Chapter 2
Gram-Negative Bacteria: The cell Membranes

Abstract This chapter presents a brief outline of our current knowledge of the structures of the bounding membranes (the inner and the outer membranes and the intermediate periplasmic layer) of a Gram-negative bacterial cell. Also the structure and chemical composition of the outer membrane vesicles (OMVs) originating from the surface of these bacteria including their proteomic profile, as obtained mainly by mass spectroscopic and related studies, have been presented in brief.

Keywords Inner membrane · Outer membrane · Peptidoglycan · OMVs · Structure · Chemical composition · Mass spectrometry · Protein profile

2.1 Inner and Outer Membranes

The Gram-negative bacteria are usually bounded by two membranous structures (Fig. 2.1). The inner one (IM), called the plasma membrane, is a trilamellar structure that bounds the bacterial protoplasm and is composed of a phospholipids bilayer. The outer membrane (OM) also presents a trilamellar structure (with two electron dense leaflets, outer and inner) in the electron micrograph and consists of proteins, including porins, receptors, and an asymmetric distribution of lipids. The outer leaflet is composed primarily of lipopolysaccharide (LPS) projecting outside and the inner leaflet containing phospholipids and lipoproteins. The LPS of a Gram-negative bacterium consists of three different sectors: (i) lipid-A, (ii) the core polysaccharide comprising the inner and the outer cores, and (iii) the O-specific polysaccharide chains (Fig. 2.1) projecting outward. The lipid portion of LPS serves as the lipid anchor and is commonly composed of fatty acids, sugars, and phosphate groups. The chemical structures of lipid-A, core polysaccharide, and O-specific polysaccharide chains of *Vibrio cholerae* are shown in Fig. 2.2 a, b,

S. N. Chatterjee and K. Chaudhuri, *Outer Membrane Vesicles of Bacteria*, SpringerBriefs in Microbiology, DOI: 10.1007/978-3-642-30526-9_2,

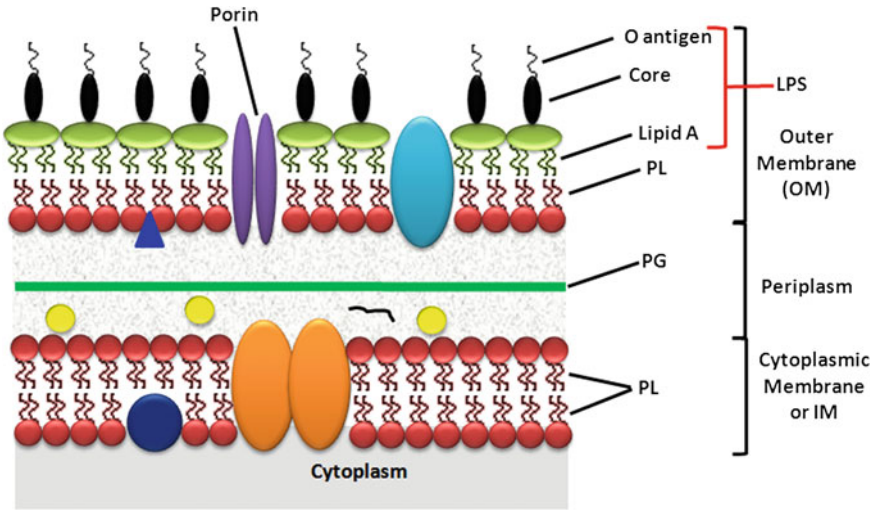

Fig. 2.1 Schematic diagram of the outer membrane (*OM*), cytoplasmic or *IM,* and the intermediate periplasmic layer containing the peptidoglycan (*PG*). The *IM* consists of the two phospholipid (*PL*) leaflets and different lipoproteins. The outer membrane consists of two leaflets, the inner leaflet being composed of one phospholipid layer and the outer leaflet of lipid-A, core polysaccharide, and the O-antigen polysaccharide chains projecting outward

and c. These two membranes, IM and OM, are separated by a gel-like layer known as the periplasm. The periplasm contains a thin layer (∼4- nm thick) of peptidoglycan (PG) which is connected to the OM and also the inner membrane (IM) through different protein–protein interactions and other proteins including the so-called resident "housekeeping" proteins and enzymes, the resident and transient components of secretory pathways and the like. In *Pseudomonas aeruginosa* there are three lipoproteins, Oprl, OprL, and OprF, that connect the OM with the PG layer. In wild- type *Salmonella spp* there are specific domains in the envelope that promote interactions between the outer membrane protein (Omp) and the peptidoglycan layer (PG) and also interactions between the Omp and the inner membrane protein (IMP) involving the PG; (Deatherage et al. 2009). Such protein–protein interactions involving the PG are primarily responsible for giving the required strength and stability to the bacterial envelope or rather the surface structures of bacteria.

A schematic description of the presence and interactions between these different proteins in wild- type *Salmonella spp* is presented in Fig. 2.3. The integral proteins in the OM include OmpC, OmpF, OmpX, and NmpC; those involved in the interactions between the OM and the PG include OmpA, LppA, and LppB, and the peptidoglycan- associated lipoprotein Pal in the OM can interact with TolA in the IM either directly or via the periplasmic protein, TolB. Pal can also interact directly with the PG. The OM contains the unique trimeric proteins known as porins. Porins are channel-forming proteins that allow small

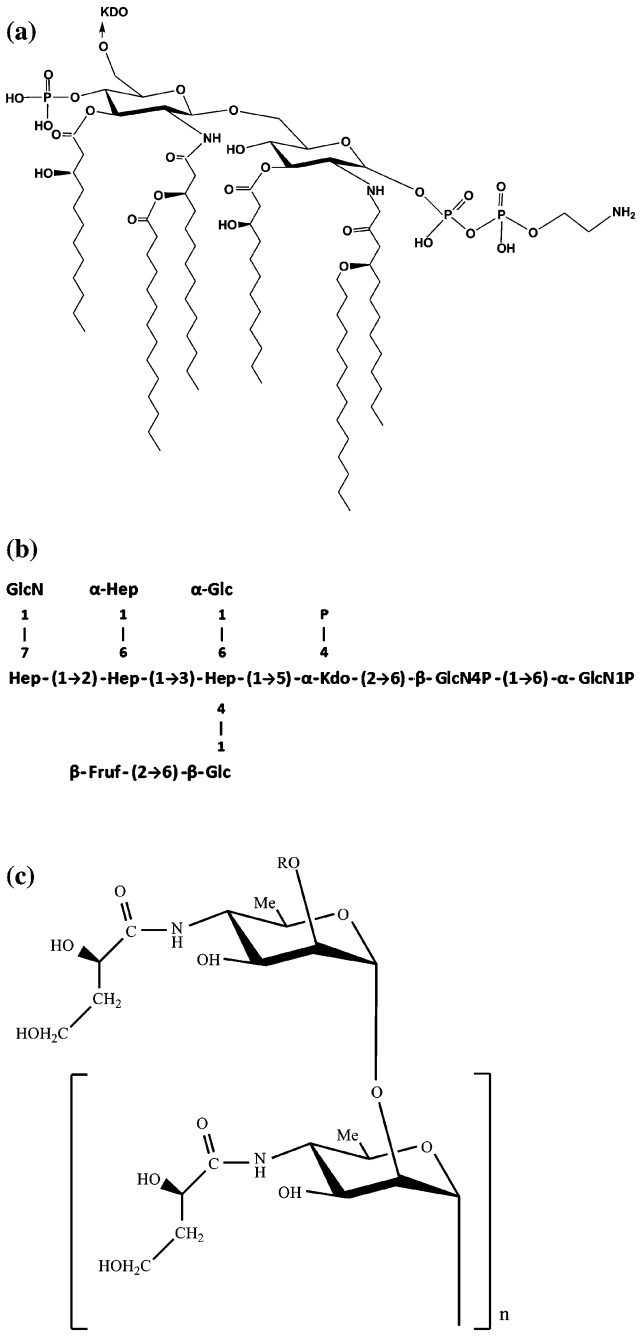

Fig. 2.2 **a** Chemical structure of lipid-A of *V. cholerae* O1. **b** Chemical structure of the core polysaccharide (core-PS) of *V. cholerae* O1strain 95R. **c** Chemical structure of the O-PS of *V. cholerae* O1. The O-PS structures of the two serotypes, Inaba and Ogawa, are the same except at the position O-2 of the upstream, terminal perosamine group; R = CH_3 in Ogawa strain and R = H only in Inaba strain; *n*, represents the number of repeating units, which may be between 12 and 18 Chatterjee and Chaudhuri (2003); and Chaudhuri and Chatterjee (2009)

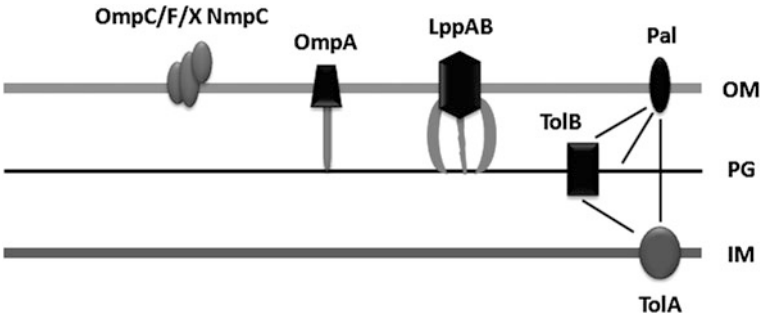

Fig. 2.3 Proteins interconnecting different layers of the cell envelope. Integral OM proteins OmpC, OmpF, OmpX, and NmpC normally do not connect to other layers of the envelope. Lpp and OmpA form an interconnection between PG and OM, whereas Pal of the OM, Tol B of the PG, and Tol A of the IM form interconnections between the different components of the envelope. The thinner straight line represents covalent interaction; the thicker straight line or the curved lines represent noncovalent interactions

molecules (<600 daltons) to pass through and enter the periplasmic space. Once in the periplasm, proteins within the plasma membrane allow transport of molecules into the cytoplasm. In *P. aeruginosa*, a specific porin, OprF, not only allows passage of small molecules, but is also associated with the underlying PG within the periplasm. Lipoproteins are also present in the periplasm. The structure of the OM of Gram-negative bacteria plays a dynamic role in the formation of outer membrane vesicles (OMVs).

2.2 Structure of OMVs

The OMVs originate by a process of bulging out and pinching off of a portion of the bacterial OM (Chatterjee and Das 1966, 1967) thereby entrapping much of the materials of the underlying periplasm. However, inclusion of periplasmic materials into the OMVs is dependent on some specific sorting mechanism. They are spherical in shape (Chatterjee and Chaudhuri 2011) with sizes varying between 50 and 250 nm as reported by most researchers (Fig. 2.4;) (Beveridge 1999; Mashburn-Warren and Whiteley 2006). In our experience, OMVs of sizes significantly smaller than 50 nm have been found and these are often lost during the procedures for isolation of OMVs. The OMVs are bounded by a trilamellar structure similar to that of the bacterial OM (Figs. 1.7 and 2.5). The bounding membrane of the OMVs also has a similar chemical structure to that of the bacterial OM and accordingly contains the antigenic LPS projecting outside.

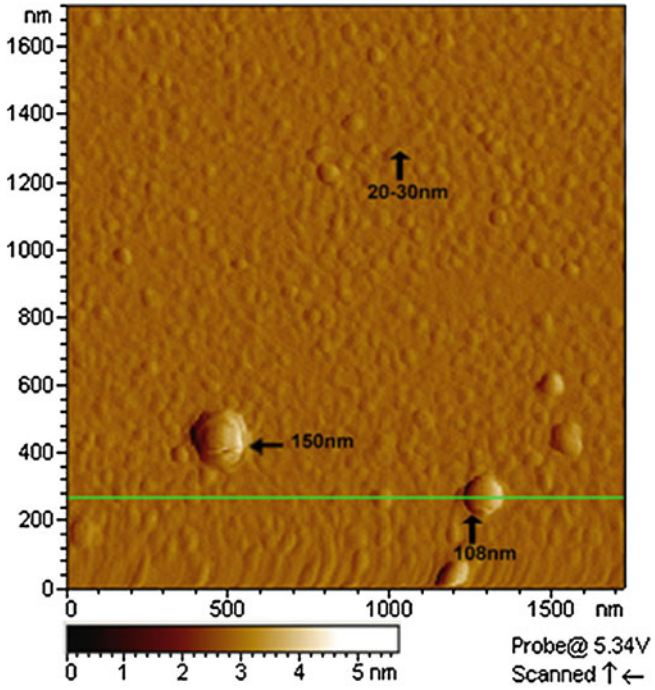

Fig. 2.4 Atomic force microscopy image of air-dried *V. cholerae* OMVs in amplitude mode (Bar = 200 nm). [From (Chatterjee and Chaudhuri 2011)]

2.3 Chemical Composition of OMVs

The OMVs originate from the bacterial surface and are, generally, known to contain OMPs, many of the periplasmic proteins, phospholipids, and LPS and other factors associated with virulence (Horstman and Kuehn 2000; Kuehn and Kesty 2005; Wai et al. 2003). By using the two-dimensional SDS-PAGE and MALDI-TOF mass spectrometric techniques, the major OMV proteins identified in *Salmonella sp.* were OmpC, OmpF, NmpC, OmpX, OmpA, LppA, LppB, Pal, and Tol B (Deatherage et al. 2009). During their formation and release from the bacterial surface, the OMVs entrap some of the underlying periplasmic constituents which may vary with bacterial growth conditions and for different bacteria. The OMVs from different bacteria can entrap toxins, enzymes, DNA, adhesins, and other virulence factors (Ellis and Kuehn 2010). Table 2.1 presents the names of different virulence factors carried by the OMVs in their lumens and the corresponding bacterial species from which they originated. The overall chemical composition of OMVs thus depends on various factors controlling growth and the species from which they originate. Recent evaluations showed that almost all OMV preparations were enriched in envelope components (Kuehn and Kesty 2005). Some of the preparations, however, were also found to contain a small

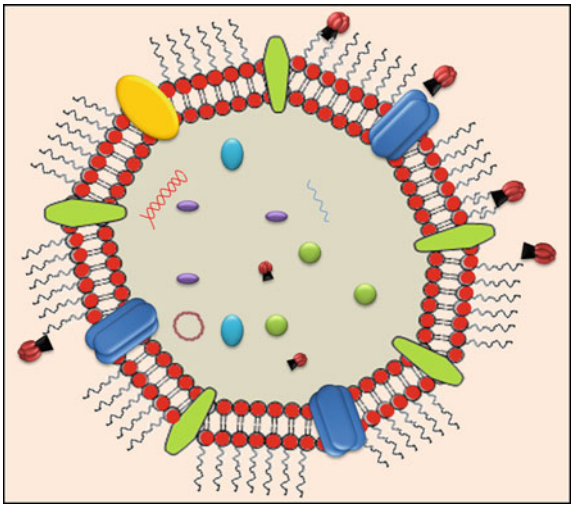

Fig. 2.5 Schematic diagram of an OMV showing the different possible luminal components (proteins, double- stranded DNA, RNA, plasmid, etc.), and the trilamellar structure of the vesicle membrane including the outwardly projecting O-PS chains, the proteins spanning the two leaflets of the membrane, and some toxin particles either bound to the LPS chains or near the outer surface of the membrane or within the lumen

amount of cytosolic and IM proteins, the basis of which remains unclear or rather controversial (Berlanda Scorza et al. 2008; Ellis and Kuehn 2010; Galka et al. 2008; Kwon et al. 2009; Lee et al. 2007; Lee et al. 2008; Sidhu et al. 2008; Xia et al. 2008). In fact, biochemical analysis of OMVs purified by density gradient centrifugation revealed that they consisted only of the proteins and lipids of the OM and periplasm and did not contain any IM and cytoplasmic components (McBroom and Kuehn 2005). Pathogenic bacteria were shown to release OMVs containing adhesins, toxins, and immunomodulatory compounds. Because of their lipid contents, such vesicles were found to fractionate into lighter density fractions than solubly secreted proteins (Allan and Beveridge 2003; Allan et al. 2003; Dorward and Garon 1989; Dorward et al. 1989; Horstman and Kuehn 2000).

2.4 Proteomic Profile of OMVs

2.4.1 Isolation and Purification

In order to have a dependable proteomic profile of OMVs of any Gram-negative bacteria, the OMVs are first required to be isolated and highly purified so that no trace of materials released from bacterial lysis or any nonvesicular component contaminates the preparation. For this, no single method of isolation and purification can serve the purpose and a combination of differential centrifugation to remove cell debris and whole cells and ultracentrifugation to pellet the OMVs is the minimum requirement (Wai et al. 2003). Furthermore, filtration of the cell culture supernatant through membrane filters (0.22–0.45 μm) before ultracentrifugation may ensure better elimination of any contamination (Berlanda Scorza et al. 2008;

Table 2.1 Virulence factors associated with OMVs derived from various bacterial species

Bacterial spp.	Associated disease	Virulence factors	Activity	Reference
Actinobacillus pleuropneumoniae	Porcine contagious pleuropneumonia	Apx toxin	Hemolytic activity, cytotoxicity	Negrete-Abascal et al. (2000)
		Proteases	Proteolytic activity, host damage	
Actinobacillus actinomycetemcomitans	Periodontal disease	Leukotoxin	Pore-forming and membranolytic activity	Goulhen et al. (1998), Karched et al. (2008), Kato et al. (2002)
		Lipopolysaccharide (LPS)	Endotoxic activity	
		GroEL	Cytotoxicity	
		Peptidoglycan associated lipoprotein (PAL)	Proinflammatory activity on human whole blood	
Bacteroides fragilis	Colon inflammation/ Colon tumor	Hemagglutinin	Hemagglutination	Patrick et al. (1996)
		Alkaline phosphatase	Enzymatic activities causing host damage	
		Esterase lipase		
		Acid phosphatase		
		Phosphohydrolase		
		α- and β- galactosidases		
		α-glucosidase		
		Glucosaminidase		
		β-glucuronidase		
Bacteroides succinogenes	Abscesses, bacteremias	Cellulase	Aryl-β-glucosidase, endoglucanase	Forsberg et al. (1981)
		Xylanase	Aryl-β-xylosidase, xylanase activities	

(continued)

Table 2.1 (continued)

Bacterial spp.	Associated disease	Virulence factors	Activity	Reference
Bordetella pertussis	Whooping cough	Adenylate cyclase hemolysin	Cytotoxicity	Hozbor et al. (1999)
		Filamentous hemaglutinin (FHA)	Adhesion, agglutinates erythrocytes	
		Pertussis toxin (Ptx)	Inhibition of eukaryotic adenylate cyclase, increases cAMP	
Borrelia burgdorferi	Lyme disease	Outer surface proteins A and B (OspA, OspB) OspD	Adherence to host cells and tissue	Dorward et al. (1991), Shoberg and Thomas (1993)
		Decorin-binding protein A (DbpA)		
Brucella melitensis	Brucellosis	Outer membrane proteins Omp25, Omp31	ND	Gamazo and Moriyon (1987)
Burkholderia cepacia	Respiratory tract infection especially in cystic fibrosis patients	Nonhemolytic phospholipase C (PLC-N)	Lipolytic activity	Allan et al. (2003)
		Lipase		
		Pseudomonas cepacia protease (PSCP)	Protease activity	
		40-kDa protease		
Campylobacter jejuni	Gastroenteritis	Cytolethal discending toxin (CDT)	Genotoxicity	Lindmark et al. (2009)
Enterohemorragic *E. coli* (EHEC)	Bloody diarrhea, hemolytic-colitis	Cytolysin (ClyA)	Pore formation, membranolytic activity	Kolling and Matthews (1999), Wai et al. (2003)
		Shiga toxin	Cytotoxic, inhibit protein synthesis	

(continued)

Table 2.1 (continued)

Bacterial spp.	Associated disease	Virulence factors	Activity	Reference
Enterotoxigenic *E. coli* (ETEC)	Diarrhea	Heat labile enterotoxin (LT)	Increases adenylate cyclase and cAMP, loss of fluid and electrolyte in host	Horstman and Kuehn (2000)
Extraintestinal pathogenic *E. coli* (ExPEC)	Extraintestinal infections such as urinary tract infections, neonatal meningitis, septicemia	Alpha-hemolysin	Hemolytic, causing detachment of cells from monolayer	Balsalobre et al. (2006)
		Cytolethal descending toxin (CDT)	Genotoxicity	
		Iron and hemin binding OMPs	Iron acquisition	
Shiga toxin producing *E. coli* (STEC)	Hemorrhagic colitis,	Shiga toxin	Inhibition of protein synthesis and death of host cells	Kolling and Matthews (1999), Yokoyama et al. (2000)
Uropathogenic *E. coli* (UPEC)	Urinary tract infection	Cytotoxic necrotizing factor type 1 (CNF1)	Cytotoxicity	Kouokam et al. (2006)
Helicobacter pylori	Gastritis and peptic ulcer, promotes gastric cancer	Vacuolating cytotoxin (VacA)	Vacuolating activity	Hynes et al. (2005), Keenan et al. (2000)
		Urease	Hydrolyzes urea	
		Helicobacter cysteine-rich proteins (Hcp)	Interferes with host cell functions	
		Lewis antigen LPS	Cytotoxic, stimulating proliferation, IL-8 secretion	

(continued)

Table 2.1 (continued)

Bacterial spp.	Associated disease	Virulence factors	Activity	Reference
Legionella pneumophila	Legionnaires' disease	Mip (lpg0791), Macrophage infectivity potentiator	Inhibition of autophagy in macrophages	Fernandez-Moreira et al. (2006), Galka et al. (2008)
		Flagellin	Evasion and spreading in host	
		LaiE/LaiF	Adhesion to and invasion of human lung epithelial cell	
		Intracellular multiplication protein K and X (IcmK/IcmX)	Involved in secretion and intracellular replication of Legionella in macrophages	
		Phospholipase C		
		Acid phosphatases		
		Diphosphohydrolase		
		Chitinase	Glycosylase, promotes persistence in the lung	
		Proteases	Interfere with immune function	
		Hsp60	Involved in adherence and invasion	
Moraxella catarrhalis	Otitis media and sinusitis, occasional cause of laryngitis	Ubiquitous surface protein A1 and A2 (UspA1/UspA2)	Binds C3 complement in serum	Tan et al. (2007)
Myxococcus xanthus	Nonpathogenic	TonB transporters	Predatory behavior and multicellularity	Kahnt et al. (2010)

(continued)

Table 2.1 (continued)

Bacterial spp.	Associated disease	Virulence factors	Activity	Reference
Neisseria gonorrheae	Sexually transmitted disease gonorrhea	Porin B	Serum resistance, B-cell activation	Zhu et al. (2005)
Neisseria meningitidis	Meningococcal disease	PorA, PorB	Adherence to host cells, serum complement resistance	Bjerre et al. (2000), Masignani et al. (2003), Schlichting et al. (1993), Vipond et al. (2006)
		NlpB	Lipoprotein, maintains membrane integrity	
		NarE	Potent toxin, ADP-ribosyltransferase activity	
Photorhabdus luminescens	Insect pathogen	Toxin AB	Insecticidal activity	Guo et al. (1999), Khandelwal and Banerjee-Bhatnagar (2003)
		GroEL	Cytotoxicity	
Porphyromonas gingivalis	Periodontal disease, gingivitis	Arg- and Lys-gingipain cysteine proteinases	Hemoglobinase activity	Duncan et al. (2004), Grenier (1992), Grenier and Mayrand (1987), Kamaguchi et al. (2003)
		Fimbriae	Interfere with immune response	

(continued)

Table 2.1 (continued)

Bacterial spp.	Associated disease	Virulence factors	Activity	Reference
P. aeruginosa	Lung infection of patients with chronic obstructive pulmonary disease (COPD), pneumonia, cystic fibrosis (CF), and bronchiectasis	Phospholipase C	Decrease of apical CFTR expression	Bauman and Kuehn (2006), Ciofu et al. (2000), Cota-Gomez et al. (1997), Kadurugamuwa and Beveridge (1995), Li et al. (1998), MacEachran et al. (2007), Mashburn and Whiteley (2005), Bomberger et al. (2009)
		Hemolysin	Lyse red blood cells	
		Alkaline phosphatase	In vitro enzyme activities	
		Cystic fibrosis transmembrane regulator (CFTR) inhibitory factor (Cif)	Degradation of CFTR	
		2-heptyl-3-hydroxy-4-quinolone (PQS)	bactericidal	
		Murein hydrolases	Lysis of peptidoglycan	
		Protease	Proteolytic activity	
		β-lactamase	IL-8 stimulation	
Salmonella typhi	Enteric fever typhoid	Cytolysin (ClyA)	Pore formation, membranolytic activity	Wai et al. (2003)
		OmpC	ND	
Salmonella typhimurium	Diarrheal disease	Protective antigens	ND	Bergman et al. (2005), Yoon et al. (2011)
Shigella dysenteriae serotype 1	Shigellosis (bacillary dysentery)	Shiga toxin 1	Toxicity	Dutta et al. (2004)
Shigella flexneri	Diarrhea	IpaB, IpaC, IpaD	Invasion	Kadurugamuwa and Beveridge (1998)
Treponema denticola	Periodontal diseases	Dentilysin	Chymotryptic activity	Chi et al. (2003), Rosen et al. (1995)
		Adhesins	Adherence to host	
		Proteases	Disruption of tight junctions	

(continued)

Table 2.1 (continued)

Bacterial spp.	Associated disease	Virulence factors	Activity	Reference
Vibrio anguillarum	Fish pathogen	Metalloprotease	Protease, metalloprotease	Hong et al. (2009)
		Hemolysin	Hemolytic activities	
		Phospholipase	Lipolytic activity	
Vibrio cholerae	Diarrhea	RTX toxin	Cell rounding, depolymerizing actin	Boardman et al. (2007), Chatterjee and Chaudhuri (2011)
		Cholera Toxin (CT)	cAMP activation, fluid accumulation	
Xanthomonas campestris	Plant pathogen	Cellulase	ND	Sidhu et al. (2008)
		β-glucosidase		
		Type 3 secretion system proteins		
		xylosidase		
		avirulence proteins		
Xenorhabdus nematophilus	Insect pathogen	Bacteriocin	Insecticidal	Khandelwal and Banerjee-Bhatnagar (2003)
		Fimbrial adhesin	Adherence to host	
		Pore-forming toxin	Cytotoxicity	
		Chitinase	Chitinase activity	

Ferrari et al. 2006; Horstman and Kuehn 2000). Better still, density gradient centrifugation is used for ensuring removal of contaminants such as pili, flagella, other protein aggregates, and so on from the OMV preparation (Bauman and Kuehn 2006; Lee et al. 2007). On the other hand, gel filtration chromatography may be the method of choice for getting a highly purified OMV preparation. A Sephacryl S500 column was used for purification of OMVs from *Neisseria meningitidis* (Post et al. 2005). Lee et al. (2007) used a slightly modified method using two sequential steps to get a highly purified preparation of OMVs from *Escheriachia coli* DH5α cells. In the first step, bacterial cells and cell debris were removed from the culture supernatant by using differential centrifugation followed by filtration through a membrane filter (0.45 μm), precentrifugation at 20,000 and 40,000 g to remove any large vesicle or vesicle aggregates or cell debris; and then ultracentrifugation at 150,000 g. The enriched OMVs, in the second step, were further purified using density gradients to remove any remaining contaminants. Subsequent electron microscopy showed the purity of OMV preparation, the OMVs having a size between 20 – 40 nm (Lee et al. 2007).

2.4.2 Protein Separation

Different methods have been tried and used for separation of proteins for their subsequent analysis by mass spectrometry to get the protein profile or proteome of the OMVs. These methods include two-dimensional gel electrophoresis (2-DE) and a combination of one dimensional (1-D) SDS-PAGE (Fig. 2.6) and liquid chromatography (LC). The purified OMVs are subjected to either 2-DE or (1-DE) SDS-PAGE followed by enzymatic digestion and LC to separate the peptides. Although 2-DE is a powerful tool for protein separation, it has its limitations, particularly in the case of membrane proteins. These proteins have very poor solubility in the nondetergent isoelectric focusing buffer that causes precipitation of the proteins at their isoelectric points. In addition, the high molecular weight, basic, or hydrophobic proteins are not properly resolved by 2-DE (Post et al. 2005; Wu and Yates 2003). The other method (1-D) SDS-PAGE can separate proteins more efficiently; its limitation, particularly in high-throughput mass analysis, is greater complexity of the proteins in each gel fraction. However, this problem can be overcome by subsequent use of LC to separate the extracted peptides based on their hydrophobicity (Nevot et al. 2006; Post et al. 2005). Lee et al. (2007) argued that because the molecular weights of vesicular proteins are quite different and the OMVs also contain less abundant proteins, they used (1-D) SDS-PAGE and then cut the gel into five slices of equal size, and subjected them to trypsin digestion to extract the peptides and got better results.

Mass spectrometric analysis of the extracted peptides from the native OMVs of *E. coli* initially identified 2,606 and 2,816 proteins with high- confidence peptide sequences. In order to eliminate the peptides shared by multiple proteins, the authors used the protein hit score (PHS) method for reliable protein identification

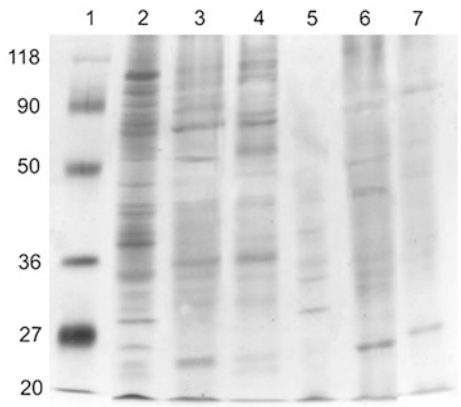

Fig. 2.6 Protein profile of the *OMV*, *OM,* and *IM* of *V. cholerae*. Proteins were separated by 12 % SDS-PAGE and visualized by silver staining; Lane 1: Molecular weight (kDa) markers, Lane 2: *OMV*, Lane 3: *OM*, Lane 4: *IM*. [From (Chatterjee and Chaudhuri 2011)]

(Lee et al. 2008; Park et al. 2006). The analyses showed that proteins with PHS > 1 were identified by multiple peptides that are unique and shared with only a few proteins. Furthermore, rigorous screening of proteins of PHS > 1 identified a total of 141 proteins, including 127 previously unknown vesicular proteins, with high confidence and reproducibility (Lee et al. 2007).

Proteomic analysis of several Gram-negative bacterial OMVs defined more than 200 vesicular proteins (Bauman and Kuehn 2006; Berlanda Scorza et al. 2008; Lee et al. 2008; Post et al. 2005). When these proteins were classified into protein families based on their sequence homology and function, several families were found to be common in OMVs derived from several species of Gram-negative bacteria: (1) the abundant OMPs, Porins (Omps, PorA, PorB and OprF), which were found in most OMVs; (2) the murein hydrolases (Met and SLT), which are responsible for the hydrolysis of certain cell wall glycopeptides, peptidoglycans in particular; (3) the multidrug efflux pumps (Mtr, Mex, and TolC), which are known to release toxic compounds (Kobayashi et al. 2000); (4) the ABC transporters (LamB and FadL); (5) the protease/chaperone proteins (DegQ/SurA) and (6) the motility proteins related to fimbriae/pili (FliC/PilQ) were found in OMVs from different strains. On the other hand, the virulence factors including hemolysin, IgA protease, and macrophage infectivity potentiator were also identified in OMVs from pathogenic strains.

Proteomic analyses of OMVs have brought out some findings that may give rise to controversy. Protein profiles of OMVs and detergent-treated OMVs (DOMVs) revealed the presence of cytoplasmic proteins as well (Ferrari et al. 2006; Henry et al. 2004; Lee et al. 2007; Molloy et al. 2000; Wei et al. 2006; Xu et al. 2006). Among the cytoplasmic proteins found in the protein profiles of OMVs of different bacteria, the proteins EF-Tu, DnaK, GroEL, and two ribosomal proteins, S1 and L7/12 (which are generally highly abundant proteins), have also been detected from cell supernatants or OM fractions (Ferrari et al. 2006). It has been suggested that the transcriptional and ribosomal proteins may be sorted into the OMVs during the informational process (Dorward and Garon 1989; Dorward et al. 1989;

Ferrari et al. 2006; Kadurugamuwa and Beveridge 1995; Yaron et al. 2000). Contrary to these findings of the proteomic analyses of OMVs, many researchers believe that the cytoplasmic proteins are excluded from the OMVs (Horstman and Kuehn 2000) and that the presence of the cytoplasmic proteins, if any, in the OMVs indicates the lysis of bacteria from which they originated (Kulp and Kuehn 2010). Kulp and Kuehn (2010) further observed that in order to use the power of proteomic analyses by Mass Spectrometry to deduce the origins or biogenesis of OMVs from their protein profiles, only very carefully purified native OMVs should be studied. In fact, Berlanda Scorza et al. (2008) did not find the cytoplasmic proteins or IMPs in the OMVs derived from a log phase culture and avoided contamination from lysed cells. The authors took great care to examine highly purified OMVs. On the other hand, Lee et al. (2007) analyzed OMV proteins from a stationary phase wild- type culture of *E. coli* cells and found the presence of cytoplasmic materials, which also could be interpreted as resulting from lysis of a fraction of the bacterial cells in the resting phase. Could it be that under certain growth conditions (not yet known) the regulation of protein synthesis in the bacterial cells loses its control, leading to excess production of some cytoplasmic proteins, which the bacteria try to get rid of by secretion through OMVs? Additional studies taking great care to eliminate bacterial lysis and adopting a very stringent method of purification of OMVs might resolve the issue.

References

Allan ND, Beveridge TJ (2003) Gentamicin delivery to *Burkholderia cepacia* group IIIa strains via membrane vesicles from *Pseudomonas aeruginosa* PAO1. Antimicrob Agents Chemother 47:2962–2965

Allan ND, Kooi C, Sokol PA, Beveridge TJ (2003) Putative virulence factors are released in association with membrane vesicles from *Burkholderia cepacia*. Can J Microbiol 49:613–624

Balsalobre C, Silvan JM, Berglund S, Mizunoe Y, Uhlin BE, Wai SN (2006) Release of the type I secreted alpha-haemolysin via outer membrane vesicles from *Escherichia coli*. Mol Microbiol 59:99–112

Bauman SJ, Kuehn MJ (2006) Purification of outer membrane vesicles from *Pseudomonas aeruginosa* and their activation of an IL-8 response. Microbes Infect 8:2400–2408

Bergman MA, Cummings LA, Barrett SL, Smith KD, Lara JC, Aderem A, Cookson BT (2005) CD4+ T cells and toll-like receptors recognize *Salmonella* antigens expressed in bacterial surface organelles. Infect Immun 73:1350–1356

Berlanda Scorza F, Doro F, Rodriguez-Ortega MJ, Stella M, Liberatori S et al (2008) Proteomics characterization of outer membrane vesicles from the extraintestinal pathogenic *Escherichia coli* DeltatolR IHE3034 mutant. Mol Cell Proteomics 7:473–485

Beveridge TJ (1999) Structures of gram-negative cell walls and their derived membrane vesicles. J Bacteriol 181:4725–4733

Bjerre A, Brusletto B, Rosenqvist E, Namork E, Kierulf P et al (2000) Cellular activating properties and morphology of membrane-bound and purified meningococcal lipopolysaccharide. J Endotoxin Res 6:437–445

Boardman BK, Meehan BM, Fullner Satchell KJ (2007) Growth phase regulation of *Vibrio cholerae* RTX toxin export. J Bacteriol 189:1827–1835

Bomberger JM, Maceachran DP, Coutermarsh BA, Ye S, O'Toole GA, Stanton BA (2009) Long-distance delivery of bacterial virulence factors by *Pseudomonas aeruginosa* outer membrane vesicles. PLoS Pathog 5:e1000382

Chatterjee D, Chaudhuri K (2011) Association of cholera toxin with Vibrio cholerae outer membrane vesicles which are internalized by human intestinal epithelial cells. FEBS Lett 585:1357–1362

Chatterjee SN, Das J (1966) Secretory activity of *Vibrio cholerae* as evidenced by electron microscopy. In: Uyeda (ed) Electron microscopy, Maruzen Co. Ltd, Tokyo

Chatterjee SN, Das J (1967) Electron microscopic observations on the excretion of cell-wall material by *Vibrio cholerae*. J Gen Microbiol 49:1–11

Chatterjee SN, Chaudhuri K (2003) Lipopolysaccharides of *Vibrio cholerae*. I. Physical and chemical characterization. Biochim Biophys Acta 1639:65–79

Chaudhuri K, Chatterjee SN (2009) Cholera toxins. Springer, Heidelberg

Chi B, Qi M, Kuramitsu HK (2003) Role of dentilisin in *Treponema denticola* epithelial cell layer penetration. Res Microbiol 154:637–643

Ciofu O, Beveridge TJ, Kadurugamuwa J, Walther-Rasmussen J, Hoiby N (2000) Chromosomal beta-lactamase is packaged into membrane vesicles and secreted from *Pseudomonas aeruginosa*. J Antimicrob Chemother 45:9–13

Cota-Gomez A, Vasil AI, Kadurugamuwa J, Beveridge TJ, Schweizer HP, Vasil ML (1997) PlcR1 and PlcR2 are putative calcium-binding proteins required for secretion of the hemolytic phospholipase C of *Pseudomonas aeruginosa*. Infect Immun 65:2904–2913

Deatherage BL, Lara JC, Bergsbaken T, Rassoulian Barrett SL, Lara S, Cookson BT (2009) Biogenesis of bacterial membrane vesicles. Mol Microbiol 72:1395–1407

Dorward DW, Garon CF (1989) DNA-binding proteins in cells and membrane blebs of *Neisseria gonorrhoeae*. J Bacteriol 171:4196–4201

Dorward DW, Garon CF, Judd RC (1989) Export and intercellular transfer of DNA via membrane blebs of *Neisseria gonorrhoeae*. J Bacteriol 171:2499–2505

Dorward DW, Schwan TG, Garon CF (1991) Immune capture and detection of *Borrelia burgdorferi* antigens in urine, blood, or tissues from infected ticks, mice, dogs, and humans. J Clin Microbiol 29:1162–1170

Duncan L, Yoshioka M, Chandad F, Grenier D (2004) Loss of lipopolysaccharide receptor CD14 from the surface of human macrophage-like cells mediated by *Porphyromonas gingivalis* outer membrane vesicles. Microb Pathog 36:319–325

Dutta S, Iida K, Takade A, Meno Y, Nair GB, Yoshida S (2004) Release of Shiga toxin by membrane vesicles in *Shigella dysenteriae* serotype 1 strains and in vitro effects of antimicrobials on toxin production and release. Microbiol Immunol 48:965–969

Ellis TN, Kuehn MJ (2010) Virulence and immunomodulatory roles of bacterial outer membrane vesicles. Microbiol Mol Biol Rev 74:81–94

Fernandez-Moreira E, Helbig JH, Swanson MS (2006) Membrane vesicles shed by *Legionella pneumophila* inhibit fusion of phagosomes with lysosomes. Infect Immun 74:3285–3295

Ferrari G, Garaguso I, Adu-Bobie J, Doro F, Taddei AR et al (2006) Outer membrane vesicles from group B *Neisseria meningitidis* delta gna33 mutant: proteomic and immunological comparison with detergent-derived outer membrane vesicles. Proteomics 6:1856–1866

Forsberg CW, Beveridge TJ, Hellstrom A (1981) Cellulase and xylanase release from *Bacteroides succinogenes* and its importance in the rumen environment. Appl Environ Microbiol 42:886–896

Galka F, Wai SN, Kusch H, Engelmann S, Hecker M et al. (2008) Proteomic characterization of the whole secretome of *Legionella pneumophila* and functional analysis of outer membrane vesicles. Infect Immun 76:1825–1836

Gamazo C, Moriyon I (1987) Release of outer membrane fragments by exponentially growing *Brucella melitensis* cells. Infect Immun 55:609–615

Goulhen F, Hafezi A, Uitto VJ, Hinode D, Nakamura R, Grenier D, Mayrand D (1998) Subcellular localization and cytotoxic activity of the GroEL-like protein isolated from *Actinobacillus actinomycetemcomitans*. Infect Immun 66:5307–5313

Grenier D, Mayrand D (1987) Functional characterization of extracellular vesicles produced by *Bacteroides gingivalis*. Infect Immun 55:111–117

Grenier D (1992) Inactivation of human serum bactericidal activity by a trypsin like protease isolated from *Porphyromonas gingivalis*. Infect Immun 60:1854–1857

Guo L, Fatig RO 3rd, Orr GL, Schafer BW, Strickland JA et al (1999) *Photorhabdus luminescens* W-14 insecticidal activity consists of at least two similar but distinct proteins. Purification and characterization of toxin A and toxin B. J Biol Chem 274:9836–9842

Henry T, Pommier S, Journet L, Bernadac A, Gorvel JP, Lloubes R (2004) Improved methods for producing outer membrane vesicles in gram-negative bacteria. Res Microbiol 155:437–446

Hong GE, Kim DG, Park EM, Nam BH, Kim YO, Kong IS (2009) Identification of *Vibrio anguillarum* outer membrane vesicles related to immunostimulation in the Japanese flounder, *Paralichthys olivaceus*. Biosci Biotechnol Biochem 73:437–439

Horstman AL, Kuehn MJ (2000) Enterotoxigenic *Escherichia coli* secretes active heat-labile enterotoxin via outer membrane vesicles. J Biol Chem 275:12489–12496

Hozbor D, Rodriguez ME, Fernandez J, Lagares A, Guiso N, Yantorno O (1999) Release of outer membrane vesicles from *Bordetella pertussis*. Curr Microbiol 38:273–278

Hynes SO, Keenan JI, Ferris JA, Annuk H, Moran AP (2005) Lewis epitopes on outer membrane vesicles of relevance to *Helicobacter pylori* pathogenesis. Helicobacter 10:146–156

Kadurugamuwa JL, Beveridge TJ (1995) Virulence factors are released from *Pseudomonas aeruginosa* in association with membrane vesicles during normal growth and exposure to gentamicin: a novel mechanism of enzyme secretion. J Bacteriol 177:3998–4008

Kadurugamuwa JL, Beveridge TJ (1998) Delivery of the non-membrane-permeative antibiotic gentamicin into mammalian cells by using *Shigella flexneri* membrane vesicles. Antimicrob Agents Chemother 42:1476–1483

Kahnt J, Aguiluz K, Koch J, Treuner-Lange A, Konovalova A et al (2010) Profiling the outer membrane proteome during growth and development of the social bacterium *Myxococcus xanthus* by selective biotinylation and analyses of outer membrane vesicles. J Proteome Res 9:5197–5208

Kamaguchi A, Nakayama K, Ichiyama S, Nakamura R, Watanabe T et al. (2003) Effect of *Porphyromonas gingivalis* vesicles on coaggregation of *Staphylococcus aureus* to oral microorganisms. Curr Microbiol 47:485–491

Karched M, Ihalin R, Eneslatt K, Zhong D, Oscarsson J et al (2008) Vesicle-independent extracellular release of a proinflammatory outer membrane lipoprotein in free-soluble form. BMC Microbiol 8:18

Kato S, Kowashi Y, Demuth DR (2002) Outer membrane-like vesicles secreted by *Actinobacillus actinomycetemcomitans* are enriched in leukotoxin. Microb Pathog 32:1–13

Keenan J, Day T, Neal S, Cook B, Perez–Perez G, Allardyce R, Bagshaw P (2000) A role for the bacterial outer membrane in the pathogenesis of *Helicobacter pylori* infection. FEMS Microbiol Lett 182:259–264

Khandelwal P, Banerjee-Bhatnagar N (2003) Insecticidal activity associated with the outer membrane vesicles of *Xenorhabdus nematophilus*. Appl Environ Microbiol 69:2032–2037

Kobayashi H, Uematsu K, Hirayama H, Horikoshi K (2000) Novel toluene elimination system in a toluene-tolerant microorganism. J Bacteriol 182:6451–6455

Kolling GL, Matthews KR (1999) Export of virulence genes and Shiga toxin by membrane vesicles of *Escherichia coli* O157:H7. Appl Environ Microbiol 65:1843–1848

Kouokam JC, Wai SN, Fallman M, Dobrindt U, Hacker J, Uhlin BE (2006) Active cytotoxic necrotizing factor 1 associated with outer membrane vesicles from uropathogenic *Escherichia coli*. Infect Immun 74:2022–2030

Kuehn MJ, Kesty NC (2005) Bacterial outer membrane vesicles and the host-pathogen interaction. Genes Dev 19:2645–2655

Kulp A, Kuehn MJ (2010) Biological functions and biogenesis of secreted bacterial outer membrane vesicles. Annu Rev Microbiol 64:163–184

Kwon SO, Gho YS, Lee JC, Kim SI (2009) Proteome analysis of outer membrane vesicles from a clinical *Acinetobacter baumannii* isolate. FEMS Microbiol Lett 297:150–156

Lee EY, Bang JY, Park GW, Choi DS, Kang JS et al (2007) Global proteomic profiling of native outer membrane vesicles derived from *Escherichia coli*. Proteomics 7:3143–3153

Lee EY, Choi DS, Kim KP, Gho YS (2008) Proteomics in gram-negative bacterial outer membrane vesicles. Mass Spectrom Rev 27:535–555

Li Z, Clarke AJ, Beveridge TJ (1998) Gram-negative bacteria produce membrane vesicles which are capable of killing other bacteria. J Bacteriol 180:5478–5483

Lindmark B, Rompikuntal PK, Vaitkevicius K, Song T, Mizunoe Y et al (2009) Outer membrane vesicle-mediated release of cytolethal distending toxin (CDT) from *Campylobacter jejuni*. BMC Microbiol 9:220

MacEachran DP, Ye S, Bomberger JM, Hogan DA, Swiatecka-Urban A, Stanton BA, O'Toole GA (2007) The *Pseudomonas aeruginosa* secreted protein PA2934 decreases apical membrane expression of the cystic fibrosis transmembrane conductance regulator. Infect Immun 75:3902–3912

Mashburn-Warren LM, Whiteley M (2006) Special delivery: vesicle trafficking in prokaryotes. Mol Microbiol 61:839–846

Mashburn LM, Whiteley M (2005) Membrane vesicles traffic signals and facilitate group activities in a prokaryote. Nature 437:422–425

Masignani V, Balducci E, Di Marcello F, Savino S, Serruto D et al (2003) NarE: a novel ADP-ribosyltransferase from *Neisseria meningitidis*. Mol Microbiol 50:1055–1067

McBroom AJ, Kuehn MJ (2005) Outer membrane vesicles In: III RC (ed) EcoSal—*Escherichia coli* and *Salmonella* : cellular and molecular biology. American Society for Microbiology Press, Washington

Molloy MP, Herbert BR, Slade MB, Rabilloud T, Nouwens AS, Williams KL, Gooley AA (2000) Proteomic analysis of the *Escherichia coli* outer membrane. Eur J Biochem 267:2871–2881

Negrete-Abascal E, Garcia RM, Reyes ME, Godinez D, de la Garza M (2000) Membrane vesicles released by *Actinobacillus pleuropneumoniae* contain proteases and Apx toxins. FEMS Microbiol Lett 191:109–113

Nevot M, Deroncele V, Messner P, Guinea J, Mercade E (2006) Characterization of outer membrane vesicles released by the psychrotolerant bacterium *Pseudoalteromonas antarctica* NF3. Environ Microbiol 8:1523–1533

Park GW, Kwon KH, Kim JY, Lee JH, Yun SH et al (2006) Human plasma proteome analysis by reversed sequence database search and molecular weight correlation based on a bacterial proteome analysis. Proteomics 6:1121–1132

Patrick S, McKenna JP, O'Hagan S, Dermott E (1996) A comparison of the haemagglutinating and enzymic activities of *Bacteroides fragilis* whole cells and outer membrane vesicles. Microb Pathog 20:191–202

Post DM, Zhang D, Eastvold JS, Teghanemt A, Gibson BW, Weiss JP (2005) Biochemical and functional characterization of membrane blebs purified from *Neisseria meningitidis* serogroup B. J Biol Chem 280:38383–38394

Rosen G, Naor R, Rahamim E, Yishai R, Sela MN (1995) Proteases of *Treponema denticola* outer sheath and extracellular vesicles. Infect Immun 63:3973–3979

Schlichting E, Lyberg T, Solberg O, Andersen BM (1993) Endotoxin liberation from *Neisseria meningitidis* correlates to their ability to induce procoagulant and fibrinolytic factors in human monocytes. Scand J Infect Dis 25:585–594

Shoberg RJ, Thomas DD (1993) Specific adherence of *Borrelia burgdorferi* extracellular vesicles to human endothelial cells in culture. Infect Immun 61:3892–3900

Sidhu VK, Vorholter FJ, Niehaus K, Watt SA (2008) Analysis of outer membrane vesicle associated proteins isolated from the plant pathogenic bacterium *Xanthomonas campestris* pv. campestris. BMC Microbiol 8:87

Tan TT, Morgelin M, Forsgren A, Riesbeck K (2007) *Hemophilus influenzae* survival during complement-mediated attacks is promoted by *Moraxella catarrhalis* outer membrane vesicles. J Infect Dis 195:1661–1670

Vipond C, Suker J, Jones C, Tang C, Feavers IM, Wheeler JX (2006) Proteomic analysis of a meningococcal outer membrane vesicle vaccine prepared from the group B strain NZ98/254. Proteomics 6:3400–3413

Wai SN, Lindmark B, Soderblom T, Takade A, Westermark M et al (2003) Vesicle-mediated export and assembly of pore-forming oligomers of the enterobacterial ClyA cytotoxin. Cell 115:25–35

Wei C, Yang J, Zhu J, Zhang X, Leng W et al (2006) Comprehensive proteomic analysis of *Shigella flexneri* 2a membrane proteins. J Proteome Res 5:1860–1865

Wu CC, Yates JR 3rd (2003) The application of mass spectrometry to membrane proteomics. Nat Biotechnol 21:262–267

Xia XX, Han MJ, Lee SY, Yoo JS (2008) Comparison of the extracellular proteomes of *Escherichia coli* B and K-12 strains during high cell density cultivation. Proteomics 8:2089–2103

Xu C, Lin X, Ren H, Zhang Y, Wang S, Peng X (2006) Analysis of outer membrane proteome of *Escherichia coli* related to resistance to ampicillin and tetracycline. Proteomics 6:462–473

Yaron S, Kolling GL, Simon L, Matthews KR (2000) Vesicle-mediated transfer of virulence genes from *Escherichia coli* O157:H7 to other enteric bacteria. Appl Environ Microbiol 66:4414–4420

Yokoyama K, Horii T, Yamashino T, Hashikawa S, Barua S et al (2000) Production of shiga toxin by *Escherichia coli* measured with reference to the membrane vesicle-associated toxins. FEMS Microbiol Lett 192:139–144

Yoon H, Ansong C, Adkins JN, Heffron F (2011) Discovery of *Salmonella* virulence factors translocated via outer membrane vesicles to murine macrophages. Infect Immun 79:2182–2192

Zhu W, Thomas CE, Chen CJ, Van Dam CN, Johnston RE, Davis NL, Sparling PF (2005) Comparison of immune responses to gonococcal PorB delivered as outer membrane vesicles, recombinant protein, or Venezuelan equine encephalitis virus replicon particles. Infect Immun 73:7558–7568

http://www.springer.com/978-3-642-30525-2

Outer Membrane Vesicles of Bacteria

Chatterjee, S.N.; Chaudhuri, K.

2012, IX, 161 p. 29 illus., 10 illus. in color., Softcover
ISBN: 978-3-642-30525-2