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Significances of OMV and Extracellular Vesicle Proteomics

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Abstract

Outer Membrane Vesicle (OMV) proteome has been involved into the pathogenesis of diseases and resistance of microorganisms against a number of antibiotics, mechanism of action of probiotics and host-pathogen interaction etc. We have enlightened the role played by extracellular vesicles proteomics in the pathogenesis of different diseases related to human. Isolation succeeded by purification of ample amount of OMV from biological samples, is one of the most important steps for further proteome related analysis. With the development of both labelled and label-free methods used in proteomics, significant progress has been made in previous years in membrane proteomics. Hence, it is important to review the biological significance of proteins found in the OMV fractions using membrane proteomics approach. We have also explained methods used for isolation, purification and quantification of OMV. In the present review, it can be concluded that proteomic study of outer membrane and extracellular vesicles has now gained priority for the detailed study of disease pathogenesis, drug resistance, vaccine development, cell signalling etc.

Keywords: Membrane proteomics; Outer membrane vesicles proteomics; Extracellular vesicle proteomics, Proteomics; Methods for OMV purification

Introduction

Microorganisms communicate among themselves and with their surrounding environment via membrane vesicles found in their Outer Membrane Vesicle (OMV) for trafficking bacterial cell signalling molecules to target cells around them. These molecules include nucleic acids, proteins, some endotoxins and allied virulence factors. Gram-negative bacteria are known to constitutively secrete OMVs in their extracellular space. Their production is the response of bacterial stress and crucial for taking up nutrients from the environment, carrying endotoxic lipopolysaccharide, biofilm formation, quorum sensing, genetic transformations, and pathogenesis. They facilitate enzymes to reach their targets in a concentrated and protected irrespective of the distance from its source. Adhesions, toxins, and immune modulatory compounds containing OM vesicles that are synthesized naturally by pathogenic bacteria, facilitates the spread of pathogenesis of bacteria by enhancing its binding and invasion, resulting in cytotoxicity, and modifying immune response of host [1]. OMVs are also reported to behave as secretion system for factors related to virulence and toxins inside a host, facilitate survival in the inter-species environmental competition, intra- and inter-species communication, secretion of proteins which are misfolded and aggregated (a novel stress response mechanism) etc.[2]. Both pathogenic and non-pathogenic species of Gram-negative bacteria are known to release vesicles [3-6], including *Escherichia coli* [7,8], *Borrelia burgdorferi* [9], *Neisseria spp.* [10,11], *Bacteroides* (including *Porphyromonas*) *spp.* [12-14], *Shigella* spp. [15,16], *Helicobacter pylori* [17], *Salmonella* spp. [18,19], *Brucella melitensis* [20], *Campylobacter jejuni* [21,22], *Xenorhabdus nematophilus* [23], *Vibrio spp.* [24,25] *Actinobacillus actinomycetemcomitans* [26], and *Pseudomonas aeruginosa* [27]. Studies related to the vesicles released by various origins of bacteria suggest that these vesicles facilitate interactions between prokaryotic or eukaryotic cells with and bacteria. Artificial outer membrane vesicles have also been designed to study the role of OMVs such as OMV of *Pseudomonas aeruginosa* [28]. OMVs reduce the toxic compound levels like toluene resulting in the release of attacking phage thereby protecting the bacteria [29,30]. OMVs based vaccines are capable of conferring immunity against the various genotypes of *Acellular pertussis* [31], *Neisseria meningitis* [32], *Pseudomonas aeroginosa* [33], *Bordetella parapertussis* and *Bordetella pertussis* infection [34]. OMV and detergent extracted outer membrane vesicles (DOMV) are also used as vaccine delivery system [35-39]. In this review, we have discussed the significance of the different methods used for isolation and characterization of the outer-membrane and extra-cellular proteome.

Membrane vesicles

Prokaryotes, eukaryotes (archaea [40,41], Gram-negative and Gram-positive bacteria [42-45], fungi [45-46] and parasites [50,51] produce the spherical, membranous vesicles from the outer cell surface. Archaeal membrane vesicles (diameter 90-230 nm) contain S-layer proteins and membrane lipids, which has been obtained from the surface of archaeal cell [41,52]. Archaea MVs produces both toxin [52] and non-toxin [53] releasing vesicles. Microbial vesicles obtained from parasites and fungi; include two types of vesicles [48,54]. Exosomes (diameter of 50-100 nm) are known to be produced from intracellular compartments and ectosomes, which are shed from plasma membrane [55]. Exosomes are typically homogeneously shaped vesicles produced during exocytosis of multivesicular bodies within the cell, while ectosomes are ubiquitous in nature that assembled at and released out from plasma membrane of cell [56]. Microvesicles (100-1,000 nm in diameter), similar to OMV are produced from plasma membrane and endosomal membrane compartment by hematopoietic cells, epithelial cells, and cells of tumor. Proteins, mRNAs, microRNAs, and lipids make up the bilayer proteolipids named as extracellular vesicles [55]. It

is also important to note that Extracellular Vesicles (EVs) (similar to bacterial OMV in component), which are released by various types of mammalian cells, facilitates communication between different cells [57]. Bacterial EV are proposed to contribute to intercellular communication, bacterial survival and human pathogenesis as a novel secretion system [58]. EVs can transport proteins, nucleic acids like mRNAs and microRNAs [57]; transport membrane components including receptors [59]; and protect the stuffs they are carrying from getting degraded by many enzymes present in the extracellular region [57]. Quantitative proteomic study of *S. typhimurium* revealed that membrane vesicles released by bacteria are modulated by the envelope proteins [60]. The location of membrane vesicle formation is the protein enriched regions of envelope characterized by temporary decrease in OM-PG and/ OM-PG-IM interconnections density [60] or slight modulation in LPS structure [61] leads to the change in MV formation. High conservation of protein domains enhances the interconnections of OM-PG-IM and OM-PG envelope among different Gram-negative bacteria [60] portray that these connections play a crucial role in the vesicle release pathway of bacteria. Although fungi, parasites, and archaea are not related taxonomically but several basic features of vesicle production are same perhaps upon which lots of other microorganisms have adapted additional mechanisms. Archaeal MVs are synthesized and secreted by pinching the cell surface off, a phenomenon reminiscent of eukaryotic SMVs and bacterial MVs.

Formation of Outer membrane vesicles

Outer Membrane Vesicles (OMV) biogenesis without disrupting the viability of bacteria still remains intangible. Various vesiculation mechanism theories based on genetic and biochemical data have been studied [62]. The ubiquitous process of vesiculation helps in the survival of Gram-negative bacteria in different conditions of environments [3]. Naturally produced membrane vesicles are very distinct, closed blebs of outer membrane released by budding cells, rather due to cell lysis or death [14,62-64]. The utmost rate of membrane vesicle formation takes place at the end of log phase and is plentiful at the sites of cell division, as explained for *B. melitensis, E. coli and Vibrio cholera* [8,20-24]. The outer membranes of Gram-negative bacteria consist of proteins, asymmetric lipid distribution, phospholipids, lipoproteins and lipopolysaccharide (LPS). The periplasm comprises of the peptidoglycan layer, occupant housekeeping enzymes and proteins, and temporary intermediates of secretory pathways. Native OMVs contain only lipids and protein of the outer membrane as well as periplasm but do not contain components of cytoplasm and inner membrane. This was confirmed from density gradient-purified OMV analysis [62]. To understand the mechanism of vesicle formation and release, deletion of *yfgL gene,* leads to remarkable decline in formation and release of OMV and in an adherent-invasive strain of *E. coli* K12 and *E. coli* (AIEC) [65]. A lipoprotein is encoded by *yfgL* gene, which plays role in the formation and/or degradation of peptidoglycan [66]. yfgL resulted in rise of the peptidoglycan production and decrease in the lytic transglycosylase synthesis which in turn reduce the turgor pressure on outer membrane of cell [65]. This experiment revealed that in the synthesis of the OMVs, LPS structure plays an important role directly and indirectly. In general, non-pathogenic bacteria produce less number of vesicles than their pathogenic counterparts [67,68]. PAO1 strain of *P. aeruginosa, has two types of O-antigen side chain, produce the highly charged and long* "B-band" form vesicles [27,69,70]. This strain also increases the vesicle formation with the increment in B-band LPS under the different condition (oxygen stress) [71]. *Salmonella* and *P. aeruginosa* mutants also show the increase in vesicle production in which LPS O-antigen side chain is missing [72,73] whereas mutation in core of LPS result in decreased OMP expression [73-75]. Some recent studies demonstrated that various pathways that manage different environmental stresses that are faced by a pathogen inside host body influence activation of pathways involved in OMV formation of *P. aeruginosa*. These stresses increase the OMV production, which does not depend on modulators of OMV formation identified from previous studies, such as PQS, MucD and, AlgU, homolog modulators of OMV synthesis in *E. coli*. It was also revealed that B-band instead of A-band LPS was responsible for OMV biogenesis induced by oxidative-stress. However, the process by which increased OMV formation helps *P. aeruginosa* to adjust with stressful conditions inside host body and survival, is yet to be studied [76].

Proteomic analysis of OMV and EVs

Outer membrane related proteins that are exposed on the surface and extracellular proteins, play a role in adhesion, entry, transport of nutrients, toxicity, suppression of host cell immune system and also resistance to the antibiotic. Consequently, identifying and characterizing the proteins associated with OMV can improve the diagnosis of diseases and result in the development of new vaccine and drug targets. Proteomics is considered as a major technique to study membrane proteomics in detail under different diverse conditions [77]. With the development of proteomic tools [77-80], considerable progress has been done in recent years in the area of membrane proteomics. Various methods that are employed in the membrane and differential proteomics are unlabelled methods, labelled method and most recent label free quantification methods [79,80]. Isotope labels can be integrated into peptides metabolically, chemically or enzymatically. In label free proteomics the mass spectrometry reveals the mass differences and their proteomic quantifications can be done by analyze their respective signal intensities. Every isolation and purification method has its own merits and demerits and they are paired to each other [79]. Based on the above introduction, it is also important to explain the significance and use of OMV and extracellular vesicle proteomics. Nowadays, numerous studies are being performed on proteomic analysis of bacterial OMVs. Recent progress in the studies of Gram-negative bacterial extracellular vesicles implies that OMVs may function as intercellular communicasomes in bacteria-bacteria and bacteria-host interactions [81]. Some studies show that 70-80% OM- associated proteins make up the OMVs [82]. Hence, the surface proteomes have been analyzed to get a better understanding of the mechanism related to virulence, drug resistance, biofilm formation and development of new drug and vaccine targets. Different steps of isolation, purification, quantification of OMV and its significances have been outlined in Figure 1.

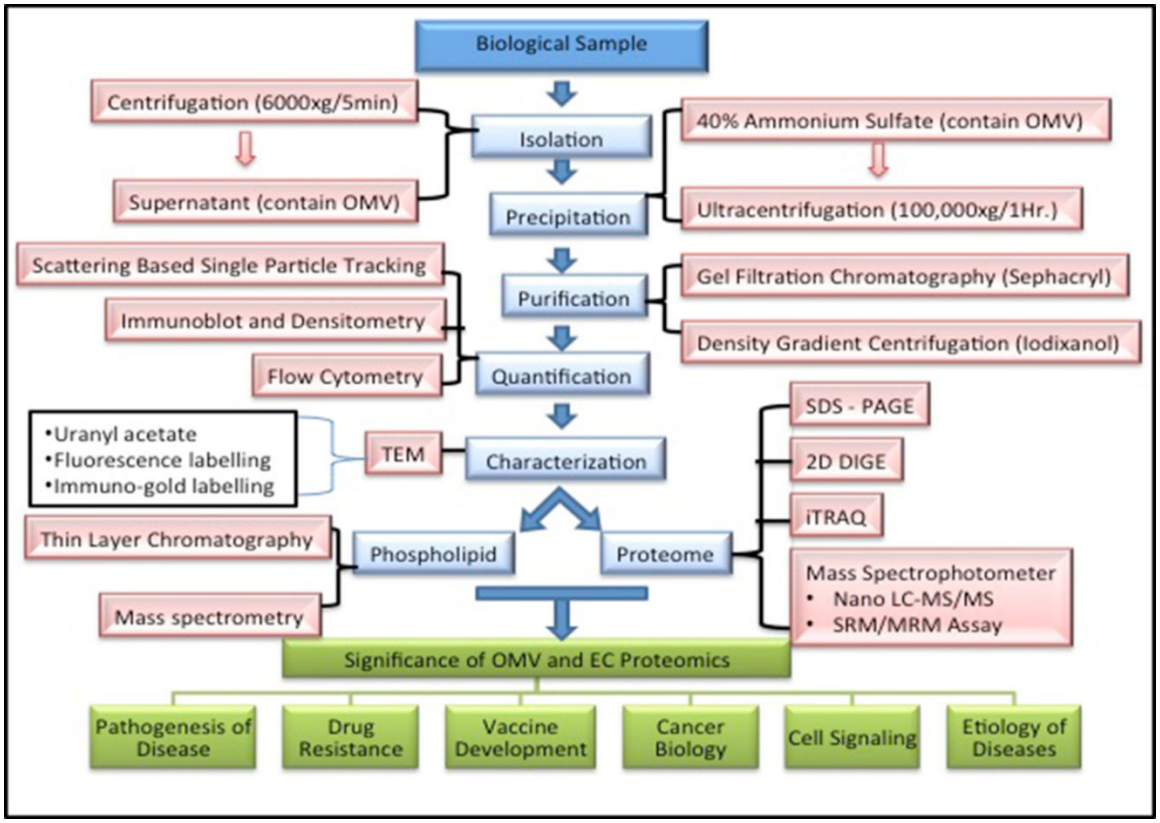

Figure 1: Outline of different steps of isolation, purification and quantification of OMV and significances of OMV.

Isolation, purification and quantification of OMV and EV

Before performing the proteomics, it is important to review the methods for the isolation and purification of the OMV. Methods used for the isolation of the OMV and EV include ultracentrifugation and precipitation [2]. The isolation of OMV starts from culture supernatant after removal of cultured bacteria by centrifugation at 6,000 × g for 5 min. Supernatant was filtered via 0.22 µm sterilization vacuum filter (V25) [83]. The non-OMV proteins are removed by filtration using the cut-off of 50-100 kDa membranes before ultracentrifugation. Precipitation of the OMV has been performed using ammonium sulfate. A particular concentration of ammonium sulfate is required for the isolation OMV therefore care must be taken to select the concentration of the ammonium sulfate. For example in *P. gingivalis*, OMV proteins are precipitated by 40% saturation of ammonium sulfate [12,14] followed by the isolation of non-OMV proteins at 70% saturation [84]. This shows that a proper concentration of ammonium sulfate should be resolute carefully and imprecise binding of extracellular proteins to ammonium sulfate-precipitated OMV is exceedingly feasible [1]. The increase in protease during concentration step may results in OMV associated protein degradation. Hence, addition of corresponding inhibitor into the media prior to the concentration step is required to prevent the degradation. The precipitated and centrifuged OMV should then be reconstituted by dialysis against suitable buffer before further investigations. Both these isolation methods are unable to isolate OMV from the extra cellular matrixes such as large protein complex and aggregates. These impurities are removed by the density gradient centrifugation and gel filtration. In the OMV, lipid content is more than secreted proteins and its density is lower than other protein complexes, hence OMV, move around to lighter fractions during the iodixanol density gradient centrifugation [85]. Gel filtration is also employed for the purification of the isolated OMV using Sephacryl S-500 column (i.e., *N. meningitidis* OMV) [86], Sephacryl S-300 (Meiothermus ruber OMV) [87]. Quantification of OMV can be done by light scattering based single particle tracking [88] and flow cytometric analysis [89]. Bicinchoninic acid assay [90], Lowry [91] and Bradford [92], or by image analysis of stained or immune-detected gel-separated sample, are also used for OMV component quantification. Proteins extracted OMV can also analyzed by immunoblot and densitometry [93,94]. The purified OMV are characterized by TEM microscopy [60] or by gel free and gel-based proteomics method followed by western blotting and mass spectrometry [94]. To achieve the basic TEM images of OMV, negative staining by 2% uranyl acetate has been performed [88]. Due to excessive cross-linking of the proteins, image background becomes complex and hard to study; therefore, fixation of the specimen has been done by glutaraldehyde and formaldehyde followed by Immuno-gold labelling to highlight the OMV components [27,96]. Examination by fluorescence labeling confirmed the interaction of OMV with other membranes, such as fusion with host-cell membrane before their entry into the eukaryotic cell [97,98]. Phospholipids and fatty acids forming the OMV membrane bilayers are also matter of interest which are first extracted from OMV and then analyzed by thin layer chromatography or mass spectrometry [99,100].

Significance of OMV proteomics in virulence of pathogens

Membrane of Gram-negative bacteria comprises of two polysaccharide bilayers, which consist of inner and outer membrane, the periplasm in between. This complicates the process of virulence factor secretion by the bacteria. These pathogens have developed various strategies; of which few are pathogen-specific, so that virulence factors can have admittance to the extracellular environment, especially bloodstream or tissue cells of the host [101]. Due to the cytotoxicity of *A. baumannii* OMV, virulence is induced in the host organism [102]. Kwon et al., reported that some of the proteins associated with OMVs named as chaperonin GroEL, AmpC-beta-lactamase, 6,7-dimethyl-8-ribityllumazine synthase, and AbOmpA. Among these proteins, several virulence factor linked proteins were recognized: Putative Zn-dependent protease, bacterioferritin, AbOmpA, catalase, putative protease, Cu/Zn superoxide dismutase, putative phospholipase A1 precursor, putative serine protease, and ferrichrome–iron receptor. Adaptive and innate immune responses are modulated by interaction of PAMPs, such as lipopolysaccharide, lipoproteins, outer membrane proteins, with PAMPs receptors in the host cells [103,104]. Among the 26 different outer membrane proteins, AbOmpA has been shown the interaction with the TLR-2 along with a modulated immune response in dendritic and epithelial cells [105,106]. In host epithelial cells, AbOmpA also facilitates the adhesion and invasion mechanism of the bacteria [107]. Kwon et al., also studied about OMVs as a virulent factor. In doing so, the OMVs in the absence of bacteria were treated with human cell line, which revealed that AbOmpA was present in the cytoplasm, which signifying that outer MVs directly hold multiple diverse virulence factors to host cells. Cytotoxicity assay of OMVs suggests that these virulence factors also alter the physiology of cells (elongation of the cell) [108]. Some previous studies also suggested that 151 proteins of the strain 173 of *A. actinomycetemcomitans* were identified with the help of LC-MS/MS and via OMV proteome investigation and they were found to be involved in virulence related mechanism. Identified protein such as GroEL, LtxA, TdeA, Omp100, TadZ, RcpC, TadE, BilR1, TadG, TadF, TadD, RcpC, RcpA, Pal, OmpA like protein, Omp18/16, and Omp39 are involved in the virulence related mechanism like cytotoxicity, immune-reactivity and/or pro-inflammatory activity, drug targeting, immune evasion and scavenging of iron and nutrients [109].

Proteins that are essential for the virulence of *Pseudomonas aeruginosa* [110], and that of *Helicobacter pylori* [111] have been identified through the LC-MS/MS analysis of outer membrane vesicles. Proteomic profiling of OMV of *Campylobacter jejuni* using high resolution LTQ-orbitrap spectroscopy identified 134 vesicular proteins which have the significant role in the bacterial infection and communication [112]. Virulence role of the OMV can also be understood from the proteomic study of the samples obtained from the septic human or rat serum [113,114]. Proteomic analyses of extracellular proteins in the *Acinetobacter baumannii* have revealed their role in defense machinery against the macrophagic attack and also in the state of oxidative stress [115]. The proteomic profiling of OMV of *Myxococcus xanthus* revealed that the OMV is rich in proteins with hydrolytic functions [116]. Proteomic analyses of OMV of *Francisella novicida* have also revealed its role in the pathogenesis [117]. Quantitative proteomics analyses of heat stressed *Clostridium difficile* helped to understand its physiological and metabolic functions used during upshift of temperature mimicking pyrexia [118]. Proteomic study of outer membrane of the *E. coli* revealed over expression FhuE and FhuA, and YbiL that have role in the iron transport [119]. By differential proteomics study, the role of iron in the continued existence of *A. baumannii* resistance strain (ATCC and carbapenem) in human host is revealed [120,121]. Proteome of the human host changes during *Acinetobacter baumannii* infection. Soares et al, found that the alterations in the plasma proteome using DIGE based differential proteomic analysis of the host infection with *A. baumannii* as compared to controls [122]. FhuE receptor, ferric acinetobactin receptor, ferrienterchelin receptor and ferric siderophore receptor have been played a role in iron transport in the host, identified in the membrane fraction of *A. baumannii* using DIGE based proteomics tool. Wurpal et al., studied about EDTA-heat induced outer MV biogenesis coupled with proteolytic treatment like carbonate extraction, cell lysis, ultracentrifugation and 2D-DIGE succeeded by MS/MS analysis of different uro-pathogenic *E. coli* (UPEC) strains. This UPEC surface-exposed proteome analysis demonstrated the constitutive proteins along with the various virulence related proteins [82]. Current MS-based high-throughput proteomic analyses of Gram-negative bacterial OMVs have identified thousands of vesicular proteins and provided clues to reveal the biogenesis and pathophysiological functions of Gram-negative bacterial OMVs [81].

Significance of OMV proteomics in the study of drug resistance and biofilm formation

Outer membrane act as a barrier between organism and host, hence the drugs have to cross the membrane to exert its action. Outer membrane vesicles are also known to possess chromosomal encoded β-lactamases which causes the degradation of extracellular β-lactam [123]. All Gram negative bacteria have been synthesized such as OM vesicles by which they facilitate biofilm formation and many biological functions namely virulence factors transportation and antibiotics resistance [60, 124,125]. Bacterial mobility is highly conserved in all *Xanthomonas*, and *S. maltophilia* species. Some recent studies related to OMV associated virulence factor Ax21 was found in *S. maltophilia* [126,127] while in *Xanthomonas oryzae* was found to contain the same factor (named Omp1x) [128] which play a role in virulence and biofilm formation [129]. In *Xanthomonas* pv. *oryzicola* this protein expression is controlled by DSF quorum sensing system [130]. The Diffusible Signal Factor (DSF) has a major importance in regulating biofilm production as well as cell-cell communication in *S. maltophilia* [131,132]. This factor system is also known to involve in co-colonization of the pathogens like *B. cenocepacia* and *P. aeruginosa* [131] in host lungs epithelial cells causing cystic fibrosis [133]. Devos et al., measured the OMV mediated protein secretion of the homologs of A×21 in the presence of BDSF, imipenem, PDSF and diffusible signal factors via targeted and label-free LC Multiple Reaction Monitoring technique. The results indicate large quantities of A×21 protein formation and packaging in OMVs that depicts the role played by it in the biofilm production and antibiotic resistance. Utilizing proteomic analysis, membrane vesicles of *Staphylococcus aureus* has been shown to have role in antibiotic resistance and pathology [43]. Minami et al., the changes in proteomic profiles of DRM fractions during cold acclimation using 2D-gel electrophoresis and mass analysis [134]. RND multidrug efflux pump membrane fusion proteins have been identified in the *P. aeruginosa* using membrane proteomics approach [86,106,135-137]. Proteomic analyses of *A. baumannii* explain that histidine metabolism also have major role in the biofilm formation [138]. Outer membrane comparative proteomic analysis of *P. aeruginosa* showed resistance against the ampicillin, kanamycin, and tetracycline antibiotics [139]. Production of vesicles is also influenced by antibiotics like mitomycin C, an inducer of Shiga toxin, used for

treating *Shigella dysenteriae. This* increases the OMV size, toxicity and production [15] while antibiotics like norfloxacin, ciprofloxacin, fosfomycin, etc. are not responsible for altering OMV production or toxicity. Carbapenem resistant *A. baumannii* has been already studied using outer membrane proteomics between wild type and resistance strain [108, 140-142]. Membrane proteomics result also showed that *A. baumannii* displays a vigorous and adaptable metabolism [143,144]. Similarly, differential high-end isoelectric point proteome analysis of *Acinetobacter radioresistens* reveals that with the help of aromatic compounds, we can induce the envelope stress responses [145]. Using differential quantitative proteomic analysis of OMVs from multidrug-resistant *A. baumannii*, Yun et al, reported that carbapenem suppress outer membrane proteins expression and increases the expression of resistance nodulation cell division transporters and protein kinases [146]. Using similar approach, Lee et al., explain the mechanism of hetero-resistance mechanism induced by imipenem in the multi-drug resistant *A. baumannii* [147]. Biofilm formation is one of the important reasons for the persistence of *A. baumannii* on the surface of host lung epithelial cells. Cabral et al., did the differential proteomics of multi-drug resistant *Acinetobacter* cultured in three diverse conditions (exponential, late stationary phase and biofilms stage) and they also examine the effects of salicylate on the biofilm formation which is a biofilm inhibitory compound. This multiple approach strategy explained unique lifestyle of *A. baumannii*, which are involved in both virulence and biofilms formation [148]. Differential proteomics of the *Porphyromonas gingivalis*, *Treponema denticola* suggest synergestic relationship in the polymicrobial biofilms formation [149].

Extracellular vesicles (EV) proteomics in the study of human diseases

EVs act as vehicles for intercellular communication and host manipulation [150]. Cancer has traditionally been considered as a human disease resulting from gene mutations but new finding suggest that extracellular vesicles which are derived from tumour can carry polypeptides, polysaccharides, DNA and RNA causative of cancer progression [151]. Intake of the tumor derived extracellular vesicles (EVs) by non-tumor cells can be converted into cancerous cell [152]. EV released by cancer cells also acts against the stress response [93]. Bioengineering of the membrane vesicles for the delivery of the siRNA into the cancer cell and used in the cancer therapy [153]. Exosomes secretion has been found to be key feature for the malignancy of the different stages of the cancer growth and development [59]. The OMVs released from Helicobacter pylori increases the chance of development of gastric cancer [154]. Biomarkers have been identified form the limited breast cancer tissue by membrane proteomics using SRM/MRM proteomic analysis in combination with iTRAQ shotgun analysis [155]. Proteomic analysis of purified MVs [156] from colorectal cancer cells has been done using SDS PAGE and nano LC–MS/MS analysis [157]. Tumor-derived MVs also have a role in intercellular communication known as extracellular organelles communicasomes [157]. The mitochondrial and endoplasmic reticulum fraction of breast cancer cell lines ZR-75-1 cells and MDA-MB-231 cells treatment with Dox-TRAIL identified new differentially abundant proteins using the iTRAQ labelling coupled with multidimensional LC-MS/MS [158]. Proteomic analysis also explained that serum glycoprotein can be a reliable biomolecule for the identification of biomarkers in the pancreatic cancer [159]. Extracellular vesicles are shed to the extracellular region by most eukaryotic and prokaryotic cells but recently it has been reported in Gram-positive bacteria, mycobacteria and fungi [160]. EV proteomes reflects developmental phases of *Bacillus subtilis* where it shows the formation of EV during sporulation is strongly supported by delineation of protein content that differs from proteome of EV formed by vegetative spores [58].

Significance of OMV in the development of probiotics

Probiotics are the microorganisms that provide health benefits when consume. *E. coli Nissle* 1917 (EcN) is the well-known probiotic that is used in treatment of intestinal disease. This enhances the useful microbiota and the homeostasis of the gastro-intestines. Using the LC-MS/MS analysis, Aguilera et al., identified 18 different proteins that have been found to be strain specific and others are outer membrane vesicles of pathogens. These outer membrane vesicles shows binding with the host and increase the beneficial and positive effects on the host intestinal function [161]. Moreover, OMVs are known to be strong immunomodulatory, so they can also be used as potent pathogen-free vaccines after modifying its immunogenic contents. Proteomic analysis of OMV has enabled researchers to identify new vaccine targets against various pathogens by increasing host immune responses. This includes neonatal meningitis *E. coli* (NMEC) [136], group A *Streptococcus* [160] and *Neisseria meningitidis* serogroup B [161].

Conclusion and Future Perspective

OMV proteomics gives a better and clear understanding of the role of various host micro-environments that contribute to the pathogen survival. Because of their role in communication between species, interaction of host and pathogen and adaptability to various environment conditions, OMV is potential source for the vaccine development. Isolation followed by purification of sufficient quantity of outer membrane vesicles from biological samples is the most crucial step for subsequent investigations such as quantitation and characterization. With the advancement in the proteomic methods significant progress has been made in the discipline of extracellular proteomics and OMV. Therefore, outer membrane and extra cellular proteomics has now become much more apparent as methods of choice for studying the pathogenesis of diseases, drug resistance, vaccine development, cancer biology, cell signalling etc. Various approaches [162-163] have been tried to develop effective drug against the pathogen and OMV proteomics can pay a significant role in this development. Study of these vesicles proteomics will also help in the better understanding of etiology of the diseases and hence help in the development of effective drug.

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Conflict of Interest

The authors have declared that no competing interests exist.

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