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Identification of Uropathogenic *Escherichia coli* Surface Proteins by Shotgun Proteomics

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Abstract

Uropathogenic *Escherichia coli* (UPEC) cause the majority of uncomplicated urinary tract infections in humans. In the process of identifying candidate antigens for a vaccine, two methods for the identification of the UPEC surface proteome during growth in human urine were investigated. The first approach utilized a protease to ‘shave’ surface-exposed peptides from the bacterial cell surface and identify them by mass spectrometry. Although this approach has been successfully applied to a Gram-positive pathogen, the adaptation to Gram-negative UPEC resulted in cytoplasmic protein contamination. In a more direct approach, whole-cell bacteria were labeled with a biotin tag to indicate surface-exposed peptides and two-dimensional liquid chromatography-tandem mass spectrometry (2-DLC-MS/MS) was used to identify proteins isolated from the outer membrane. This method discovered 25 predicted outer membrane proteins expressed by UPEC while growing in human urine. Nine of the 25 predicted outer membrane proteins were part of iron transport systems or putative iron-regulated virulence proteins, indicating the importance of iron acquisition during growth in urine. One of the iron transport proteins identified, Hma, appears to be a promising vaccine candidate is being further investigated. The method described here presents a system to rapidly identify the outer membrane proteome of bacteria, which may prove valuable in vaccine development.

Keywords

Outer membrane proteome; vaccine; surface-exposed peptides

1. Introduction

Urinary tract infections (UTIs) are among the most frequent bacterial infections affecting humans. It is estimated that there are 11.3 million community-acquired UTIs in the United States annually, which result in projected public health costs of over two billion dollars (Foxman *et al.*, 2000; Litwin *et al.*, 2005). Forty percent of all women will experience at least one UTI in their lifetime and one in four will have a recurrent UTI within six months (Foxman, 1990). Up to 90% of uncomplicated UTI cases in adults are caused by the extraintestinal pathogen uropathogenic *Escherichia coli* (UPEC) (Zhang and Foxman, 2003). Oftentimes, the

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UPEC bacteria originate from the patients' own intestinal flora. Infection begins when the bacteria ascend the urethra from the periurethral area and colonize the bladder leading to cystitis. If left untreated, the infection can ascend the ureters, leading to the development of pyelonephritis and potentially bacteremia.

Bacteria sense and interact with their environment using proteins expressed on their surface. Proteins on the bacterial surface are also likely to be readily accessible to host immune responses, making them attractive vaccine targets to neutralize or eradicate infecting pathogens (Grandi, 2001). The UPEC genome contains numerous surface-expressed virulence factors that aid in the colonization of the urinary tract. UPEC utilizes several surface appendages termed fimbriae or adhesins, such as P, type 1, F1C, S, M, and Dr fimbriae, to colonize the mucosal epithelium and endothelial cells of the urinary tract (Johnson, 1991; Nowicki *et al.*, 1989). Other virulence factors include secreted toxins, such as cytotoxic necrotizing factor 1 (Caprioli *et al.*, 1987), secreted autotransporter toxin (Maroncle *et al.*, 2006) and hemolysin (Smith, 1963; Welch, 1991), as well as numerous iron acquisition systems that aid in survival within the urinary tract by scavenging iron molecules from the host (Opal *et al.*, 1990; Russo *et al.*, 2001; Russo *et al.*, 2002; Torres *et al.*, 2001).

Many proteins associated with the phospholipid lipid membrane layers are hydrophobic and contain numerous transmembrane domains, making them more challenging to study than readily soluble proteins. These technical limitations have hindered the ability to define many membrane proteins at the structural and functional level and have led to an underrepresentation of particular classes of proteins. Recent advances in proteomics technology have led to a better understanding of the microbial outer membrane proteome. The use of carbonate extraction and the strong zwitterionic detergent amido sulfobetaine-14 (ASB-14) allowed for identification of 21 of the 26 predicted outer membrane proteins present in *E. coli* K-12 (Molloy *et al.*, 2000). Two-dimensional gel electrophoresis (2-DE) has improved the solubilization and separation of proteins and become a method of choice for studying membrane proteins. However, intrinsic properties of 2-DE lead to an underrepresentation of proteins that are highly hydrophobic or highly basic and proteins of low abundance, high molecular weight, or extreme isoelectric points.

Recently, gel-free methods have been developed that overcome many of the problems associated with 2-DE and provide a comprehensive analysis of bacterial membrane proteins (Wu *et al.*, 2003; Wu and Yates, 2003). Gel-free methods utilize two-dimensional liquid chromatography (2-DLC), frequently strong cation exchange in the first dimension followed by reverse-phase chromatography in the second dimension, and tandem mass spectrometry (MS/MS) to separate mixtures of proteins that have been subjected to protease digestion and individually identify them. The 2-DLC-MS/MS approach does not show the bias against membrane proteins which are highly hydrophobic or highly basic, proteins with low abundance, high molecular weight, or extreme isoelectric points typically observed with 2-DE analysis (Cordwell, 2006).

In this report, we examine two methods aimed at identifying the outer membrane proteome of UPEC cultured in pooled human urine. Our initial method attempted to isolate surface-exposed peptides from UPEC through the use of a protease to shave the extracellular domains of outer membrane proteins from the bacterial surface, similar to an approach taken with group A *Streptococcus* by Rodriguez-Ortega and colleagues (Rodriguez-Ortega *et al.*, 2006). Bacterial cell lysis proved problematic with this approach, so we utilized a more direct approach and isolated outer membrane proteins by differential centrifugation and identified them with 2-DLC-MS/MS. Sulfo-NHS-SS-Biotin was used to label lysines on extracellular loops of outer membrane proteins to identify domains that may be surface exposed and available to interact with the host immune system. This technique allowed us to identify 25 predicted outer

membrane proteins expressed during growth in human urine, one of which is currently being applied in the development of an UPEC UTI vaccine.

2. Materials and Methods

2.1 Bacterial strains, media, and growth conditions

CFT073 is a representative UPEC strain that was isolated from the urine and blood of a patient with acute pyelonephritis (Mobley *et al.*, 1990); the genome has been sequenced and fully annotated (Welch *et al.*, 2002). Overnight bacterial cultures were inoculated from isolated colonies and cultured in Luria-Bertani (LB) aerobically at 37°C. Bacteria from the overnight cultures were centrifuged (3500 x g, 10 min, 25°C) to collect the cells, washed with sterile phosphate-buffered saline (PBS), and used to inoculate human urine cultures. Human urine was collected midstream in sterile sample containers from 4–8 male and female donors. Urine was pooled and sterilized by vacuum filtration through a 0.22- μ m-pore-size filter. UPEC strain CFT073 was cultured statically at 37°C in pooled urine to exponential phase to an optical density at 600 nm of 0.25–0.28 for all studies.

2.2 ‘Shaving’ of bacterial surface proteins by trypsin

Bacterial cells were collected by centrifugation and washed 3 times with PBS, pH 7.4. Cell pellets were resuspended in 1 ml of PBS, pH 7.4; PBS containing 40% sucrose, pH 7.4 (Rodriguez-Ortega *et al.*, 2006); or 10 mM HEPES, pH 7.4. Twenty micrograms of porcine sequencing grade modified trypsin (Promega) was added to the samples and bacterial cell suspensions were incubated for 30 min at 37°C. Samples of the bacterial suspension were taken pre- and post- protease digestion to determine colony forming units (CFUs). Bacterial cells were removed by centrifugation (3500 x g, 10 min, 4°C). The supernatant was passed through a 0.22- μ m-pore-size filter to remove any remaining bacterial cells. Dithiothreitol (DTT) and trifluoroacetic acid (TFA) were added to final concentrations of 5 μ M and 0.1% respectively. The solution containing the peptides was stored at –80°C until ready for analysis.

2.3 Biotin labeling and preparation of bacterial membranes

UPEC bacteria cultured to exponential phase in pooled human urine were collected by centrifugation (3500 x g, 10 min, 4°C) and washed 3 times with PBS. Cells were resuspended in 10 ml PBS containing 0.8 mg/ml Sulfo-NHS-SS-Biotin (Pierce) and incubated on ice for 30 min. Bacterial cells were collected by centrifugation (3500 x g, 10 min, 4°C) and washed twice with 500 mM glycine-PBS to remove and quench any free biotin labeling reagent.

Outer membranes were prepared by carbonate extraction and ultracentrifugation adapted from Molloy, *et al.* (Molloy *et al.*, 2000). Briefly, bacteria were resuspended in 10 ml of 10 mM HEPES, pH 7.4, containing 100 U of Benzonase (Sigma) and lysed in a French pressure cell at 20,000 lb/in². Cell debris and unbroken whole cells were removed by centrifugation and the supernatant was diluted to 60 ml with 0.1 M sodium carbonate buffer, pH 11.0. The solution was incubated with stirring at 4°C for 1 hr. Membranes were recovered by ultracentrifugation (116,000 x g, 1 hr, 4°C) and the membrane pellet rinsed with 10 mM HEPES, pH 7.4. The pellet was resuspended in 2% sarcosine and incubated for 30 min to solubilize inner membranes. Outer membranes were subsequently collected by ultracentrifugation (116,000 x g, 30 min, 4°C).

2.4 Solubilization of membrane proteins and trypsin digestion

Proteins in the membrane pellet from the biotin-labeled UPEC were solubilized using an organic solvent followed by a detergent, similar to the approaches taken by Zhang, *et al.* (Zhang *et al.*, 2007). Briefly, membrane proteins were quantified using the BCA Protein Assay

(Pierce) and 0.5 mg protein was solubilized with 60% methanol. Protein that was insoluble in the organic solvent was recovered by ultracentrifugation (116,000 x g, 1 hr, 4°C). The supernatant was transferred to a new collection tube and the pellet was solubilized with a 1% solution of the anionic detergent sodium dodecyl sulfate (SDS). Once solubilized, the solution was diluted 10 times with 50 mM NH₄HCO₃ buffer, resulting in a final concentration of SDS of 0.1%. DTT was added to a concentration of 10 mM to both the organic and detergent fractions, and the solutions were incubated at 50°C for 1 hr to reduce disulfide bonds. Iodoacetic acid (IAA) (10 mM) was used to alkylate free thiol groups for 45 min while protected from light. Proteins in the methanol and SDS fractions were each digested with 10 µg porcine sequencing grade modified trypsin (Promega) overnight at 37°C.

2.5 Fractionation of peptides by strong cation exchange

The pH of both the trypsin cleaved surface peptides and the methanol and SDS samples was adjusted to pH 2.8 with concentrated hydrochloric acid. Samples were fractionated on strong cation exchange spin columns (Pierce) following the manufacturer's protocol. Briefly, the sample was passed through the column and washed twice with 5 mM potassium phosphate buffer, pH 2.8. Bound peptides were eluted into 5 KCl fractions (0.01 M, 0.05 M, 0.1 M, 0.5 M, and 1.0 M) containing 40% acetonitrile in 5 mM potassium phosphate buffer, pH 2.8. Fractions of the same KCl concentration from the organic- and detergent-solubilized samples were combined and stored at -80°C.

2.6 Liquid chromatography and tandem mass spectrometry

Peptides were separated by C₁₈ nano-liquid chromatography (LC) using an 1100 Series nano-HPLC equipped with µWPS autosampler, 2/10 microvalve, ultra-violet multiple wavelength detector (214 nm), and Micro-FC fraction collector/spotter (Agilent). Each fraction was reconstituted with 43 µl 0.1 % TFA, v/v, in water and 40 µl of sample was injected onto a C₁₈ cartridge (Zorbax300SB, 5 µm, 0.3 x 5 mm; Agilent) and desalted with solvent C (CH₃CN:H₂O:TFA, 5:95:0.1) at 20 µl/min for 9 min. An enrichment cartridge was placed ahead of a C₁₈ column (Zorbax300SB, 3.5 µm, 0.075 x 15 mm; Agilent) equilibrated with solvent A (CH₃CN:H₂O:TFA, 6.5:93.5:0.1). Peptides were eluted with a gradient of solvent B (CH₃CN:H₂O:TFA, 90:10:0.1) from 6.5% B to 50% B over 90 min at a flow rate of 0.3 µl/min. Column effluent was mixed (micro Tee, Agilent) with matrix (2 mg/ml α-cyano-4-hydroxy cinnamic acid) in CH₃OH:isopropanol:CH₃CN:H₂O:acetic acid (14:30:22.4:33:0.6) containing 10 mM ammonium phosphate delivered with a PHD200 infusion pump (Harvard Apparatus) at 1.0 µl/min. Fractions were spotted at 24 s intervals onto a stainless steel maldi target plate (192 wells/plate, Applied Biosystems).

Mass spectra were acquired on an Applied Biosystems 4800 MalDI TOF/TOF Analyzer (TOF/TOF) using 4000 Series Explorer. MS spectra from 800–3500 Da were acquired for each fraction. The 12 most intense peaks in each MS spectrum were selected for MS/MS analysis. Peptide fragmentation was induced by the use of atmosphere as a collision gas with a pressure of 6 x 10⁻⁷ torr, measured at the vacuum gauge, and collision energy of 2 kV.

Peptide identifications were performed using GPS Explorer (v3.6, Applied Biosystems) as a front end for the MASCOT search engine (v2.1 MatrixScience). The search parameters were (i) species, *E. coli* strain CFT073; (ii) trypsin, specificity of two missed cleavages; (iii) fixed modification, cysteines alkylated with IAA; (iv) variable modifications; oxidized methionine, deamidation, pyroglutamate, and thioacyl IAA lysine; (v) peptide tolerance, 0.7 ppm; and (vi) MS/MS tolerance, +/- 0.3 Da. Thioacyl modification of lysine resulted from the cleavage of the disulfide bond of lysine-bound Sulfo-NHS-SS-Biotin, releasing the biotin tag. Only significant hits as defined by a confidence interval (C.I. %) of 95%, as calculated by GPS Explorer, were considered.

3. Results

3.1 Buffer selection for surface-exposed protein trypsin digestion

Initially, buffers used during the trypsin digestion of surface-exposed proteins were tested for the amount of cell lysis occurring during the 30 min incubation at 37°C by plating samples pre- and post-digestion on Luria agar to determine CFUs (Table 1). The first buffer tested was PBS, pH 7.4 containing 40% sucrose as used by Rodriguez-Ortega, *et al.* in their identification of vaccine candidates from protease digestion of group A *Streptococcus* surface proteins (Rodriguez-Ortega *et al.*, 2006). This buffer resulted in the lysis of nearly half of the UPEC cells under the given conditions. Similar results were obtained when PBS, pH 7.4 containing no sucrose was used. It was found that 10 mM HEPES, pH 7.4 resulted in minimal cell lysis with approximately 0.25% of cells lysing.

3.2 Comparison of trypsin ‘shaving’ of surface-exposed proteins and organic/detergent soluble outer membrane preparation

Peptides from the 0.1 M KCl SCX fractions of the trypsin ‘shaving’ and the outer membrane preparation were analyzed by 2-DLC-MS/MS to determine which method would be better able to assess the surface proteome of UPEC during growth in human urine. The analysis identified 47 proteins in the surface protein trypsin digestion and 31 proteins in the organic and detergent soluble outer membrane preparation (Table 2). The trypsin ‘shaving’ fraction contained only one protein predicted by PSORT (Nakai and Kanehisa, 1991) to be located in the outer membrane, whereas the same fraction from the outer membrane preparation yielded 11 proteins predicted to be localized to the outer membrane by PSORT. Furthermore, significantly more proteins predicted by PSORT to be located in the cytoplasm were present in the 0.1 M fraction of the trypsin ‘shaving’ sample (26 proteins) than in the equivalent outer membrane preparation fraction (1 protein). The numerous proteins predicted to be cytoplasmic in the protease-cleaved surface peptide sample were indicative of bacterial cell lysis. Both the trypsin ‘shaving’ and outer membrane preparation samples contained a small number of proteins predicted to be located in the inner membrane or periplasm, as well as a large percentage of proteins with an unknown location based on PSORT analysis (~30% and ~45%, respectively).

3.3 Outer membrane proteome of CFT073 during culture in human urine

Based on the comparison of predicted protein locations found in the 0.1 M KCl fraction of the protease ‘shaving’ and the outer membrane preparation samples, it appeared that the second method resulted in the least amount of contaminating cytoplasmic proteins. The remaining 4 fractions from SCX separation of the outer membrane preparation were subsequently analyzed. Organic and detergent solubilization of the outer membranes prepared from CFT073 cultured in human urine allowed us to identify 102 unique proteins by 2-DLC-MS/MS. Of the proteins identified, the PSORT algorithm predicted that 25 were localized to the bacterial outer membrane, 12 resided in the inner membrane, 6 were periplasmic, 1 was extracellular, 22 were cytoplasmic, and 36 protein locations were unknown.

Ten proteins identified by 2-DLC-MS/MS contained thiolacyl modified lysines on at least one peptide as a result of biotinylation with Sulfo-NHS-SS-Biotin. The thiolacyl modification was found on 3 proteins predicted to be located in the outer membrane, 2 in the periplasm, 3 with an unknown location, and 2 predicted to be cytoplasmic. Modeling the outer membrane proteins using the Hidden Markov Model method and TMRPres2D revealed that the majority of modified lysines were present on extracellular loops of the outer membrane proteins, but not all (data not shown). The presence of biotin-labeled lysines on proteins residing in the periplasm and cytoplasm suggests that the Sulfo-NHS-SS-Biotin was able to cross the membrane of the cell during the labeling process, as proteins from lysed cells modified during the labeling step would have been removed by washing.

4. Discussion

Several new technologies for studying bacterial surface proteomics have been recently developed that surmount a number of limitations presented by the traditional method of choice, 2-DE. Identification of surface-exposed portions of the outer membrane proteome may significantly improve the efficacy of a vaccine by focusing on portions of the protein that are exposed to and recognized by the host immune system. The first method attempted to determine surface-exposed domains of outer membrane proteins through the use of a protease to cleave off exposed peptides, an approach first successfully performed in a Gram-positive bacterium without significant cytoplasmic protein contamination by Rodriguez-Ortega and colleagues (Rodriguez-Ortega *et al.*, 2006). However, adaptation of this technique to the Gram-negative UPEC proved problematic. Despite similar numbers of viable cells before and after surface protein digestion with trypsin in the 10 mM HEPES, pH 7.4 buffer (Table 1), the majority of proteins in the sample (~55%) were predicted to be cytoplasmic proteins in the 0.1 M KCl SCX fraction (Table 2). The thinner cell walls of the Gram-negative *E. coli* may make them more susceptible to lysis during the trypsin digestion of surface-exposed peptides. Although cell lysis did not appear to be significant based on determination of CFU pre- and post-digestion with the 10 mM HEPES, pH 7.4 buffer, the amount of cytoplasmic protein released into the sample from even a small number of bacteria lysing may quickly overwhelm the amount of trypsin-cleaved peptides from surface-exposed domains of outer membrane proteins. Modifications to the length of digestion, trypsin concentration, and buffer composition were unsuccessful in significantly reducing the amount of contaminating cytoplasmic proteins (data not shown) despite the fact that loss of viability could be limited.

To overcome the cytoplasmic protein contamination, a more direct method of ‘shotgun’ proteomics was used to determine the outer membrane proteome of UPEC during growth in urine. Solubilization of the outer membrane was carried out in a stepwise progression to maximize the number of proteins extracted from the membrane. The first step used was an organic solvent (60% methanol), which has been previously shown to result in the LC-MS/MS identification of more proteins than SDS-solubilized samples (Zhang *et al.*, 2007) and can be easily removed from the sample after protease digestion. The second step used the anionic detergent SDS to solubilize the proteins that were insoluble in 60% methanol. SDS dissolves a wider range of proteins, as well as precipitated proteins, but has the drawbacks of needing to be used at high concentrations and difficulty removing the detergent from the sample. The high concentration of detergents may also lead to interference with enzymes, such trypsin used prior to 2-DLC-MS/MS. The use of both organic and detergent methods to solubilize the outer membrane should result in the majority of proteins being extracted from the membrane for analysis by 2-DLC-MS/MS.

This approach was able to identify 25 proteins predicted to be located in the outer membrane. The number of contaminating cytoplasmic proteins was also significantly reduced (~22% of the proteins identified) compared to the protease ‘shaving’ method (~55% of the proteins identified). Nine of the 25 predicted outer membrane proteins that were identified either belonged to iron transport systems or were a putative iron-regulated virulence protein. Similar to other pathogens, iron acquisition has been shown to be important for UPEC pathogenesis (Russo *et al.*, 2001; Russo *et al.*, 2002; Torres *et al.*, 2001). The prototypic UPEC strain CFT073 encodes at least 14 outer membrane iron receptors (Welch *et al.*, 2002), illustrating the importance of iron acquisition during growth in the urinary tract. The results presented here further contribute evidence that iron acquisition plays a crucial role in the ability of UPEC to grow in human urine. One of the iron-related proteins identified in this screen was the recently characterized heme receptor Hma (Hagan and Mobley, 2008). This protein has been shown to be more prevalent in UPEC strains than commensal *E. coli* strains (Lloyd *et al.*, 2007) and to be expressed *in vivo* in a mouse model of an ascending UTI (Hagan and Mobley, 2007). These

properties make Hma an attractive vaccine target, and its development is actively being pursued.

Since the digestion of surface peptides from the outer membrane was unsuccessful, the membrane impermeable Sulfo-NHS-SS-Biotin was used to label surface-exposed lysines of outer membrane proteins to determine peptides likely to be surface-exposed and accessible to the host immune system. In this study, 3 predicted outer membrane proteins and 7 other proteins identified by 2-DLC-MS/MS contained thiolacyl modified lysines indicating that they had been bound by the biotin tag. The presence of labeled proteins residing in the cytoplasm and periplasm indicate that the Sulfo-NHS-SS-Biotin was able to cross the membrane in these experiments. The biotin label was not utilized for affinity purification with immobilized-Neutravidin (Pierce) as a substrate because of the solubilization methods employed in this approach, specifically the organic fraction. The 60% methanol would likely precipitate Neutravidin, leading to the loss of its ability to bind the biotin label. One possible improvement of these techniques might be to utilize the Sulfo-NHS-SS-Biotin tag to label outer membrane proteins, cleave the surface-exposed portions of the proteins with a protease, and separate the surface-exposed peptides from the supernatant utilizing the interaction between biotin and Neutravidin.

This work examined two methods of identifying the outer membrane proteome of UPEC during growth in pooled human urine. It was found that the novel method of using a protease to ‘shave’ the surface of the bacteria resulted in cytoplasmic protein contamination with UPEC. The more direct method of ‘shotgun’ sequencing the outer membrane fraction of UPEC allowed us to identify 25 predicted outer membrane proteins expressed by UPEC while growing in human urine. Information gained from the identity of these proteins found on the bacterial surface during growth in human urine may allow for the development of a vaccine aimed at preventing or reducing the severity of UTIs caused by UPEC. To this end, work is underway on creating a vaccine based on the outer membrane heme receptor, Hma. The method reported here offers a promising technique to identify the membrane proteome of bacteria expressed under a given condition that may prove valuable in vaccine creation and could aid in the development of further applications to determine the surface-exposed portions of proteins.

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Table 1

Colony forming units pre- and post-trypsin digestion

	Pre-digestion	Post-digestion
PBS + 40% Sucrose	5.89×10^9	3.01×10^9
PBS	6.60×10^9	3.75×10^9
10 mM HEPES	7.58×10^9	7.56×10^9

Table 2

PSORT-Predicted locations of proteins from the 0.1 M KCl fractions

PSORT-Predicted Location	Trypsin 'shaving'	Organic / detergent soluble
Outer Membrane	1	11
Inner Membrane	4	2
Periplasmic	1	1
Cytoplasmic	26	3
Extracellular	1	0
Unknown	14	14

Table 3

Proteins from outer membrane preparation identified by 2-DLC-MS/MS

Protein	Function	PSORT Location	Biotinylated
Imp	Organic solvent tolerance protein precursor	Outer Membrane	+
FhuA	Ferriochrome-iron receptor	Outer Membrane	
YaeT	Unknown protein	Outer Membrane	
Tsx	Nucleoside-specific channel-forming protein	Outer Membrane	
OmpT	Outer Membrane protease	Outer Membrane	
FepA	Ferrienterobactin receptor	Outer Membrane	
Pal	Cell envelope biogenesis, Outer Membrane	Outer Membrane	
OmpF	Porin	Outer Membrane	
OmpA	Porin	Outer Membrane	+
FocD	F1C fimbrial usher	Outer Membrane	
IroN	Siderophore receptor	Outer Membrane	
C2348	Porin	Outer Membrane	
Hma	Heme/hemoglobin receptor	Outer Membrane	
OmpC	Porin	Outer Membrane	
FadL	Long-chain fatty acid transport	Outer Membrane	
C3610	Putative receptor	Outer Membrane	
IutA	Ferric Aerobactin Receptor	Outer Membrane	
C3655	Type V secretion/autotransporter, adhesin	Outer Membrane	
TolC	Efflux of hydrophobic/amphipathic molecules	Outer Membrane	
ChuA	Heme/hemoglobin receptor	Outer Membrane	
YiaD	Hypothetical lipoprotein	Outer Membrane	
BtuB	Vitamin B12 receptor	Outer Membrane	
C5174	Putative iron-regulated virulence protein	Outer Membrane	
C5389	Hypothetical protein	Outer Membrane	
TonB	Uptake of chelated Fe(2+) and cyanocobalamin	Outer Membrane	+
YgjD	O-sialoglycoprotein endopeptidase	Extracellular	
YagU	Hypothetical protein yagU	Inner Membrane	
AcrA	Acriflavine resistance and efflux protein	Inner Membrane	
CydA	Cytochrome D ubiquinol oxidase subunit I	Inner Membrane	
C0970	Hypothetical protein	Inner Membrane	
LoID	Lipoprotein transporter ATP-binding subunit	Inner Membrane	
XasA	Amino acid antiporter	Inner Membrane	
GlpT	Glycerol-3-phosphate transporter	Inner Membrane	
SdaC	Serine transporter	Inner Membrane	
PstC	Phosphate transport system permease	Inner Membrane	
AtpE	ATP synthase C chain	Inner Membrane	
YijP	Hypothetical protein	Inner Membrane	
CycA	D-serine/D-alanine/glycine transporter	Inner Membrane	
RcsF	Outer Membrane lipoprotein sensor kinase	Unknown	
SdhA	Succinate dehydrogenase flavoprotein	Unknown	
YaeC	D-methionine-binding lipoprotein metQ precursor	Unknown	

Protein	Function	PSORT Location	Biotinylated
RlpA	Rare lipoprotein A coli	Unknown	
RlpB	Assembly of LPS in the Outer Membrane	Unknown	
SdhB	Succinate dehydrogenase iron-sulfur protein	Unknown	
YbhC	Pectinesterase lipoprotein	Unknown	
YbjP	Putative lipoprotein	Unknown	
C1171	Conserved hypothetical protein	Unknown	
MltE	Membrane-bound lytic murein transglycosylase	Unknown	
LolB	Incorporates lipoproteins in the Outer Membrane	Unknown	
C1874	Putative conserved protein	Unknown	
SlyB	Cell envelope biogenesis, Outer Membrane	Unknown	
Lpp	Cell wall, membrane, envelope biogenesis	Unknown	+
YdiB	Quinate/shikimate dehydrogenase	Unknown	
YeaY	Hypothetical lipoprotein	Unknown	
YfaZ	Hypothetical protein	Unknown	
VacJ	Lipoprotein	Unknown	
XseA	Single-strand-specific exonuclease	Unknown	
MltA	Membrane-bound lytic murein transglycosylase	Unknown	
RplQ	Translation, 50S ribosomal protein L17	Unknown	
RpsK	Translation, 30S ribosomal protein S11	Unknown	
RplN	Translation, 50S ribosomal protein L14	Unknown	
RplB	Translation, 50S ribosomal protein L2	Unknown	
RpsL	Translation, 30S ribosomal protein S12	Unknown	
DamX	Hypothetical protein	Unknown	
C4182	Hypothetical protein	Unknown	
YhhA	Hypothetical protein	Unknown	
Slp	Starvation-inducible Outer Membrane lipoprotein	Unknown	
C4729	Conserved hypothetical protein	Unknown	
C5196	Transposase <i>insC</i>	Unknown	
C4533	Putative pduB protein, propanediol utilization	Unknown	
HflK	FtsH protease regulator	Unknown	
HflC	FtsH protease regulator	Unknown	
IroE	Esterase for <i>apo</i> and Fe ³⁺ -bound siderophores	Unknown	+
C5426	Conserved hypothetical protein	Unknown	+
GlnH	Part of glutamine ABC transporter	Periplasmic	
OppA	Periplasmic oligopeptide-binding protein	Periplasmic	+
DmsA	Anaerobic dimethyl sulfoxide reductase chain A	Periplasmic	
TonB	Membrane spanning protein in TonB-ExbB-ExbD	Periplasmic	+
KpsD	Polysialic acid transport protein	Periplasmic	
FdnG	Formate dehydrogenase	Periplasmic	
RpsB	Ribosomal protein S2	Cytoplasm	
C0276	Hypothetical protein	Cytoplasm	
YcaJ	recombination factor protein	Cytoplasm	

Protein	Function	PSORT Location	Biotinylated
YdfR	Hypothetical protein	Cytoplasm	
C1513	Putative Nudix hydrolase	Cytoplasm	
YdhV	Hypothetical protein	Cytoplasm	+
PpsA	Phosphoenolpyruvate synthase	Cytoplasm	
YfiA	Stress-response protein	Cytoplasm	
RpsU	Translation, 30S ribosomal protein	Cytoplasm	
AgaY	Carbohydrate transport/metabolism	Cytoplasm	+
DeaD	ATP-dependent RNA helicase DeaD	Cytoplasm	
SspA	Stringent starvation protein A	Cytoplasm	
RpsI	Translation, 30S ribosomal protein S9	Cytoplasm	
RpsD	Translation, 30S ribosomal protein S4	Cytoplasm	
RplO	Translation, 50S ribosomal protein L15	Cytoplasm	
RpsC	Translation, 30S ribosomal protein S3	Cytoplasm	
EF-Tu	GTP-binding translation factors	Cytoplasm	
GlgC	Glucose-1-P adenylyltransferase	Cytoplasm	
AtpD	ATP synthase beta chain	Cytoplasm	
TufB	GTPases - translation elongation factor	Cytoplasm	
RpoC	DNA-directed RNA polymerase	Cytoplasm	
RpsR	Translation, 30S ribosomal protein S18	Cytoplasm	