-L-ribosyl-formimino)-5-amino-1- (5'-phosphoribosyl)-4-imidazolecarboxamide isomerase		histidine biosynthetic process	cytoplasm	
	imidazole glycerol phosphate synthase, catalytic subunit with HisH	imidazole glycerol phosphate synthase, catalytic subunit with HisH	imidazole glycerol phosphate synthase subunit in heterodimer with HisH = imidazole glycerol phsphate	histidine biosynthetic process	cytoplasm	

			synthase holoenzyme			
	fused phosphoribosyl-AMP cyclohydrolase/phosphoribosyl-ATP pyrophosphatase	fused phosphoribosyl-AMP cyclohydrolase/phosphoribosyl-ATP pyrophosphatase	phosphoribosyl-amp cyclohydrolase; phosphoribosyl-ATP pyrophosphatase	histidine biosynthetic process	cytoplasm	
glutamine biosynthetic process						
	arginine transporter subunit	arginine transporter subunit	arginine 3rd transport system periplasmic binding protein	glutamine biosynthetic process	periplasmic space	
glutamate biosynthetic process						
	glutamate synthase, large subunit	glutamate synthase, large subunit		glutamate biosynthetic process, nitrogen compound metabolic process		
	glutamate synthase, 4Fe-4S protein, small subunit	glutamate synthase, 4Fe-4S protein, small subunit	glutamate synthase, small subunit	glutamate biosynthetic process, nitrogen compound metabolic process		
aspartate biosynthetic process						
	aspartate aminotransferase, PLP-dependent	aspartate aminotransferase, PLP-dependent	aspartate aminotransferase	aspartate biosynthetic process, cellular amino acid catabolic process	cytoplasm	
	glutamate/aspartate:proton symporter	glutamate/aspartate:proton symporter	glutamate-aspartate symport protein	aspartate biosynthetic process, glutamate biosynthetic process	organelle inner membrane, peptidoglycan-based cell wall	
L-serine						

biosynthetic process						
	3-phosphoserine/phosphohydroxythreonine aminotransferase	3-phosphoserine/phosphohydroxythreonine aminotransferase	3-phosphoserine aminotransferase	L-serine biosynthetic process	cytoplasm	
tryptophan biosynthetic process						
	NAD(P)H:quinone oxidoreductase	predicted flavoprotein in Trp regulation	flavoprotein WrbA (Trp repressor binding protein)	tryptophan biosynthetic process	cytoplasm	
	tryptophan synthase, beta subunit	tryptophan synthase, beta subunit	tryptophan synthase, beta protein	tryptophan biosynthetic process	cytoplasm	
	fused indole-3-glycerolphosphate synthetase/N-(5-phosphoribosyl)anthranilate isomerase	fused indole-3-glycerolphosphate synthetase/N-(5-phosphoribosyl)anthranilate isomerase	N-(5-phosphoribosyl)anthranilate isomerase and indole-3-glycerolphosphate synthetase	tryptophan biosynthetic process	cytoplasm	
	fused glutamine amidotransferase (component II) of anthranilate synthase/anthranilate phosphoribosyl transferase	fused glutamine amidotransferase (component II) of anthranilate synthase/anthranilate phosphoribosyl transferase	anthranilate synthase component II, glutamine amidotransferase and phosphoribosylanthranilate transferase	tryptophan biosynthetic process	cytoplasm	
	component I of anthranilate synthase	component I of anthranilate synthase	anthranilate synthase component I	tryptophan biosynthetic process		
	tryptophan transporter of low affinity	tryptophan transporter of low	low affinity tryptophan	tryptophan biosynthetic process	organelle inner membrane,	

		affinity	permease		peptidoglycan-based cell wall	
NUCLEIC ACID METABOLISM						
purine ribonucleotide biosynthetic process						
	formyltetrahydrofolate hydrolase	formyltetrahydrofolate hydrolase	formyltetrahydrofolate deformylase; for purT-dependent FGAR synthesis	purine ribonucleotide biosynthetic process	cytoplasm	
pyrimidine nucleotide biosynthetic process						
	aspartate carbamoyltransferase, regulatory subunit	aspartate carbamoyltransferase, regulatory subunit		pyrimidine nucleotide biosynthetic process	cytoplasm	
nucleotide-sugar biosynthetic process						
	bifunctional UDP-sugar hydrolase/5'-nucleotidase	bifunctional UDP-sugar hydrolase/5'-nucleotidase	UDP-sugar hydrolase (5'-nucleotidase)	nucleotide-sugar biosynthetic process	cytoplasm	
CARBOHYDRATE CATABOLIC PROCESS						
	predicted transporter	predicted transporter	probable carnitine transporter	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-	

					based cell wall	
	L-arabinose isomerase	L-arabinose isomerase		carbohydrate catabolic process	cytoplasm	
	L-ribulokinase	L-ribulokinase		carbohydrate catabolic process	cytoplasm	
	predicted DNA-binding transcriptional regulator	predicted DNA-binding transcriptional regulator	probable regulator for maltose metabolism	carbohydrate catabolic process		
	beta-D-galactosidase	beta-D-galactosidase		carbohydrate catabolic process	cytoplasm	
	manno(fructo)kinase	manno(fructo)kinase	possible NAGC-like transcriptional regulator	carbohydrate catabolic process		
	maltodextrin glucosidase	maltodextrin glucosidase		carbohydrate catabolic process, polysaccharide catabolic process	cytoplasm	
	anaerobic C4-dicarboxylate transport	anaerobic C4-dicarboxylate transport	transport of dicarboxylates	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	phosphoglucomutase	phosphoglucomutase		carbohydrate catabolic process, glucose metabolic process	cytoplasm	
	pyruvate dehydrogenase (pyruvate oxidase), thiamin-dependent, FAD-binding	pyruvate dehydrogenase (pyruvate oxidase), thiamin-dependent, FAD-binding	pyruvate oxidase	carbohydrate catabolic process, pyruvate catabolic process	cytoplasm	
	fused glucose-specific PTS enzymes: IIB component/IIC component	fused glucose-specific PTS enzymes: IIB component/IIC component	PTS system, glucose-specific IIBC component	carbohydrate catabolic process, protein modification process	organelle inner membrane, peptidoglycan-based cell wall	
	glucose-1-phosphate uridylyltransferase	glucose-1-phosphate uridylyltransferase		carbohydrate catabolic process, colanic acid		

				biosynthetic process, galactose metabolic process, glucose metabolic process, response to desiccation		
	galactose-1-epimerase (mutarotase)	galactose-1-epimerase (mutarotase)		carbohydrate catabolic process	periplasmic space	
	galactokinase	galactokinase		carbohydrate catabolic process, colanic acid biosynthetic process, galactose metabolic process, response to desiccation	cytoplasm	cell surface antigen activity, host-interacting
	galactose-1-phosphate uridylyltransferase	galactose-1-phosphate uridylyltransferase		carbohydrate catabolic process, colanic acid biosynthetic process, galactose metabolic process, response to desiccation	cytoplasm	cell surface antigen activity, host-interacting
	ring 1,2-phenylacetyl-CoA epoxidase subunit	predicted multicomponent oxygenase/reductase subunit for phenylacetic acid degradation		carbohydrate catabolic process		
	ring 1,2-phenylacetyl-CoA epoxidase possible subunit, not required for in vitro activity	predicted multicomponent oxygenase/reductase subunit for phenylacetic acid degradation		carbohydrate catabolic process		
	ring 1,2-phenylacetyl-CoA epoxidase subunit	predicted multicomponent oxygenase/reductase subunit for phenylacetic acid		carbohydrate catabolic process		

		degradation				
	ring 1,2-phenylacetyl-CoA epoxidase subunit	predicted multicomponent oxygenase/reductase subunit for phenylacetic acid degradation		carbohydrate catabolic process		
	altronate oxidoreductase, NAD-dependent	altronate oxidoreductase, NAD-dependent	altronate oxidoreductase	carbohydrate catabolic process		
	mannose-6-phosphate isomerase	mannose-6-phosphate isomerase		carbohydrate catabolic process, colanic acid biosynthetic process, nucleotide-sugar biosynthetic process, response to desiccation	cytoplasm	
	6-phosphofructokinase II	6-phosphofructokinase II	6-phosphofructokinase II; suppressor of pfkA	carbohydrate catabolic process	cytoplasm	
	N,N'-diacetylchitobiose-specific enzyme IIB component of PTS	N,N'-diacetylchitobiose-specific enzyme IIB component of PTS	PTS family enzyme IIB, cellobiose/arbutin/salicin sugar-specific	carbohydrate catabolic process		
	fused mannose-specific PTS enzymes: IIA component/IIB component	fused mannose-specific PTS enzymes: IIA component/IIB component	PTS enzyme IIAB, mannose-specific	carbohydrate catabolic process	cytoplasm	
	mannose-specific enzyme IIC component of PTS	mannose-specific enzyme IIC component of PTS	PTS enzyme IIC, mannose-specific	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	mannose-specific enzyme IID component of PTS	mannose-specific enzyme IID component of PTS	PTS enzyme IID, mannose-specific	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-	

					based cell wall	
	multifunctional 2-keto-3-deoxygluconate 6-phosphate aldolase and 2-keto-4-hydroxyglutarate aldolase and oxaloacetate decarboxylase	multifunctional 2-keto-3-deoxygluconate 6-phosphate aldolase and 2-keto-4-hydroxyglutarate aldolase and oxaloacetate decarboxylase	2-keto-3-deoxygluconate 6-phosphate aldolase and 2-keto-4-hydroxyglutarate aldolase	Entner-Doudoroff pathway, carbohydrate catabolic process, glyoxylate catabolic process	cytoplasm	
	6-phosphogluconate dehydratase	6-phosphogluconate dehydratase		Entner-Doudoroff pathway		
	glucose-6-phosphate 1-dehydrogenase	glucose-6-phosphate dehydrogenase		carbohydrate catabolic process, pentose-phosphate shunt, oxidative branch	cytoplasm	
	fused L-arabinose transporter subunits of ABC superfamily: ATP-binding components	fused L-arabinose transporter subunits of ABC superfamily: ATP-binding components	ATP-binding component of high-affinity L-arabinose transport system	carbohydrate catabolic process	cytoplasm	
	L-arabinose transporter subunit	L-arabinose transporter subunit	L-arabinose-binding periplasmic protein; periplasmic-binding component of ABC superfamily	carbohydrate catabolic process	periplasmic space	
	6-phosphogluconate dehydrogenase, decarboxylating	gluconate-6-phosphate dehydrogenase, decarboxylating		carbohydrate catabolic process, pentose-phosphate shunt, non-oxidative branch, pentose-phosphate shunt, oxidative branch		
	galactitol-specific enzyme IIC component of PTS	galactitol-specific enzyme IIC	PTS system galactitol-specific	carbohydrate catabolic process	organelle inner membrane,	

		component of PTS	enzyme IIC		peptidoglycan-based cell wall	
	D-tagatose 1,6-bisphosphate aldolase 2, subunit	D-tagatose 1,6-bisphosphate aldolase 2, subunit	putative tagatose 6-phosphate kinase 1	carbohydrate catabolic process		
	fused fructose-specific PTS enzymes: IIBcomponent/IIC components	fused fructose-specific PTS enzymes: IIBcomponent/IIC components	PTS system, fructose-specific transport protein	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	fused fructose-specific PTS enzymes: IIA component/HPr component	fused fructose-specific PTS enzymes: IIA component/HPr component	PTS system, fructose-specific IIA/fpr component	carbohydrate catabolic process	cytoplasm	
	glucokinase	glucokinase		carbohydrate catabolic process	cytoplasm	
	PEP-protein phosphotransferase of PTS system (enzyme I)	PEP-protein phosphotransferase of PTS system (enzyme I)	PEP-protein phosphotransferase system enzyme I	carbohydrate catabolic process		
	glucose-specific enzyme IIA component of PTS	glucose-specific enzyme IIA component of PTS	PTS system, glucose-specific IIA component	carbohydrate catabolic process	cytoplasm	
	transketolase 2, thiamin-binding	transketolase 2, thiamin-binding	transketolase 2 isozyme	carbohydrate catabolic process, nucleobase-containing small molecule interconversion, pentose-phosphate shunt, non-oxidative branch		
	DNA-binding transcriptional activator of 3-phenylpropionic acid catabolism	DNA-binding transcriptional activator of 3-phenylpropionic acid catabolism	transcriptional activator of hca cluster	carbohydrate catabolic process	cytoplasm	transcription activator activity, transcription repressor activity
	glucitol/sorbitol-specific	glucitol/sorbitol-	PTS system,	carbohydrate catabolic		

	enzyme IIC component of PTS	specific enzyme IIC component of PTS	glucitol/sorbitol-specific IIC component, one of two	process		
	glucitol/sorbitol-specific enzyme IIB component of PTS	glucitol/sorbitol-specific enzyme IIB component of PTS	PTS system, glucitol/sorbitol-specific IIB component and second of two IIC components	carbohydrate catabolic process		
	glucitol/sorbitol-specific enzyme IIA component of PTS	glucitol/sorbitol-specific enzyme IIA component of PTS	PTS system, glucitol/sorbitol-specific enzyme IIA component	carbohydrate catabolic process		
	sorbitol-6-phosphate dehydrogenase	sorbitol-6-phosphate dehydrogenase	glucitol (sorbitol)-6-phosphate dehydrogenase	carbohydrate catabolic process		
	DNA-binding transcriptional activator of glucitol operon	DNA-binding transcriptional activator of glucitol operon	glucitol operon activator	carbohydrate catabolic process		
	DNA-bindng transcriptional repressor	DNA-bindng transcriptional repressor	regulator for gut (srl), glucitol operon	carbohydrate catabolic process, regulation of transcription, DNA-dependent	cytoplasm	
	(D)-glucarate dehydratase 1	(D)-glucarate dehydratase 1	(D)-glucarate dehydratase 1 (L)-idarate dehydratase (L)-idarate epimerase (D)-glucarate epimerase	carbohydrate catabolic process		
	predicted D-glucarate transporter	predicted D-glucarate transporter	putative D-glucarate permease (MFS family)	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	6-phospho-beta-glucosidase	6-phospho-beta-	6-phospho-beta-	carbohydrate catabolic		

	A	glucosidase A	glucosidase A; cryptic	process		
	fructose-bisphosphate aldolase, class II	fructose-bisphosphate aldolase, class II		carbohydrate catabolic process		
	transketolase 1, thiamin-binding	transketolase 1, thiamin-binding	transketolase 1 isozyme	carbohydrate catabolic process, nucleobase-containing small molecule interconversion, pentose-phosphate shunt, non-oxidative branch		
	glycolate oxidase subunit, FAD-linked	glycolate oxidase subunit, FAD-linked	glycolate oxidase subunit D	carbohydrate catabolic process		
	altronate hydrolase	altronate hydrolase		carbohydrate catabolic process		
	uronate isomerase	uronate isomerase		carbohydrate catabolic process		
	hexuronate transporter	hexuronate transporter	transport of hexuronates	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	(D)-galactarate dehydrogenase	(D)-galactarate dehydrogenase		carbohydrate catabolic process		
	4-alpha-glucanotransferase (amylomaltase)	4-alpha-glucanotransferase (amylomaltase)		carbohydrate catabolic process, polysaccharide catabolic process	cytoplasm	
	maltodextrin phosphorylase	maltodextrin phosphorylase		carbohydrate catabolic process, polysaccharide catabolic process		
	glycogen phosphorylase	glycogen phosphorylase		carbohydrate catabolic process, polysaccharide biosynthetic process	cytoplasm	
	glycogen debranching enzyme	glycogen debranching enzyme	part of glycogen operon, a glycosyl hydrolase, debranching enzyme	carbohydrate catabolic process, polysaccharide biosynthetic process	cytoplasm	

	glyoxylate/hydroxypyruvate reductase B	2-keto-D-gluconate reductase (glyoxalate reductase) (2-ketoaldonate reductase)		carbohydrate catabolic process	cytoplasm	
	xylulokinase	xylulokinase		carbohydrate catabolic process		
	D-xylose isomerase	D-xylose isomerase		carbohydrate catabolic process, glucose metabolic process	cytoplasm	
	alpha-amylase	alpha-amylase		carbohydrate catabolic process, polysaccharide catabolic process, proteolysis	periplasmic space	
	fused mannitol-specific PTS enzymes: IIA components/IIB components/IIC components	fused mannitol-specific PTS enzymes: IIA components/IIB components/IIC components	PTS system, mannitol-specific enzyme IIABC components	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	mannitol-1-phosphate dehydrogenase, NAD-dependent	mannitol-1-phosphate dehydrogenase, NAD(P)-binding	mannitol-1-phosphate dehydrogenase	carbohydrate catabolic process		
	phosphoglycero mutase III, cofactor-independent	phosphoglycero mutase III, cofactor-independent	putative 2,3-bisphosphoglycerate-independent phosphoglycerate mutase	carbohydrate catabolic process	cytoplasm	
	predicted cytoplasmic sugar-binding protein	predicted cytoplasmic sugar-binding protein	D-ribose high-affinity transport system; membrane-associated protein	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	D-ribose transporter subunit	D-ribose transporter subunit	D-ribose periplasmic binding protein; periplasmic-	carbohydrate catabolic process	periplasmic space	

			binding compoent of ABC superfamily			
	fused predicted PTS enzymes: Hpr component/enzyme I component/enzyme IIA component	fused predicted PTS enzymes: Hpr component/enzyme I component/enzyme IIA component	PEP-protein phosphotransferase system enzyme I	carbohydrate catabolic process		
	maltose transporter subunit	maltose transporter subunit	part of maltose permease, inner membrane; membrane component of ABC superfamily	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	maltose transporter subunit	maltose transporter subunit	part of maltose permease, periplasmic; membrane component of ABC superfamily	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	maltose transporter subunit	maltose transporter subunit	periplasmic maltose-binding protein; substrate recognition for transport and chemotaxis; periplasmic-binding component of ABC superfamily	carbohydrate catabolic process, protein folding	periplasmic space	
	fused maltose transport subunit, ATP-binding component of ABC superfamily/regulatory protein	fused maltose transport subunit, ATP-binding component of ABC superfamily/regulatory protein	ATP-binding component of transport system for maltose	carbohydrate catabolic process	cytoplasm	
	maltose regulon periplasmic	maltose regulon	periplasmic	carbohydrate catabolic	periplasmic space	

	protein	periplasmic protein	protein of mal regulon	process		
	ribose 5-phosphate isomerase B/allose 6-phosphate isomerase	ribose 5-phosphate isomerase B/allose 6-phosphate isomerase	ribose 5-phosphate isomerase B	carbohydrate catabolic process, pentose-phosphate shunt, non-oxidative branch		
	C4-dicarboxylate transporter, anaerobic; DcuS co-sensor	C4-dicarboxylate antiporter	anaerobic dicarboxylate transport	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
	predicted DNA-binding transcriptional regulator of abgABT operon	predicted DNA-binding transcriptional regulator	putative transcriptional regulator LYSR-type	carbohydrate catabolic process	cytoplasm	transcription activator activity, transcription repressor activity
	5-keto-D-gluconate-5-reductase	5-keto-D-gluconate-5-reductase		carbohydrate catabolic process	cytoplasm	
	L-idonate 5-dehydrogenase, NAD-binding	L-idonate 5-dehydrogenase, NAD-binding		carbohydrate catabolic process		
	fused L-arabinose transporter subunits of ABC superfamily: membrane components	fused L-arabinose transporter subunits of ABC superfamily: membrane components	high-affinity L-arabinose transport protein (ABC superfamily, membrane)	carbohydrate catabolic process	organelle inner membrane, peptidoglycan-based cell wall	
AMINO ACID CATABOLIC PROCESS						
	Isoaspartyl peptidase	L-asparaginase	putative asparaginase	cellular amino acid catabolic process		
	D-amino acid dehydrogenase	D-amino acid dehydrogenase	D-amino acid dehydrogenase subunit	cellular amino acid catabolic process		
	glutamate decarboxylase B, PLP-dependent	glutamate decarboxylase B, PLP-dependent	glutamate decarboxylase isozyme	cellular amino acid catabolic process, cellular amino acid	cytoplasm	

				metabolic process		
	succinylglutamic semialdehyde dehydrogenase	succinylglutamic semialdehyde dehydrogenase		cellular amino acid catabolic process		
	arginine succinyltransferase	arginine succinyltransferase		cellular amino acid catabolic process		
	succinylornithine transaminase, PLP-dependent	succinylornithine transaminase, PLP-dependent	acetylornithine delta-aminotransferase	cellular amino acid catabolic process		
	L-serine deaminase I	L-serine deaminase I	L-serine deaminase	cellular amino acid catabolic process		
	DNA-binding transcriptional repressor, regulatory protein accessory to GcvA	DNA-binding transcriptional repressor, regulatory protein accessory to GcvA	transcriptional regulation of gcv operon	cellular amino acid catabolic process	cytoplasm	transcription repressor activity
	glycine decarboxylase, PLP-dependent, subunit (protein P) of glycine cleavage complex	glycine decarboxylase, PLP-dependent, subunit (protein P) of glycine cleavage complex	glycine decarboxylase, P protein of glycine cleavage system	cellular amino acid catabolic process		
	glycine cleavage complex lipoylprotein	glycine cleavage complex lipoylprotein	in glycine cleavage complex, carrier of aminomethyl moiety via covalently bound lipoyl cofactor	cellular amino acid catabolic process		
	aminomethyltransferase, tetrahydrofolate-dependent, subunit (T protein) of glycine cleavage complex	aminomethyltransferase, tetrahydrofolate-dependent, subunit (T protein) of glycine cleavage complex	aminomethyltransferase (T protein; tetrahydrofolate-dependent) of glycine cleavage system	cellular amino acid catabolic process		
	catabolic threonine dehydratase, PLP-dependent	catabolic threonine dehydratase, PLP-dependent	threonine dehydratase, catabolic	cellular amino acid catabolic process		

	propionate kinase/acetate kinase C, anaerobic	propionate kinase/acetate kinase C, anaerobic	propionate kinase/acetate kinase II, anaerobic	cellular amino acid catabolic process		
	glutamate decarboxylase A, PLP-dependent	glutamate decarboxylase A, PLP-dependent	glutamate decarboxylase isozyme	cellular amino acid catabolic process, cellular amino acid metabolic process	cytoplasm	
	dipeptide transporter	dipeptide transporter	dipeptide transport protein; periplasmic-binding component of ABC superfamily	cellular amino acid catabolic process	periplasmic space	
	tryptophanase leader peptide	tryptophanase leader peptide		cellular amino acid catabolic process		
	tryptophanase/L-cysteine desulfhydrase, PLP-dependent	tryptophanase/L-cysteine desulfhydrase, PLP-dependent	tryptophanase	cellular amino acid catabolic process		
	cystathionine gamma-synthase, PLP-dependent	cystathionine gamma-synthase, PLP-dependent	cystathionine gamma-synthase	cellular amino acid catabolic process, methionine biosynthetic process	cytoplasm	
	lysine decarboxylase, acid-inducible	lysine decarboxylase 1		cellular amino acid catabolic process, lysine biosynthetic process via diaminopimelate	cytoplasm	
	L-serine dehydratase 3	L-serine dehydratase 3	L-serine dehydratase	cellular amino acid catabolic process		
CENTRAL DOGMA						
DNA replication						
	regulatory protein for replication initiation	regulatory protein for replication initiation	negative modulator of initiation of	DNA-dependent DNA replication	organelle inner membrane, peptidoglycan-	

			replication		based cell wall	
	deoxyribodipyrimidine photolyase, FAD-binding	deoxyribodipyrimidine photolyase, FAD-binding	deoxyribodipyrimidine photolyase (photoreactivation)	DNA repair	cytoplasm	
	DNA topoisomerase I, omega subunit	DNA topoisomerase I, omega subunit	DNA topoisomerase type I, omega protein	DNA-dependent DNA replication	cytoplasm	
transcription						
	global DNA-binding transcriptional dual regulator H-NS	global DNA-binding transcriptional dual regulator H-NS	DNA-binding protein HLP-II (HU, BH2, HD, NS); pleiotropic regulator	regulation of transcription, DNA-dependent, taxis	cytoplasm, flagellum	
translation						
	seryl-tRNA synthetase, also charges selenocysteinyl-tRNA with serine	seryl-tRNA synthetase, also charges selenocysteinyl-tRNA with serine	serine tRNA synthetase; also charges selenocystein tRNA with serine	tRNA aminoacylation for protein translation	cytoplasm	
	purine nucleoside phosphoramidase, dadA activator protein	purine nucleoside phosphoramidase		translation		
	selenophosphate synthase	selenophosphate synthase	selenophosphate synthase, H(2)Se added to acrylyl-tRNA	tRNA aminoacylation for protein translation	cytoplasm	
	elongation factor P-like protein	predicted elongtion factor	putative elongation factor	translation		
	glutamyl-tRNA synthetase	glutamyl-tRNA synthetase	glutamate tRNA synthetase, catalytic subunit	tRNA aminoacylation for protein translation	cytoplasm	
	cold shock protein associated with 30S ribosomal subunit	cold shock protein associated with 30S ribosomal subunit	putative yhbH sigma 54 modulator	translation		
	glycine tRNA synthetase,	glycine tRNA		tRNA aminoacylation for	cytoplasm	

	beta subunit	synthetase, beta subunit		protein translation		
	glycine tRNA synthetase, alpha subunit	glycine tRNA synthetase, alpha subunit		tRNA aminoacylation for protein translation	cytoplasm	
	lysine tRNA synthetase, inducible	lysine tRNA synthetase, inducible	lysine tRNA synthetase, inducible; heat shock protein	tRNA aminoacylation for protein translation	cytoplasm	
	Stationary-phase-induced ribosome-associated protein	30S ribosomal subunit protein S22	30S ribosomal subunit protein S22; stationary phase-induced ribosome-associated protein	translation	cytoplasm	structural constituent of ribosome
NUCLEIC ACID METABOLISM						
DNA catabolic process						
	exonuclease I	exonuclease I	exonuclease I, 3' -> 5' specific; deoxyribophosphodiesterase	DNA catabolic process		
RNA catabolic process						
	ribonuclease I	ribonuclease I	RNase I, cleaves phosphodiester bond between any two nucleotides	RNA catabolic process	cytoplasm	
	pleiotropic regulatory protein for carbon source metabolism	pleiotropic regulatory protein for carbon source metabolism	carbon storage regulator; controls glycogen synthesis,	RNA catabolic process, carbohydrate catabolic process, gluconeogenesis,	cytoplasm	

			gluconeogenesis, cell size and surface properties	glycolysis, taxis, translational attenuation		
	exoribonuclease R, RNase R	exoribonuclease R, RNase R		RNA catabolic process		
DNA recombination						
	integration host factor (IHF), DNA-binding protein, alpha subunit	integration host factor (IHF), DNA-binding protein, alpha subunit	integration host factor (IHF), alpha subunit; site specific recombination	DNA recombination	cytoplasm	
RNA modification						
	16S rRNA U516 pseudouridine synthase	16S rRNA pseudouridylate 516 synthase	16S pseudouridylate 516 synthase	RNA modification	cytoplasm	
	selenocysteine synthase	selenocysteine synthase	selenocysteine synthase: L-seryl-tRNA (Ser) selenium transferase	RNA modification, pyridoxine biosynthetic process	cytoplasm	
	delta(2)-isopentenylpyrophosphate tRNA-adenosine transferase	delta(2)-isopentenylpyrophosphate tRNA-adenosine transferase		RNA modification	cytoplasm	
nucleobase-containing small molecule interconversion						
	cytosine/isoguanine deaminase	cytosine deaminase		nucleobase-containing small molecule interconversion	cytoplasm	
	adenosine deaminase	adenosine deaminase		nucleobase-containing		

				small molecule interconversion		
	AMP nucleosidase	AMP nucleosidase		nucleobase-containing small molecule interconversion	periplasmic space	
	nucleoside (except guanosine) transporter	nucleoside (except guanosine) transporter	permease of transport system for 3 nucleosides	nucleobase-containing small molecule interconversion	organelle inner membrane, peptidoglycan-based cell wall	
	uracil phosphoribosyltransferase	uracil phosphoribosyltransferase		nucleobase-containing small molecule interconversion		
	nucleoside transporter	nucleoside transporter	transport of nucleosides, permease protein	nucleobase-containing small molecule interconversion	organelle inner membrane, peptidoglycan-based cell wall	
	guanylate kinase	guanylate kinase		nucleobase-containing small molecule interconversion, purine ribonucleotide biosynthetic process	cytoplasm	
	uridine phosphorylase	uridine phosphorylase		nucleobase-containing small molecule interconversion		
	2':3'-cyclic-nucleotide 2'-phosphodiesterase	2':3'-cyclic-nucleotide 2'-phosphodiesterase		nucleobase-containing small molecule interconversion	cytoplasm	
	2-deoxyribose-5-phosphate aldolase, NAD(P)-linked	2-deoxyribose-5-phosphate aldolase, NAD(P)-linked	2-deoxyribose-5-phosphate aldolase	nucleobase-containing small molecule interconversion	cytoplasm	
	thymidine phosphorylase	thymidine phosphorylase		nucleobase-containing small molecule interconversion		
	phosphopentomutase	phosphopentomutase		nucleobase-containing small molecule interconversion		
PROTEIN						

MODIFICATION and FOLDING						
protein folding						
	DNA-binding transcriptional regulator of rRNA transcription, DnaK suppressor protein	DNA-binding transcriptional regulator of rRNA transcription, DnaK suppressor protein	dnaK suppressor protein	protein folding	cytoplasm	
	periplasmic chaperone	periplasmic chaperone	periplasmic molecular chaperone for outer membrane proteins	protein folding	peptidoglycan-based cell wall, periplasmic space	
	ribosome modulation factor	ribosome modulation factor		protein folding	cytoplasm	
	curved DNA-binding protein, DnaJ homologue that functions as a co-chaperone of DnaK	curved DNA-binding protein, DnaJ homologue that functions as a co-chaperone of DnaK	curved DNA-binding protein; functions closely related to DnaJ	protein folding	cytoplasm	
	protein disaggregation chaperone	protein disaggregation chaperone	heat shock protein	protein folding	cytoplasm	
	protease involved in processing C-terminal end of HycE	protease involved in processing C-terminal end of HycE	protease involved in processing C-terminal end of the large subunit of hydrogenase 3	protein folding		
	heat shock protein	heat shock protein	phage lambda replication; host DNA synthesis; heat shock protein; protein repair	protein folding	cytoplasm	
	FKBP-type peptidyl-prolyl	FKBP-type peptidyl-		protein folding	periplasmic space	

	cis-trans isomerase (rotamase)	prolyl cis-trans isomerase (rotamase)				
	peptidyl-prolyl cis-trans isomerase A (rotamase A)	peptidyl-prolyl cis-trans isomerase A (rotamase A)		protein folding		
	protein export chaperone	protein export chaperone	protein export; molecular chaperone; may bind to signel sequence	protein folding	cytoplasm	
	Cpn60 chaperonin GroEL, large subunit of GroESL	Cpn60 chaperonin GroEL, large subunit of GroESL		protein folding	cytoplasm	
	methionine sulfoxide reductase A	methionine sulfoxide reductase A	peptide methionine sulfoxide reductase	protein folding		
BIOSYNTHETIC PROCESS						
biosynthetic process						
	agmatinase	agmatinase		polyamine biosynthetic process		
	DNA-binding transcriptional dual regulator, O-acetyl-L-serine-binding	DNA-binding transcriptional dual regulator, O-acetyl-L-serine-binding	positive transcriptional regulator for cysteine regulon	cysteine biosynthetic process	cytoplasm	transcription activator activity, transcription repressor activity
	gamma-Glu-putrescine synthase	gamma-Glu-putrescine synthase	putative glutamine synthetase	glutamine biosynthetic process, nitrogen compound metabolic process		
	pyridoxine 5'-phosphate oxidase	pyridoxine 5'-phosphate oxidase	pyridoxinephosphate oxidase	pyridoxine biosynthetic process		
	GTP cyclohydrolase I	GTP cyclohydrolase I		folic acid biosynthetic process		
	cystathionine beta-lyase,	cystathionine beta-	cystathionine	methionine biosynthetic	cytoplasm	

	PLP-dependent	lyase, PLP-dependent	beta-lyase (beta-cystathionase)	process		
	L-threonine/L-serine transporter	L-threonine/L-serine transporter	anaerobically inducible L-threonine, L-serine permease	threonine biosynthetic process	organelle inner membrane, peptidoglycan-based cell wall	
cofactor biosynthetic process						
	DNA-binding transcriptional repressor for the molybdenum transport operon modABC	DNA-binding transcriptional dual regulator	molybdate uptake regulatory protein	Mo-molybdopterin cofactor biosynthetic process	cytoplasm	transcription repressor activity
	molybdate transporter subunit	molybdate transporter subunit	molybdate-binding periplasmic protein; permease; periplasmic-binding component of ABC superfamily	Mo-molybdopterin cofactor biosynthetic process	periplasmic space	
NAD biosynthetic process						
	UDP-galactose-4-epimerase	UDP-galactose-4-epimerase		NAD biosynthetic process, carbohydrate catabolic process, colanic acid biosynthetic process, galactose metabolic process, response to desiccation	cytoplasm	cell surface antigen activity, host-interacting
	NAD synthetase, NH3/glutamine-dependent	NAD synthetase, NH3/glutamine-dependent	NAD synthetase, prefers NH3 over glutamine	NAD biosynthetic process		

APPENDIX E

Table 6: *Total Number of Genes According to Their Biological Processes, Functions and Molecular Functions*

	NUMBER of GENES	
BIOLOGICAL PROCESS	**UP**	**DOWN**
stress response	22	20
metabolism	65	20
metabolic process	26	7
biosynthesis	21	54
aminoacid biosynthesis	44	5
catabolic process	114	30
nucleic acid&central dogma	30	31
protein modification	13	20
flagellum assembly, taxis	7	8
FUNCTIONS		
stress proteins	8	
drug resistance	2	
acid resistance	2	
ATP system	2	
chaperones (modulator)	3	
carrier proteins	1	
membrane proteins	19	75
transporter proteins	9	38
enzymes	99	48
lipoproteins	5	4

prophage	7	17
regulatory proteins	2	12
cell division	1	2
hypothetical proteins	3	4
mix	14	29
adhesin proteins		5
efflux proteins		8
antitoxin proteins		2
transposae		9
secretory pathway		7
pseudogene		8
predicted proteins	35	55
conserved proteins	54	58
MOLECULAR FUNCTIONS	13	20

CV

Education Status:

Graduate (2009 –):Marmara University, Faculty of Engineering, Department of Bioengineering (Istanbul, Turkey)

Undergraduate (2005 – 2009): Istanbul University, Faculty of Science, Department of Biology (Istanbul, Turkey)

High School Education (2000 – 2004): Hasköy Güner Akýn High School (Istanbul, Turkey)

Work Experience:

Laboratory Intern: Yildiz Technical University, Faculty of Chemical-Metallurgy, Deparment of Bioengineering, Cell and Tissue Culture Laboratory (July, 2009).

Presented Announcements

- Karaosmanoğlu K., Özbalci Ç., Aksan Kurnaz I., Kazan D., Sariyar Akbulut B. : "Comparative Transcriptome and Proteome Analysis for the Effect of Berberine", 35th Federation of European Biochemical Societies Congress 2010,Gothenburg, Sweden (Poster Presentation).
- Karaosmanoğlu K., Kazan D., Sariyar Akbulut B. : "Proteomic Analysis of the Effect of Berberine on Escherichia coli ", Mass Spectrometry in Biotechnology and Medicine 2011, Dubrovnik, Croatia (Poster Presentation).
- Karaosmanoğlu K., Yurtsever Aksoy İ., Coşkun A., Akgöz M. : " Decomposition of Liver Tissue During Storage in Hydroxyethyl Starch" , Mass Spectrometry in Biotechnology and Medicine 2011, Dubrovnik, Croatia (Poster Presentation).
- Karaosmanoğlu, K., Coskun, A., Kazan, D., Akgöz, M., Berber, İ., Yazici, C., Bilsel, G., Aksoy, İ.: "Proteomics Profiling Of Preservation Solution Prior To Renal Transplantation" , Central and Eastren European Proteomics Congress 2011, Prague, Czech Republic. (Poster Presentation)

Attended Courses& Symposiums

- Proteomics Workshop With International Participation "Proteomistanbul" , August 2010, Istanbul University, Faculty of Science, Deparment of Biology and Genetics, Istanbul,Turkey.

- "5th Summer School on Mass Spectrometry in Biotechnology and Medicine " , 3-10 July, Center for Advanced Academic Studies, Dubrovnik, Croatia.
- "RNA Tabanlı Moleküler Yöntemler Uygulamalı Eğitim Kursu" , Boğaziçi University&Istanbul Technical University, September, 2011.
- 2nd Symposium of the Proteomics Society "Small Molecule Design and Protein Interactions", May 3 2012, School of Medicine, Acıbadem University, Istanbul, Turkey.

Awards:

"Proteomics Profiling Of Preservation Solution Prior To Renal Transplantation" , Central and Eastren European Proteomics Congress 2011, Prague, Czech Republic. (Best Poster Presentation).