Bacterial outer membrane vesicles (OMVs)

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Outline

- Introduction of OMVs
- Formation mechanism and content of OMVs
- Entry mechanism into host cells of OMVs
- Functions of OMVs
- Application: OMV-based vaccines
- Conclusions and perspectives
Outer membrane vesicles (OMVs)

- **Bacterial OMVs**: nano-sized spherical structures 20-250 nm in diameter derived from the bacterial cell envelopes
- produced by almost all Gram-negative bacteria during all growth phases and in all environmental conditions
  - Pathogenic and nonpathogenic bacteria

TEM image of OMVs in *Aeromonas hydrophila*

META J. KUEHN AND NICOLE C. KESTY. GENES DEV, 2005
ANDREA GUERRERO MANDUJANO, ET AL. EC MICROBIOLOGY, 2015
How do bacteria produce OMVs?

----a general mechanism of OMVs formation
OMVs formation

**VacJ/Yrb transport system:**
- highly conserved in Gram-negative bacteria
- affects the migration of membrane phospholipids

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**VacJ/Yrb-mediated phospholipids migration**

Prevention of phospholipids accumulation in the outer leaflet of the outer membrane

**OMVs formation mechanism**

**Step 1:**
Inhibition of VacJ/Yrb system-related genes expression results in phospholipids accumulation in the outer leaflet of the outer membrane, thus initiates an outward bulging of the outer membrane through the asymmetric expansion of the outer leaflet.

**Step 2:**
Further enrichment of positive and negative curvature-inducing phospholipids in both leaflets supports the budding of the outer membrane, which finally pinches off to form an OMV.

**Step 3:**
The released OMV is enriched in phospholipids incorporated into the outer leaflet of the vesicle membrane.

Structure and content of OMVs

Detailed proteomic and biochemical analyses have shown that OMVs contain diverse components derived from the parent bacteria:

- lipopolysaccharide (LPS)
- periplasmic and membrane-bound proteins
- enzymes
- toxins
- DNA
- RNA

A secretion and delivery system

How do OMVs enter host cells?
Mechanisms of OMVs entry

Four main pathways of endocytosis have all been implicated in mediating OMVs entry into host cells.

Maria Kaparakis-Liaskos and Richard L. Ferrero.
Nature Reviews Immunology, 2015
Inhibition of OMVs entry

Pharmacological inhibition of key components of the endocytic pathways prevents OMVs entry:

Eloise J. O’Donoghue and Anne Marie Krachler.
Cellular Microbiology, 2016
Functions of OMVs in bacterial physiology and pathogenesis
Stress response >>>> bacterial survival
Nutrient acquisition >>>> bacterial survival
Defense and resistance >>>> bacterial pathogenicity
**Delivery of virulence factors >>>> bacterial pathogenicity**

Pathogenic bacteria can use OMVs to mediate the delivery of virulence factors, such as toxins, into host cells, including immune cells.
### OMVs-related virulence factors

<table>
<thead>
<tr>
<th>OMV-associated proteins</th>
<th>Species</th>
<th>Activity</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Apx toxin</td>
<td>Actinobacillus pleuropneumoniae</td>
<td>Hemolysis, Cytolysis</td>
<td>(59)</td>
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<tr>
<td>BabA, SabA</td>
<td>Helicobacter pylori</td>
<td>Adhasin</td>
<td>(60)</td>
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<tr>
<td>CagA</td>
<td>Helicobacter pylori</td>
<td>Cytotoxicity-associated immunodominant antigen</td>
<td>(61)</td>
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<tr>
<td>Cholera toxin (CTX)</td>
<td>Vibrio cholera</td>
<td>Adenylate cyclase activation</td>
<td>(62)</td>
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<td>Cif</td>
<td>Pseudomonas aeruginosa</td>
<td>Cystic fibrosis transmembrane conductance regulator (CFTR) inhibition</td>
<td>(63)</td>
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<tr>
<td>Cytotoxic necrotizing factor type 1 (CNF1)</td>
<td>Uropathogenic Escherichia coli</td>
<td>Cytotoxic</td>
<td>(66)</td>
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<tr>
<td>Gingipains (KgpA, KgpB, Kgp)</td>
<td>Campylobacter jejuni, Porphyromonas gingivalis</td>
<td>Trypsin-like cysteine proteinases</td>
<td>(22, 67)</td>
</tr>
<tr>
<td>Heat-labile enterotoxin (LT)</td>
<td>Enterotoxigenic Escherichia coli</td>
<td>Enterotoxic and vacuolating activities</td>
<td>(68)</td>
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<tr>
<td>HmuY</td>
<td>Porphyromonas gingivalis</td>
<td>Sequestering heme from host carriers</td>
<td>(19)</td>
</tr>
<tr>
<td>HtrAb</td>
<td>Borrelia burgdorferi</td>
<td>Proteolytic activity</td>
<td>(69)</td>
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<tr>
<td>IpaB, IpaC, IpaD</td>
<td>Shigella flexeri</td>
<td>Invasins</td>
<td>(70)</td>
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<tr>
<td>Leukotoxin (Ltx)</td>
<td>Aggregatibacter actinomycetemcomitans, Campylobacter jejuni</td>
<td>Pore-forming</td>
<td>(71, 72)</td>
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<td>NarE</td>
<td>Neisseria meningitidis</td>
<td>Iron-containing ADP-ribosyltransferase</td>
<td>(73)</td>
</tr>
<tr>
<td>OmpA</td>
<td>Aggregatibacter actinomycetemcomitans</td>
<td>Adhasin/invasion, immune evasion, biofilm formation</td>
<td>(71)</td>
</tr>
<tr>
<td>OspA, OspB</td>
<td>Borrelia burgdorferi</td>
<td>Outer membrane surface antigens</td>
<td>(74)</td>
</tr>
<tr>
<td>PaAP</td>
<td>Pseudomonas aeruginosa</td>
<td>Aminopeptidase</td>
<td>(36)</td>
</tr>
<tr>
<td>PagC</td>
<td>Salmonella enterica serovar Choleraesuis</td>
<td>Required for survival</td>
<td>(75)</td>
</tr>
<tr>
<td>PagJ, PagK1, PagK2</td>
<td>Salmonella enterica serovar Typhimurium</td>
<td>Required for survival</td>
<td>(45)</td>
</tr>
<tr>
<td>PorA</td>
<td>Neisseria meningitidis</td>
<td>Outer membrane protein (OMP) antigens</td>
<td>(73)</td>
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<tr>
<td>RTX (repeat-in-toxin) tox</td>
<td>Vibrio cholera</td>
<td>Cross-linking of actin cytoskeleton</td>
<td>(76)</td>
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<tr>
<td>Serralysin</td>
<td>Pseudomonas aeruginosa</td>
<td>Extracellular protease</td>
<td>(60)</td>
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<tr>
<td>Shiga toxins (Stx, Stx1, Stx2)</td>
<td>Pseudomonas aeruginosa, E. coli</td>
<td>Protein synthesis inhibition</td>
<td>(13, 77)</td>
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<tr>
<td>UspA1, UspA2</td>
<td>Moraxella catarrhalis</td>
<td>Surface adhesion protein</td>
<td>(39)</td>
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<tr>
<td>VacA</td>
<td>Helicobacter pylori</td>
<td>Vacuolating cytotoxin</td>
<td>(78)</td>
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<tr>
<td>α-Hemolysin (HlyC)</td>
<td>Enterohemorrhagic E. coli</td>
<td>Acytransferase inducing hemolysin</td>
<td>(68)</td>
</tr>
<tr>
<td>β-Lactamase</td>
<td>Pseudomonas aeruginosa</td>
<td>Antibiotics resistance</td>
<td>(79)</td>
</tr>
</tbody>
</table>

Over 30 virulence factors......
**association with biofilm formation >>>>> bacterial pathogenicity**

<table>
<thead>
<tr>
<th>Species</th>
<th>Factor</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>22-kDa protein</td>
<td>Plays an important role in biofilm formation.</td>
</tr>
<tr>
<td><em>Francisella</em></td>
<td>OMV</td>
<td>Involved in biofilm formation and forming part of biofilm matrix.</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>CPA</td>
<td>Its absence causes structural defects which limit the development of mature biofilms.</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>OMV-associated protein DegP</td>
<td>Required for the secretion of biofilm matrix components and the activity strongly influences biofilm formation.</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>OMV</td>
<td>Lead to an increased hydrophobicity of cells surface which enhanced their ability to form biofilms</td>
</tr>
</tbody>
</table>

OMVs have been shown to participate in biofilm formation.

Application: OMV-based vaccines

OMVs are attractive vaccine candidates:

1. high immunogenicity
   closely reflect the native conformation of their parent bacteria
   that induce both innate and adaptive immunity after entering
   eukaryotic cells
2. non-replicative and hence safe

✓ A success: OMV-based vaccine against serogroup B
   Neisseria meningitides (approved by the European Commission)
   ➢ The OMV-based 4CMenB vaccine contains three highly
     immunogenic proteins that induce protective antibody
     responses.
Conclusions and perspectives

1. The production of OMVs in Gram-negative bacteria allows bacteria to interact with a wide area of their environment.

2. OMVs play important roles in bacterial physiology and pathogenesis, ranging from secretion and delivery of biomolecules over stress response and biofilm formation to immunomodulation and adherence to host cells.

3. Due to the multifunctional activities, OMVs have been an attractive platform for bioengineering applications. (OMV-based vaccines, drug delivery vehicles)
Conclusions and perspectives

Some aspects of OMVs need to be illustrated

• What is the energy source for the vesiculation process in Gram-negative bacteria?
• How do bacteria regulate OMVs formation?
• How is protein cargo selected?

➢ Gram-positive bacteria also release membrane vesicles: *Staphylococcus aureus* and *Mycobacterium tuberculosis* ........

◆ biogenesis mechanisms?
◆ functions in bacterial physiology and pathogenesis??
Thank you