Comparative Analysis of Envelope Proteomes in *Escherichia coli* B and K-12 Strains

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Received: October 28, 2011 / Revised: December 7, 2011 / Accepted: December 10, 2011

Recent genome comparisons of *E. coli* B and K-12 strains have indicated that the makeup of the cell envelopes in these two strains is quite different. Therefore, we analyzed and compared the envelope proteomes of *E. coli* BL21(DE3) and MG1655. A total of 165 protein spots, including 62 nonredundant proteins, were unambiguously identified by two-dimensional gel electrophoresis and mass spectrometry. Of these, 43 proteins were conserved between the two strains, whereas 4 and 16 strain-specific proteins were identified only in *E. coli* BL21(DE3) and MG1655, respectively. Additionally, 24 proteins showed more than 2-fold differences in intensities between the B and K-12 strains. The reference envelope proteome maps showed that *E. coli* envelope mainly contained channel proteins and lipoproteins. Interesting proteomic observations between the two strains were as follows: (i) B produced more OmpF porin with a larger pore size than K-12, indicating an increase in the membrane permeability; (ii) B produced higher amounts of lipoproteins, which facilitates the assembly of outer membrane β-barrel proteins; and (iii) motility- (FliC) and chemotaxis-related proteins (CheA and CheW) were detected only in K-12, which showed that *E. coli* B is restricted with regard to migration under unfavorable conditions. These differences may influence the permeability and integrity of the cell envelope, showing that *E. coli* B may be more susceptible than K-12 to certain stress conditions. Thus, these findings suggest that *E. coli* K-12 and its derivatives will be more favorable strains in certain biotechnological applications, such as cell surface display or membrane engineering studies.

Keywords: Envelope protein, *Escherichia coli*, outer membrane protein, proteomics, two-dimensional gel electrophoresis

The cell envelope of a Gram-negative bacterium is a complex macromolecular structure that is essential to the organism’s viability. It is a permeable barrier whose particular physical and structural properties contribute to maintaining the cell’s morphology and protecting the bacteria from various environmental stresses. The Gram-negative cell envelope, for example, in *Escherichia coli*, can be defined as an organelle composed of the outer membrane (OM), the periplasmic peptidoglycan layer, and the inner membrane (IM), also referred to as the cytoplasmic membrane.

The intrinsic resistance of Gram-negative bacteria to various stresses has often been attributed entirely to the presence of the OM. Indeed, the OM does contribute to the resistance, as the narrow porin channels slow down the penetration of even small, hydrophilic solutes, and the low fluidity of the lipopolysaccharide (LPS) leaflet decreases the rate of transmembrane diffusion of lipophilic solutes [30]. There are two major groups of proteins present in the OM [21]: (i) lipoproteins that are present in the periplasm but are anchored by a lipid moiety to the inner leaflet of the OM and (ii) β-barrel proteins that are integral membrane proteins. The β-barrel proteins are involved in a wide range of functions, including adhesion, transport of small molecules, and enzymatic activities. Some of the β-barrel proteins, such as OmpA, OmpC, OmpF, and LamB, are called “porins” as they produce relatively nonspecific pores that allow the passage of small, hydrophilic molecules across the OM.

In particular, OM proteins play an important role in the adaptation of bacteria to changes in the external environment owing to their location at the outermost region of the cell.
Several studies have shown that the synthesis of OM proteins changes when bacteria are exposed to environmental changes in various stresses [30, 45]. For example, a number of genes encoding OM and IM proteins are differently expressed by pH changes under aerobic [25] and anaerobic conditions [13]. Interestingly, recent genome sequence comparisons of E. coli B and K-12 strains have revealed that the makeup of the OM in the two strains was quite different [17, 40]. Differences in the composition of the LPS core and expression of OM proteins may influence the permeability and integrity of the cell envelope. This could potentially make a difference in cellular susceptibility or resistance to various stress conditions. As a result, we became interested in the envelope compositions in the two E. coli strains.

With the recent advent of advanced proteomic technologies and continued efforts toward the isolation and solubilization of bacterial membrane proteins prior to isoelectric focusing, the separation of membrane proteins by two-dimensional gel electrophoresis (2DE) has been significantly improved. Two methods, sodium carbonate and sodium lauryl sarcosinate (sarcosine) enrichments, have been widely used for subproteome analysis of the membrane fraction. For example, Stenberg et al. [39] identified certain protein complexes, including 34 distinct IM proteins and 9 different OM proteins, in the E. coli cell envelope by using blue native-PAGE in combination with SDS-PAGE and mass spectrometry (MS). Huang et al. [16] also identified 31 OM proteins enriched in the sarcosine-insoluble fraction that included previously unrecognized membrane-interacting protein complexes, such as the complex consisting of OmpW and fumarate reductase. More recently, we identified 50 IM proteins and 61 OM proteins from the E. coli B REL606 strain, which is a parent strain of E. coli BL21(DE3) [10]. These results indicated that the improved proteomic methodologies could be utilized for the investigation of differential patterns of envelope proteins in E. coli.

In this study, reference maps of envelope proteomes from two representative E. coli strains, B strain BL21(DE3) and K-12 strain MG1655, were constructed and compared to investigate the E. coli cell envelope, especially the OM, because this is the contact point of the cell to its environment.

Materials and Methods

Bacterial Strains and Growth Conditions

The E. coli B and K-12 strains used in this study were BL21(DE3) and MG1655, respectively. E. coli cells were grown in a potassium-modified Luria broth (LBK) (10 g/l of tryptone, 5 g/l of yeast extract, and 7.45 g/l of KCl) in a shaking incubator at 37°C and 200 rpm [38]. Cell growth was monitored by measuring the OD$_{600}$ of the cultures using a spectrophotometer (Ultraspec3000; Pharmacia Biotech, Uppsala, Sweden).

Preparation of Envelope Proteins

Cell envelope proteins were isolated using a method described by Molloy et al. [27] with slight modifications. Culture broth (~0.2 g of dry cell weight) was centrifuged at 3,500 × g for 5 min at 4°C, and the pellet was washed with 100 ml of 50 mM Tris-HCl (pH 7.3) and resuspended in 15 ml of 50 mM Tris-HCl (pH 7.3) containing 1% (v/v) protease inhibitor cocktail (Complete Mini EDTA-free; Roche Diagnostics GmbH, Germany). The cells were ruptured by more than 30 cycles of sonication (each for 5 s at 30% of maximum output with a 10 s pause time; High-intensity ultrasonic liquid processors; Sonics & Material Inc., Newtown, CT, USA), and the unbroken cells were removed by centrifugation at 2,500 × g for 10 min. The supernatant was diluted with cold 100 mM Na$_2$CO$_3$ (> pH 11) to a final volume of 150 ml and stirred slowly on ice for 1 h. The carbonate-treated membranes were collected by ultracentrifugation in a Beckman 70 Ti rotor at an average of 115,000 × g for 1 h at 4°C. The supernatant was discarded, and the membrane pellet was resuspended and washed with 2 ml of 50 mM Tris-HCl (pH 7.3). The pellet was collected by centrifugation at an average of 115,000 × g for 20 min at 4°C and solubilized for 2DE with a sample rehydration buffer composed of 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 1% (w/v) DTT, and 1% (v/v) protease inhibitor cocktail (Roche Diagnostics GmbH).

Two-Dimensional Gel Electrophoresis (2DE)

2DE experiments were conducted with the IPGphor IEF system (GE Healthcare, Chalfont St. Giles, UK) and Protein II xi xi Cell (Bio-Rad, Hercules, CA, USA) as described previously [9]. Isolated proteins (300 µg) were quantified using a Bradford assay and were diluted with the sample rehydration buffer containing 1% (v/v) IPG buffer (pH 4–7; GE Healthcare). The samples were then carefully loaded onto IPG strips (18 cm, pH 4–7; GE Healthcare). The loaded IPG strips were rehydrated for 12 h and focused at 20°C for 15 min at 250 V, followed by 8,000 V until a total of 60 kV·h was reached. The strips were equilibrated for 15 min in equilibration buffer 1, which was composed of 6 M urea, 0.375 M Tris-HCl (pH 8.8), 20% (w/v) glycerol, 2% (w/v) SDS, and 130 mM DTT. This step was followed by incubation in equilibration buffer II, which was composed of 6 M urea, 0.375 M Tris-HCl (pH 8.8), 20% (w/v) glycerol, 2% (w/v) SDS, and 135 mM iodoacetamide, for 15 min. The strips were then placed on 12% (w/v) SDS-PAGE gels. Proteins spots were visualized using a silver staining kit (GE Healthcare), and the stained gels were scanned using a UMAX PowerLook 2100XL Scanner (UMAX Technologies, Inc., TX, USA). PDQuest 2-D Analysis Software (Bio-Rad) was used to automate the process of finding protein spots within the image and quantifying the spot densities as a percentage of volume. Features resulting from non-protein sources (e.g., dust particles and scratches) were filtered out, protein spots were normalized, and pairwise image comparisons were performed. At a minimum, triplicate gels of each sample were analyzed to check the reproducibility.

Protein Identification by LC-MS/MS Analysis

Samples for the MS/MS analysis were prepared as described previously [20]. Briefly, protein spots were excised and destained by incubating in 30 mM potassium ferricyanide and 65 mM sodium thiosulfate for 10 min. Gel pieces were washed in Milli-Q water until they were colorless and transparent, and then they were vacuum-dried. These pieces were proteolyzed overnight with
0.02 µg/µl of modified trypsin (Promega, Madison, WI, USA) in 40 mM ammonium bicarbonate at 37°C. Tryptic peptides (10 µl aliquots) were analyzed by a nano-LC/MS system consisting of an Ultimate HPLC system (LC Packings, Amsterdam, The Netherlands) and a quadrupole-time-of-flight (Q-TOF) MS (Micromass, Manchester, UK) equipped with a nano-ESI source, as described previously [20]. The acquired MS/MS spectra were processed using MassLynx v.3.5 (Waters, Milford, MA, USA). The MASCOT search server (version 2.0, Matrix Science, London, UK) was used for the identification of protein spots by querying the sequences of the tryptic peptide fragments. Reference databases used for the identification of target proteins were NCBI (http://www.ncbi.nlm.nih.gov/) and UniProt Knowledgebase (Swiss-Prot and TrEMBL; http://kr.expasy.org/).

Computational Analysis

The subcellular localization of identified proteins was analyzed using the Swiss-Prot database and was also predicted using PSORTb v. 3.0.2 (http://www.psort.org/psortb/) [8]. Transmembrane β-barrel OM proteins and the numbers of transmembrane domains (TMDs) were also predicted using PRED-TMBB (http://bioinformatics.biol.uoa.gr/ PRED-TMBB/) [2]. Proteins that showed higher scores than the threshold (2.965) were presumably not OM proteins.

RESULTS AND DISCUSSION

Envelope Proteome Reference Maps of E. coli B BL21(DE3) and K-12 MG1655 Strains

The cell envelope is the principal stress-bearing and shape-maintaining element in E. coli, and its integrity is of critical importance to cell viability. Each cell has a unique composition and quantity of envelope proteins according to its function. The cell’s surface proteins might therefore be expected to have rather unusual structures and properties to allow the cell’s continued survival and function in a harsh environment. Recent genome sequence comparisons have revealed that the envelope makeup of E. coli B is quite different from that of K-12 [17, 40]. Moreover, it has been demonstrated that the E. coli B strain displays higher membrane permeability than does K-12 [14], as both strains have very different clusters of genes for the synthesis of the core oligosaccharide and O-antigen polysaccharide of LPS. However, a comparative analysis of the envelope proteins in E. coli B and K-12 strains has not been thoroughly studied. Thus, an envelope proteome analysis was performed for the two strains during the exponential growth phase.

Two representative strains, E. coli B BL21(DE3) and K-12 MG1655, were cultured in LBK media in a flask culture as described in Materials and Methods. The representative reference maps of the envelope proteomes of the two E. coli strains are shown in Fig. 1. Protein spots that appeared reproducibly in 2D gels of samples taken from three independent flask cultures were chosen as targets for MS/MS analysis. A total of 165 protein spots, 77 spots from the E. coli BL21(DE3) gels and 88 spots from the E. coli MG1655 gels, were unambiguously identified with LC-MS/MS, representing 62 nonredundant proteins. Detailed

![Fig. 1. Representative 2D gel maps of envelope proteins of E. coli B BL21(DE3) (A) and K-12 MG1655 (B) grown at pH 7.0 in LBK culture medium.](image-url)

Most of the envelope proteins identified by LC-MS/MS are annotated on the 2D gel and are listed in Table S1. The positions of the molecular mass standards are indicated in kilodaltons on the vertical axes, and the approximate pl scales are indicated on the horizontal axes.
information, including protein name, protein description, accession number, MS score, sequence coverage, theoretical pI, molecular mass, subcellular localization, and the number of TMDs, is presented in Table S1. Of the 62 different proteins, 23 proteins (37%) were found to exist in more than one form, at an average of 2.5 forms per protein in the 2D gels, which was likely due to post-translational modifications, partial degradation, or incomplete denaturation prior to electrophoresis.

The PSORTb search engine predicted that the locations of the identified proteins were mainly within the membrane, including 10 IM proteins and 19 OM proteins. However, it should be mentioned that some proteins did not match to their predicted locations, which was due to misprediction by the computational tools. Some proteins predicted to be unknown or localized to other locations were actually membrane-associated proteins involved in ATP synthesis (AtpA and AtpF), lipoproteins (BamB, BamC, Lpp, SlyB, and YajG), or others (HybC, SdhA, and YfgM) after comparing to the data in the curated Swiss-Prot database (Table S1). In particular, lipoproteins may be difficult to classify correctly if they span from the IM to the OM. In addition, examination of the relative hydrophobicities of proteins based on the number of TMDs provides information on the propensities of proteins to be membrane-associated [10]. The number of TMDs, which was based on the presence of transmembrane β-barrel OM domains, was predicted using PRED-TMBB [2]. These results revealed that a total of 43 proteins (69%) had a minimum of one TMD (ranging from 1 to 32), which indicated that most of the identified proteins were OM proteins.

Comparison of Envelope Components Between E. coli BL21(DE3) and K-12 MG1655 Strains

Of the nonredundant proteins, 43 proteins (69%) were commonly identified in both BL21(DE3) and MG1655 samples. Sixteen proteins (BtuB, CheA, CheW, FluA, FecA, FlIC, Flu, GatY, HybC, HybO, OmpC, OmpT, OmpW, ProV, YbhC, and YchF) were uniquely detected in K-12, whereas four proteins (BorD, GatA, RplL, and SlyB) were only found in B (Fig. 2, Table S1); these were strain-specific envelope proteins. The findings for four proteins (FliC, Flu, OmpT, and OmpW) were consistent with genome sequence data that the corresponding (fliC, flu, ompT, and ompW) genes were missing in the B genome [17]. It has previously been observed that E. coli B strains lack the flagellar biosynthesis gene cluster, because a 38 kb region found in K-12 from yecF to yedS, containing the fltYZACDSTEGFHJKLNMOPQR genes, was deleted from the B genome, providing evidence that E. coli B is non-motile [17]. It was further shown that E. coli B did not swarm in a swarming motility assay [1]. It is known that flagellar biosynthesis genes are differentially expressed at low pH [25], where the low pH contributes to the proton motive force that drives flagellar rotation [26]. This is reflected in the surprisingly different compositions and protein interaction patterns of flagella from different species, which may reflect adaptations to species-specific motility needs.

In addition, 24 proteins exhibited differences in intensities greater than 2-fold between the B and K-12 strains (Fig. 2). The levels of six proteins (AceA, CusB, FadL, SdhA, UspF, and YdgA) were high in K-12, whereas 18 proteins (AcrB, AtpB, BamA, BamC, BamD, DcrB, FepA, MipA, OmpF, OmpX, Pal, RlpA, RpsB, TolC, Tsr, YceB, YcfM, and YiaF) were significantly upregulated in B. These results showed that both strains have somewhat different types and expressions of protein components in the cell envelope.

The reference envelope proteome maps showed that the E. coli envelope mainly contained channel proteins, such as porins, and lipid-modified proteins, the so-called lipoproteins. The channel proteins span the OM, whereas lipoproteins are anchored to the OM through their attached lipids. Thus, we classified the E. coli envelope components into two major categories (Table S1); channel proteins (BamA, BtuB, FadL, FecA, FepA, LamB, OmpA, OmpC, OmpF, OmpT, OmpW, OmpX, TolC, and Tsr) and lipoproteins (Acra, BamB, BamC, BamD, DcrB, HslJ, Lpp, Pal, RlpA, SlyB, YajG, YbhC, YceB, YcfM, YiaF, and YraM), which are described in detail below. Other proteins, such as proteins involved in F0F1 proton-translocating systems, cell mobility, stress response, and some unknown proteins, were also present in the E. coli envelope.

Channel Proteins

Channel proteins, including nonspecific channels (so-called porins), specific channels, and high-affinity siderophore receptors, are typical OM proteins that contain a β-barrel structure [30]. These proteins generally form pore-like structures for the purpose of allowing an influx of nutrients or extrusion of waste products. Consistent with our findings, the channel proteins comprised the most abundant proteins detected in the envelope of the E. coli B and K-12 strains (Fig. 1), and they were all predicted as localization assignments of the OM (Table S1). These included BamA, BtuB, FadL, FecA, FepA, LamB, OmpA, OmpC, OmpF, OmpT, OmpW, OmpX, TolC, and Tsr, although they were not all present at the same levels, depending on the strain. An interesting finding was that OmpT and OmpW were synthesized only in E. coli MG1655 and were not detected in E. coli BL21(DE3) owing to the absence of the corresponding genes in the E. coli B strain genome. In addition, E. coli B produced significantly more OmpF porin than K-12 because the B genome lacks micF, which post-transcriptionally prevents the production of OmpF [36, 49]. This finding is
further supported by the result of a 1D gel that showed high levels of OmpF in B, whereas high levels of OmpC and OmpA were found in K-12 (Fig. 2A).

The expression of the two major porins, OmpF and OmpC, is exquisitely regulated. It has previously been demonstrated that OmpF produces a slightly larger channel than OmpC [11, 30]. Thus, noxious agents, such as antibiotics and bile acids, diffuse better through the larger OmpF channel. The loss of the more efficient \textit{ompF} channel has been found to reduce the permeability of the OM and
to allow the slow uptake of nutrients for survival [11]. In its natural habitat, such as the intestinal tract, E. coli encounters 4 to 16 mM bile salts [3], and it is very important to minimize their influx. The prevailing conditions in the intestinal tract, namely, high osmotic strength and temperature, favor the production of the narrower channel OmpC and repress the production of OmpF [12]. In another study in which acid stress was used to induce cadaverine synthesis, the decrease in OM permeability was interpreted as the result of an increase in OmpC and a decrease in OmpF, a component of the acidic pH response. Indeed, E. coli acid-adapted cells showed a significant reduction in proton permeability, and this finding correlated well with the increase in low pH tolerance associated with these cells [18]. These results indicated that a decrease in porin-mediated permeability might increase the degree of resistance to various stresses. It suggested that the E. coli B strain, which showed a significantly increased production of OmpF, may be more susceptible than K-12 to various stresses.

OmpA was found to be the most abundant protein, as it was found in more than 5 spots in the reference maps of both E. coli strains (Fig. 1 and 2). The function of OmpA is thought to contribute to the structural integrity of the OM along with murein lipoprotein and peptidoglycan-associated lipoprotein. OmpA is highly conserved and well regulated to maintain nearly constant levels throughout the OM, and it contains a nonspecific diffusion channel, allowing various small molecules to be transported into the cell [41]. However, the proteomic results showed that the levels of OmpA were slightly higher in K-12 than in B (Fig. 1). Additionally, the results from the SDS-PAGE gel clearly indicated that OmpA was present at significant levels in K-12 (Fig. 2). E. coli OmpA contributes to serum resistance and pathogenicity by binding to C4b-binding protein, a complement fluid-phase regulator [34]. In contrast, an ompA mutant strain was previously found to reduce the stress resistance of E. coli [46] to sodium dodecyl sulfate (SDS), cholate, acidic environments, and high osmolality. The ompA mutant was also revealed to be significantly more sensitive to acid, both in the mid-exponential and the stationary phases of growth [37]. It is possible that the role of this protein in maintaining the structural integrity of the cell envelope contributes in some way to protecting the cell from stress conditions.

E. coli OmpW, a small OM protein, is a receptor for colicin S4 [33], but otherwise nothing is known about its function. This protein belongs to a homology group that includes Pseudomonas putida AlkL, which is hypothesized to be an alkane channel [44], and NahQ, which is part of the cluster coding for naphthalene utilization and is also likely related to an OM channel. These sequence similarities suggest an unproven role for OmpW in the transport of various hydrophobic molecules across the OM. Proteomic analysis of Stenotrophomonas sp. OK-5 revealed that OmpW is, together with stress-shock proteins, such as DnaK and OsmC, one of the few proteins that become significantly induced by the presence of trinitrotoluene in the growth media [15]. In another report, the expression of OmpW from Vibrio cholerae was found to be dependent on in vitro culture conditions, such as temperature, salinity, and the availability of nutrients or oxygen [28]. The expression of the OmpW homolog from V. alginolyticus, a marine pathogen, was induced dramatically (at least 10-fold) under conditions of high salinity (4% NaCl), implying a role of OmpW in osmoregulation [50, 51]. Interestingly, the OmpW protein was uniquely expressed in E. coli K-12 MG1655. This finding suggests that members of this protein family (OmpW and its homologs) may be involved in bacterial adaptation in response to various stress conditions.

Among several siderophore receptors of E. coli, 3 proteins (enterobactin receptor FepA, ferric citrate receptor FecA, and the vitamin B12 receptor BtuB) were detected in this study. BtuA and FecA were only expressed to a higher degree in K-12, whereas FepA was more highly expressed in B than in K-12. Siderophores (iron chelators of microbial origin) and vitamin B12 are too large to pass through the classical porin channels of E. coli, and it would be difficult to design specific channels for them without making the pores nonspecifically permeable to many large solutes, thus compromising the bacterial resistance to environmental toxic compounds.

In E. coli, a major multidrug efflux transport complex composed of the AcrB antiporter protein, the AcrA periplasmic protein of the membrane fusion protein family, and the TolC OM channel is largely responsible for the intrinsic resistance to a wide variety of antimicrobial agents [29]. Sulavik et al. [42] showed that null AcrAB mutant strains became more susceptible to 29 of the 35 compounds tested. Conversely, Nishino and Yamaguchi [31] showed that expression of plasmidic AcrAB conferred significant resistance to 19 of the 24 compounds tested. In this study, AcrA and TolC showed increased levels in B than in K-12. The enhanced levels of the multidrug efflux system may be needed to compensate for the increased membrane permeability due to considerable levels of OmpF in E. coli B.

Indeed, there are several ways for E. coli to contribute to its overall degree of intrinsic resistance. In the E. coli K-12 strain, it mainly contributes to a reduction in the permeability of the OM. In contrast, the E. coli B strain positively controls the expression of the efflux AcrAB pump to make up for lack of resistance relative to K-12. Thus, these channel proteins might be important for dealing with external stresses in the cell envelope. In particular, based on these channel proteins and earlier studies [14], the E.
coli B strain has a higher membrane permeability than *E. coli* K-12, suggesting that the K-12 strain possesses a greater tolerance than the B strain.

**Lipoproteins**

Lipoproteins, with their Type II signal peptides, are universal components of eubacterial membranes. In *E. coli*, a majority of lipoproteins are transported to the OM via the LolCDEAB system, whereas those having an avoidance signal remain in the IM, along with a minority of lipoproteins [24, 43]. The ability of lipoproteins to decorate bacterial membranes provides for a wide variety of essential structural and functional roles, such as those involved with adhesins, enzymes, transporters, binding proteins, toxins, and others essential for virulence [22]. Proteome profiling showed the expression of 17 lipoproteins in either the *E. coli* B or K-12 strain (AcrA, BamB, BamC, BamD, BorD, DerB, HslJ, Lpp, Pal, RlpA, SlyB, YajG, YbhC, YceB, YcfM, YiaF, and YraM). Among them, most of the lipoproteins (particularly the Bam complex) were strongly induced in B rather than in K-12.

The major lipoprotein Lpp (murein lipoprotein) is one of the most abundant OM proteins [47]. Murein lipoprotein anchors the inner face of the Gram-negative OM to the peptidoglycan cell wall. It is responsible for the bacterial resistance to osmotic and mechanical stresses. Moreover, the peptidoglycan-associated lipoprotein Pal is anchored to the OM. It has previously been shown that lpp and tol-pal mutations perturb the OM permeability barrier, and they cause the release of periplasmic proteins and the formation of OM vesicles. Thus, these lipoproteins play a critical role in maintaining the cell wall organization. However, because *E. coli* strains with null mutations in lpp, nlpA, bamC (nlpB), nlpC, nlpD, nlpE, pal, and slp genes can survive [47], these genes are not essential for the growth of *E. coli* under normal laboratory conditions.

Significantly, lipoproteins have been found to be part of various OM assembly machineries. The Bam complex, which is responsible for the assembly and insertion of β-barrel proteins into the OM, contains a central core protein, BamA (YaeT), and four different lipoproteins in *E. coli*, namely BamB (YfgL), BamC (NlpB), BamD (YfiO), and BamE (SmpA) [19, 48]. The genes for all lipoproteins and BamA are regulated by the σE extracellular stress response pathway [32, 35]. All of the Bam lipoproteins have roles in OM protein biogenesis, as their depletion leads to varying degrees of OM protein assembly defects, from OM permeability defects to antibiotic hypersensitivity, but only BamA and BamD are crucial for cell viability and OM protein biogenesis [23, 48]. It is not known how BamA inserts β-barrel proteins into the OM, but polypeptide transport-associated domains that are present in the periplasmic part of the protein are likely to play an important role in this process [4]. Malinverni *et al.* [23] demonstrated that the OM proteins affected by BamA or BamD depletion include β-barrel proteins that require LPS for proper assembly, including LamB, OmpF, OmpC, and OmpA, and β-barrel proteins that assemble in an LPS-independent fashion, such as TolC. In addition, OMs isolated from BamA- or BamD-depleted cells have dramatically reduced protein content, and this reduction lowers the OM buoyant density significantly, presumably by decreasing the protein-to-lipid ratio. Interestingly, three proteins (BamA, BamC, and BamD) were highly expressed in *E. coli* BL21(DE3). Likely, increased levels of the Bam complex might be required for the assembly of high-expression level OM β-barrel proteins, including OmpF, TolC, and BamA itself.

However, cells lacking the core components of the BamA complex do not exhibit severe defects in the assembly of LPS [7]. This finding demonstrates that the OM proteins and LPS assembly machines can operate independently. However, in growing cells, these assembly processes appear to be coordinated. Wild-type cells maintain a homeostatic LPS-to-protein ratio under dynamic environmental conditions [30]. It has been reported that acid-challenged *E. coli* changes the lipid composition of its membranes. Specifically, *E. coli* cells adapted to pH 5.0 were found to have elevated levels of cyclopropane fatty acids [5]. *E. coli* cells are also known to increase the cyclopropane fatty acid content of their membranes as they enter into the stationary phase [6, 52]. The acid tolerance levels of individual strains have been shown to correlate well with membrane cyclopropane fatty acid content [5]. It may be that changes in the lipid composition of membranes affect their proton conductance, perhaps reducing the leakage of protons across the membrane when the external proton concentration is high. Therefore, it may be that both lipid and protein alterations are necessary to confer elevated levels of protection to the cell in acidic environments.

Differences in the composition of the LPS core and synthesis of OM proteins may influence the permeability and integrity of the cell envelope, which presumably results in changes to screening barriers that control the import and export of materials, such as antibiotics, nutrients, and proteins. Thus, proper coordination of the LPS and OM protein assembly is necessary to maintain the barrier function of the OM.

**Acknowledgments**

We gratefully thank Yu Hyun Lee for the technical assistance. This work was supported by the Basic Science Research Program (2010-0008826) and Converging Research Center Program (2009-0093652) through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology.
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