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Tiny but mighty: bacterial membrane vesicles in food biotechnological applications

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Membrane vesicle (MV) production is observed in all domains of life. Evidence of MV production accumulated in recent years among bacterial species involved in fermentation processes. These studies revealed MV composition, biological functions and properties, which made us recognize the potential of MVs in food applications as delivery vehicles of various compounds to other bacteria or the human host. Moreover, MV producing strains can deliver benefits as probiotics or starters in fermentation processes. Next to the natural production of MVs, we also highlight possible methods for artificial generation of bacterial MVs and cargo loading to enhance their applicability. We believe that a more in-depth understanding of bacterial MVs opens new avenues for their exploitation in biotechnological applications.

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Introduction

Production of extracellular membrane vesicles (MVs or EVs) is a conserved phenomenon in Eukarya, Archaea and Bacteria [1,2•]. Being lumen-containing spheres enclosed by lipid-bilayers, MVs are identified in different sizes ranging from 20 nm to 500 nm in diameter, and with various cargo components, including proteins, DNA, RNA, signalling molecules, and so on. Moreover, MVs are considered to have essential functions in cellular life, that is, cell-to-cell communication, competition, survival or stress response of cells [3•,4–6].

In bacteria, MV production was first observed in Gram-negatives over fifty years ago, and there have been an increasing number of studies performed on these MVs in recent decades [2•]. Gram-negative bacterial MVs are often referred to as outer-membrane vesicles (OMVs), since they are derived from the outer membrane of the bacteria via budding processes [7]. By contrast, due to the presence of a thick cell wall in Gram-positive bacteria, MV production was expected to be absent. This assumption resulted in a three-decade delay in the discovery of Gram-positive MVs compared to their Gram-negative counterparts, although in recent years the evidence of MV production in Gram-positive bacteria accumulated rapidly [2•,3•].

Recent advances in the study of MV production in Gram-positive bacteria are particularly interesting for the food biotechnological field. The Gram-positive lactic acid bacteria (LAB) are key players in various food and feed fermentation processes as starter cultures, probiotics and producers of vitamins [8,9]. Among these, evidence has so far been collected for MV production from *Lactococcus lactis* (S. Alexeeva *et al.*, unpublished) and *Lactobacillus plantarum* [10••]. *Bacillus subtilis* was shown to secrete MVs as well [11], and certain strains of *B. subtilis* are involved in the production of fermented soybeans known as 'natto' while boosting this food product with high levels of vitamin K2 [12]. Among Gram-positive Actinobacteria, species of the genus *Bifidobacterium* were associated with probiotic effects as well as with MV production [13••].

To date, comprehensive studies have been mostly focusing on MVs produced by pathogenic bacteria [14•,15–22]. One of the best-identified functions of MVs is indeed associated with pathogenesis, where they serve as the vehicles for delivering virulence factors and toxins to the host cells [3•,5,22]. As MVs are able to trigger responses of the host immune system, the application of vaccines against meningitis was realized using OMVs derived from the Gram-negative bacterium *Neisseria meningitidis* [23]. The potential of vaccine development based on Gram-positive MVs was recognized as well: vaccinations with MVs derived from *Clostridium perfringens*, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis* and *Staphylococcus aureus* were demonstrated to be effective in mice models against infections by respective bacteria [24–27]. However, the possibility of applying microbial MVs in other areas of biotechnology remains largely unexplored. In this paper, we provide an overview on current understanding of microbial MVs, with a focus on potential applications of MVs from food-associated bacteria.

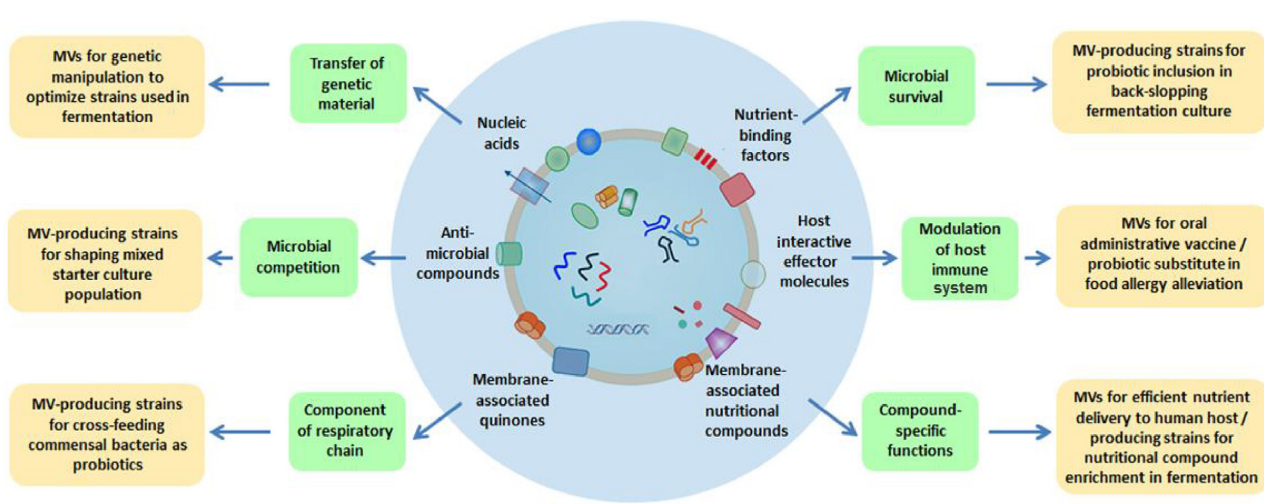
Natural functions and biotechnological applications of bacterial MVs

The composition of various MVs has been studied and has supported the formulation of hypotheses regarding their natural function. Although so far the studies of Gram-positive MVs are not as extensive, the available data already suggest that Gram-positive MVs have similar roles to their Gram-negative counterparts [28]. This knowledge served as a basis for us to propose potential applications of microbial MVs in food biotechnology, in which Gram-positive bacteria are intensively involved (Figure 1).

Nucleic acids, including chromosomal DNA, extracellular DNA, plasmids, phage DNA, rRNA, tRNA, mRNA and intragenic RNA species are frequently found as cargo in bacterial MVs (Figure 1) [16,31–34]. MVs protect the nucleic acids from degradation by nucleases and are proposed to facilitate horizontal gene transfer in the microbial community. Gene transfer by MVs within or between species was demonstrated in various genera of Gram-negative bacteria and certain Gram-positive species [35,36]. As only a number of genes were retrieved from the MVs, the incorporation of nucleic acids into MVs might be a selective mechanism instead of a random process [20], although extra assessments are needed for a conclusive statement. In this regard, MVs may provide the opportunity to deliver designed or natural (large) DNA or RNA molecules to recipient bacteria in a controlled manner. Similarly, membrane-derived vesicles have been considered as novel tools for delivering therapeutic genome editing elements (i.e., CRISPR/Cas cassettes) to human cells [37]. Interestingly, natural MV-mediated gene transfer is not considered to result in genetically modified organisms (GMOs) under current legislation (Directive 2001/18/EC [38]), and is therefore especially interesting for food biotechnological applications. MV producing strains with favourable traits could be used to naturally transfer nucleic acids to recipient bacteria, providing them resistance to environmental stresses or bacteriophages, enhanced productivity and so on, thus optimizing strains used in starter cultures and other fermentation processes (Figure 1).

In addition to nucleic acids, MVs can also carry other cargo that as well plays a role in microbial competition, survival and fitness increase in a microbial community (Figure 1). Antimicrobial compounds and bacteriolytic enzymes including autolysins and extracellular bacteriolytic enzymes, carried by MVs of particular bacterial species are suggested to exert an inhibitory effect on sensitive co-existing bacteria or fungi [14•,39,40]. MVs derived from several species were found to contain factors such as iron-binding proteins, contributing to increased survival of bacteria under nutrient limiting conditions [41,42]. A few cases also revealed a protective effect of MVs that carry enzymes which degrade certain antimicrobial compounds [3•,43,44]. This type of protection can also benefit other bacterial species in the same community. Moreover, bacterial cross-feeding through trafficking of membrane-associated compounds, that is, vitamin K2 (menaquinone), has been observed between *L. lactis* and another species [45]. In bacteria, menaquinones function as electron carriers in the membrane-embedded respiratory electron transport chain [46]. As a result of menaquinones cross-feeding, the respiratory chain was completed in the recipient bacteria resulting in stimulated growth [45]. Although the cross-feeding mechanism

Figure 1



Microbial MV cargos, functions and potential applications. Examples of MV cargos (blue circle), corresponding natural function of the MV carrying the cargo (green box) and proposed applications of the MVs or MV-producing strains (yellow box) are presented. MV scheme modified from EVpedia [29••,30].