Eco-evolutionary feedbacks mediated by bacterial membrane vesicles

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One sentence summary: This review aims to highlight the role of the bacterial membrane vesicles in different eco-evolutionary processes in which bacteria are subjects and operators.

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ABSTRACT

Bacterial membrane vesicles (BMVs) are spherical extracellular organelles whose cargo is enclosed by a biological membrane. The cargo can be delivered to distant parts of a given habitat in a protected and concentrated manner. This review presents current knowledge about BMVs in the context of bacterial eco-evolutionary dynamics among different environments and hosts. BMVs may play an important role in establishing and stabilizing bacterial communities in such environments; for example, bacterial populations may benefit from BMVs to delay the negative effect of certain evolutionary trade-offs that can result in deleterious phenotypes. BMVs can also perform ecosystem engineering by serving as detergents, mediators in biochemical cycles, components of different biofilms, substrates for cross-feeding, defense systems against different dangers and enzyme-delivery mechanisms that can change substrate availability. BMVs further contribute to bacteria as mediators in different interactions, with either other bacterial species or their hosts. In short, BMVs extend and deliver phenotypic traits that can have ecological and evolutionary value to both their producers and the ecosystem as a whole.

Keywords: bacterial membrane vesicles; ecology; evolution

INTRODUCTION: ‘EVERYTHING IS EVERYWHERE, [BUT] THE ENVIRONMENT SELECTS’

What goes on in nature, according to the father of comparative biochemistry Albert Jan Kluyver, can be explained by the rules established by the founder of the Delft School Martinus Willem Beijerinck (Van Iterson, De Jong and Kluyver 1940; Kluyver 1953).

These rules, summarized by another Delft scholar, Baas Becking, state that ‘everything is everywhere, [but] the environment selects’ (Becking 2016 [1934]). The rules represent the relationship of bacteria with their milieu cosmique as a result of their different trophic modes, combined with different adaptive traits (i.e. phenotypes whose values increase the fitness of the bacteria in a given environment). Not only has this combination allowed bacteria to inhabit virtually all the ecosystems of our planet, but
it has also provided bacteria with the capacity to modify the structure and function of the different ecological systems they inhabit (Van Iterson, De Jong and Kluyver 1940; Kluyver 1953; Becking 2016 [1934]).

Based on this relationship, Kluyver suggested that the bacterial adaptation to a change in environmental conditions or nutrients is ‘an evaluation of the actual situation as [it results from] evolution’ (Kluyver 1953). If inherited, that change will lead to a stepwise evolution due to mutation and selection; in other words, he suggested the link between bacterial adaptation and the causes of evolution (Kluyver 1953). The phenotypic and genetic diversity found among different taxa, the short generation time and existence in vast populations all provide bacteria with the opportunity for rapid evolutionary changes that can occur at contemporary time scales (Bohanan and Lenski 2000; Hansen et al. 2007; Heilmann, Sneppen, Krishna 2010; Andrade-Dominguez et al. 2014). Bacteria can also enter a non-evolving state in the form of persisters, spores and viable but non-culturable bacteria (Bohanan and Lenski 2000; Lewis 2010; Oliver 2010). The fast-evolving and non-evolving states of bacteria can be a direct, simultaneous and non-result from both ecology affecting evolution (i.e. the survival of the conditionally fittest) and evolution affecting ecology (i.e. the structure of ecosystems), collectively referred to as eco-evolutionary (or ‘eco-evo’) feedbacks. Besides being subject to eco-evolutionary cycles, bacteria can also be the operators of these cycles. A group of examples demonstrates the importance of the microbiota as drivers of the eco-evo developmental processes of the multicellular organisms: symbionts can help with morphogenesis and species maintenance, provide selectable allelic variation or potentiate reproductive isolation (Gilbert, Bosch and Ledon-Rettig 2015). Finally, whether the bacterial symbionts may also trigger contemporary transitions toward multicellularity, such as in the Choanoflagellates (Alegado et al. 2012; Gilbert, Bosch and Ledon-Rettig 2015), is part of a much bigger and unsettled question on the role of the symbionts in ‘evolutionary progression’ from unicellular organisms to multicellularity.

The extracellular bacterial membrane vesicles (BMVs) hold an interesting place in biology. They are currently viewed as extracellular spherical structures, with an average diameter between 20 and 250 nm, in which a hydrosol is enclosed by one or two biological membranes. Their structure is indirectly encoded and is the result of the assembly of their biological membrane, a phospholipid (PL) bilayer carrying embedded membrane proteins, and the accumulation of the vesicular cargo. Not only can bacteria release these subcellular structures, but they can also integrate naturally selected functionalities in their vesicles as a response to environmental (extrinsic) stimuli or to intrinsic factors. Thus, the secreted BMVs extend and deliver ‘phenotypic traits’ that can have adaptive value to the individual organism, its population, and to the ecosystem as a whole.

This review, which follows the same approach outlined by these Delft-based scholars, presents the role of BMVs in different eco-evo dynamics. The specialized functions mediated via BMVs depend on the type of bacteria and their evolutionary history, as well as on the bacterial population’s specific state and ecological niche. For this reason, we will first briefly outline certain evolutionary aspects; in the second part of the introduction we will then consider the different aspects of BMV biogenesis.

Lessons from the ‘pre-cells’ and various evolutionary aspects linked to BMVs

Beijerinck’s rules bear another deep meaning: the environment was what also selected when, where and how life once began. According to the notable biochemist Alexander I. Oparin, life on earth began with the formation of coacervates (the original spelling referring to Oparin’s work was “coazervates”), or ‘pre-cells’: vesicles with a certain degree of individuality due to their spatially concentrated organic substance, isolated from the surrounding environment by a semi-permeable membrane (Oparin 1961 [1924]). This membrane has opened the door to the existence of unique assemblies of organic and inorganic molecules, and for their possibility to interact with each other (Oparin 1961 [1924]). To achieve this degree of individuality, the coacervates had to obey the laws of physics and chemistry, as well as those of biology (Oparin 1961 [1924]). And since life presumably started with the formation of vesicles, the logical continuation of this supposition is that life is to be also maintained by vesicles.

An interesting parallel may be drawn between BMVs and coacervates, because both exist on the border of living and non-living biological structures. We will present the basic characteristics of BMVs through previous studies obtained from evolutionary experiments on lipid vesicles that lack the protein component of the biological membrane. These characteristics both provide clues about the origin of life and explain the functionalities brought by the lipid component of the secreted BMVs. To self-maintain in the environment, the pre-cells have a certain degree of stability because of their membranes’ composition of mixed amphiphilic and hydrophobic lipids (Namani and Deamer 2008; Jordan et al. 2019). The evolutionary experiments that Szostak and Luisi conducted in their respective laboratories have demonstrated that the fatty acid vesicles are capable of primordial quasi-biological processes (such as growth, division, environmental interaction and intra- and intervesicular competition for resources), which eventually may have led to primeval natural selection for stable vesicle populations with heterogeneous lipid membranes over others with homogeneous membranes (Berclez et al. 2001; Cheng and Luisi 2003; Hanczyc, Fujikawa and Szostak 2003; Chen and Szostak 2004; Chen, Roberts and Szostak 2004; Luisi et al. 2004). Researchers have also suggested that the osmotic pressure exerted by the vesicular aqueous phase and its content on the composite membrane led to the redistribution of the lipid species that define membrane asymmetry, and size and curvature of the vesicle (Fig. 1A, Box 1)—all of which represent important factors for lipid-facilitated vesicular fusion and fission (Cheng and Luisi 2003; Chen, Roberts and Szostak 2004). As with any physical object, the membrane vesicles are characterized by properties, including size, shape and the total energy of the system, which can be transformed for processes such as fusion and fission (Fig. 1A). In Box 1, we briefly outline the physical properties of the vesicles to help demonstrate the bacterial vesicle release as a spontaneous, indirectly energy-dependent process that can be further controlled by the state of the bacteria and the external environment.

Box 1. Physical properties of the vesicles

For most of the history of science, physicists have been fascinated by the way nature integrates complex physiological processes in simple figures (Thompson 1992 [1917]). The

![Figure 1A](https://example.com/figure1a.png)

**Box 1.**

**Physical properties of the vesicles**

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Life likely started under hydrothermal and chemiosmotic conditions (such as those found in black smokers and/or Lost City systems) that consecutively allowed for chemical, biochemical, biological and ecosystem evolution that then developed the tree of life (Baross and Hoffman 1985; Martin et al. 2008). Even though some biogenic activities may have existed 4.1 billion years (Byr) ago (Bell et al. 2015), based on fossils records, primordial life presumably did not arise until 3.5 Byr ago (Tashiro et al. 2017). Since recent phylogenetic studies have placed the eukaryotes in a monophyletic group with Lokiararchaeota (Domain Archaea), the tree of life most probably was developed by the prokaryotes—Domain Archaea and Domain Bacteria (Cavalier-Smith 2002; Yutin et al. 2008; Williams et al. 2012; Spang et al. 2015; Eme et al. 2018; Williams et al. 2020). (Phyla appear in bold text in this review.)

The ancient bacteria appeared around 3.5 Byr ago, based on Cavalier-Smith’s (2002) theory, the microfossil records and the presence of deep-sea vent phototrophic bacterial life, and are presumed to have been anaerobic green (sulfur) photosynthetic with a double-membrane envelope, porins, acylster phospholipids and a chromosome (Ochman and Wilson 1987; Rasmussen 2000; Cavalier-Smith 2002; Beatty et al. 2005; Wacey et al. 2011). The lineages of the deep-branching bacteria Aquifex and Thermotoga may have appeared 3.5–3.3 Byr ago, while the land colonizers (Actinobacteria, Deinococcus-Thermus and Cyanobacteria) branched off 2.5–3 Byr ago from the lineages that also produced the Proteobacteria and other bacterial phyla (Feng, Cho and Doolittle 1997; Hedges et al. 2001; Sheridan, Freeman

Figure 1. Physical properties of the vesicles (A) (see also Box 1) and the proposed mechanisms for vesicle genesis—single cell-based vesicle release (B, C), and cell death-mediated vesicle release (D, E). (A) The radii (R1, R2) and the curvatures (C1, C2) of a centrally symmetric spherical system. (B) The process of vesicle release (modified from Kühl and Kuehn 2010). Phase I represents the bulging of the outer membrane (OM) due to membrane-bending proteins (blue cylinder), to the presence of non-bilayer lipids (blue eclipse) or to a change in the osmotic pressure in the presence of selected compounds (red circles). Phase II represents the release phase in which the membrane tension and the osmotic pressure of the bilayer and the vesicle are balanced. (C) A hypothetical model for the outer-inner membrane vesicle (O-IMV) biogenesis based on Kadurugamuwa and Beveridge (1995) and Pérez-Cruz et al. (2013). The bulging region that involves the two membranes is stabilized by the rigidity of the mature lipopolysaccharide (LPS; dark red) and results from a process that involves the formation of close contacts between the IM and OM (step I) surrounding a region on which the cytoplasmic osmotic pressure can act (step II). Upon close proximity of the two zones of close contact, the presence of bilayer-disturbing amphiphiles leads to a ‘recombination event’ between the PLs of the two zones, which is resolved in the release of an O-IMV (step III). (D) Explosive cell death-mediated vesicle release based on Toyofuku et al. (2016). It takes place within a bacterial community, e.g. biofilm (panel I), and involves the production of a holin/endolysin dyad (panel II), holins are represented with blue and purple squares, while the endolysin is shown as a red packman) that acts upon the cell wall of the bacteria resulting in their rounding (panel II), followed up by their ‘explosive’ death that results in the release of membrane fragments that can form vesicles (panel III). (E) Prophage-triggered vesiculation based on Toyofuku et al. (2017). The mechanism also takes place in a bacterial community (panel I) and involves the production of holin/endolysin dyad (holins are represented with blue and purple squares, while the endolysin is shown as a red packman) that disrupts the membrane potential and osmotic integrity of the bacterial membrane (panel II) resulting in vesiculation without cell rounding and in the death of the bacteria (panel III).

form of the vesicle can be described in a centrally symmetrical system with curvature radii (R1, R2) of the two perpendicular curvatures (C1, C2), as shown in Fig. 1A. When the force lines in all axes of the vesicle are equally distributed, the shape becomes spherical (R1 = R2); see Fig. 1A. In terms of physics, membrane vesicles are often thought of as structures in which a 2D membrane encloses a 3D aqueous phase. Membrane vesicles have also been defined as low-energy shapes (E0,min) that depend on the bending energy (E0) constrained by a given area (A), the enclosed volume and the mean curvature. The link between the shape and energy, here for simplicity E0,min can be expressed with the simplified version of Helfrich’s equation, where k stands for the static bilayer bending rigidity: E0,min = (k/2) \int dA(C1 + C2)^2.

For further details, see Seifert et al. (1991) and Jarić et al. (1995). Overall, the E0,min is maintained by the balanced interaction of the membrane tension and the osmotic pressure of the aqueous phase exerted on the membrane. If a change is introduced (due to molecular crowding or lipid clustering, for example), in order to restore the mechanical equilibrium, the vesicle may enter into another E0,min state by changing shape in this transition from spherical to a dumbbell, pear-like, oval or tubular shape (Seifert et al. 1991; Jarić et al. 1995). The other option for this system is to change: it can either increase its volume or undergo processes such as fusion or fission, such as by vesicle release (Fig. 1B).
and Brenchley 2003; Battistuzzi, Feijao and Hedges 2004). The prokaryotes maintained life and evolution on their own for almost 2 Byr until the emergence of the eukaryotes, with which they have co-existed ever since (Ochman and Wilson 1987; Schopf 1994).

For all this time, the membrane vesicle release may have remained a common feature of all domains of life (Deatherage and Cookson 2012; Gill, Catchpole and Forterre 2019). And the contribution of how vesicle release evolved—combined with the drastic changes in cell wall structure, membranes and secretion machineries—needs to be considered as a feature of prokaryotic evolution, among other factors (Cavalier-Smith 2002; Makarova et al. 2010; Gould, Garg and Martin 2016).

**Single cell-based, bona fide, biogenesis and cell death-mediated formation of BMVs**

The type of secreted BMVs depends on bacterial physiology and the cell envelope—a mechanical structure dynamically composed of extracellularly sugar-coated biological membrane(s). The cell envelope defines the borders of bacteria as discrete biological units, provides them with physicochemical plasticity and is the frontline of interaction between the cells and the environment. The cell envelope is also where BMV biogenesis takes place.

A typical bacterial cell may have a Gram-positive or Gram-negative organization of its cell envelope. The cellular content of both types is delineated by a cytoplasmic membrane (CM). The lipid bilayer of the CM exists in a dynamic liquid-crystalline state that is often (but not always) maintained by the composition of the phosphatidylethanolamine (PE), lyso-PE, phosphatidyglycerol (PGl) and cardiolipin (CL), as well as by the type of acylsterified fatty acids (Morein et al. 1996; Parsons and Rock 2013; Sohlenkamp and Geiger 2016; Rajagopal and Walker 2017). In the case of Gram-positive cell wall, on the outer side the CM carries a relatively thick peptidoglycan (PG) composed of 25–30 layers of linear polysaccharide chains of N-acetylglucosamyl-β-1,4-N-acetylmuramyl parental unit cross-linked via oligopeptide chains (Schleifer and Kandler 1972; Vollmer, Blanot and de Pedro 2008; Rohde 2019). Recent atomic force microscopy experiments with Staphylococcus aureus and Bacillus subtilis have shown that the PG of the Gram-positive bacteria displays two distinct architectures: the inner PG layer is denser, while the mature outer layer represents a disordered gel carrying large (around 60 nm in diameter) and up to 23 nm deep pores (Pasquina-Lemonche et al. 2020). Exterior to the CM, a typical Gram-negative cell wall is surrounded by an outer membrane (OM) (Beveridge 1981; Silhavy, Kahne and Walker 2010).

Most of the extracellular leaflet of the OM is built by lipid A—the hydrophobic anchor of the lipopolysaccharide (LPS) molecular complex—linked to a glycan polymer via a core oligosaccharide (Nikaido 2003; Kalnychny, Morona and Cygler 2014; Putker, Bos and Tommassen 2015; Henderson et al. 2016). The inner leaflet of the OM often contains relatively large amounts of PGl, lyso-PE, CL and relatively little PE compared with the IM (White, Lennarz and Schnaitman 1972; Hoekstra et al. 1976). Kinetic experiments with radioactively labeled LPS from Escherichia coli have demonstrated that the LPS distribution in the OM is non-random: the mature LPS clusters in rigid localized domains, while the newly produced cluster is inserted at specific zones characterized by increased fluidity (Leive 1977). The difference between the inner and outer phospholipid layers of the OM defines the OM asymmetry, which protects the bacteria from the dangerous activity of some lipophilic and polarly charged molecules and allows the passage of the useful molecules (Nikaido 2003; Henderson et al. 2016). Upon exposure to membrane-disrupting agents, such as chelators or antimicrobial peptides, bacteria maintain OM integrity by incorporating PLs instead of LPS on the OM (Nikaido 2003). To prevent the incorporation of PLs instead of LPS into the external OM layer and thus maintain OM asymmetry, bacteria use the Mla (Maintenance of OM/lipid asymmetry) system, which is an ABC transporter that in a retrograde fashion flips the surface-exposed PLs back into the inner leaflet of the OM (Malinverni and Silhavy 2009; Henderson et al. 2016).

Experiments with fluorescence probes in E. coli have further demonstrated that the OM is more rigid than the IM (Overath and Trauble 1973; Chang, Thomas and Kulpa 1974; Leive 1977; Nikaido 2003). While the proteins represented in the OM are predominantly α-helical, the OM is embedded with trans-membrane β-barrel proteins collectively referred to as outer membrane proteins (OMPs). Most OMPs function as porins, allowing the passive diffusion of different metabolites, ions and other compounds, but they can also have other properties, for example by serving as receptors or facilitating adhesion (Beveridge 1981; Silhavy, Kahne and Walker 2010). The PG layer of Gram-negative bacteria is thin (1–3 layers) and is located between the IM and the OM in an oxidizing, ATP-devoid environment called the periplasmic space (Beveridge 1981; Silhavy, Kahne and Walker 2010). Some OMPs, such as OmpF and OmpC, are embedded only within the OM, while others either interact with the PG layer (e.g. OmpA and Lpp), or, as is the case with the Tol-Pal system, they interact with the PG and the IM (Braun and Sieglin 1970; De Mot and Vanderleyden 1994; Clavel et al. 1998; Walburger, Lazardnzi and Corda 2002; Cascales and Llob`es 2004; Parsons, Lin and Orban 2006). Electron microscopy (EM) studies with E. coli and Salmonella enterica demonstrated the existence of membrane adhesion zones where the OM comes in close proximity to the IM, known as ‘Bayer bridges’ (Bayer 1968, 1991; Crowlesmith, Schindler and Osborn 1978; Smit and Nikaido 1978; Lopez and Webster 1985; Bayer and Bayer 1986; Walderich et al. 1988; Leduc and Frehel 1990). The number of these bridges varies between 200 and 400 per cell and increases when the bacteria are challenged with UV irradiation or when they are under attack by certain bacteriophages (Walderich et al. 1988; Bayer 1991). Although Kellenberger (1990) has challenged the existence of Bayer bridges, others have suggested that they serve as zones where the transportation of OM components (such as LPS, capsular polysaccharide and OMPs) as well as the attachment and/or release of some bacteriophages, all take place (Smit and Nikaido 1978; Lopez and Webster 1985; Bayer and Bayer 1986; Kellenberger 1990; Henderson et al. 2016).

Reasoning from the fact that adaptations for the benefit of a community may markedly differ from adaptations for the benefit of an individual, we employ two approaches to define vesiculation and its mechanisms. The first approach is single cell-based and considers the BMV release by individual free-living bacteria, which eventually leads to the preservation of the integrity of the bacterium and its vesicles (Fig. 1B and C). While the first type of vesiculation implies that the BMV release is not adapted for death of the bacterium, the second approach involves vesiculation that results from a genetically encoded cell death (CD) triggered in a subpopulation sacrificed for the survival of the community (Fig. 1D and E).

Based on previous studies on vesicle release, pre-cell models and membrane biochemistry, the following description is
how we envision vesicle release, i.e. bona fide vesicle biogenesis, according to the single cell-based approach (Fig. 1B and C).

For a BMV to be released, there must be an extracellular protrusion of PG-detached membrane areas, as shown in Fig. 1B (Kulp and Kuehn 2010). The protrusion may be triggered by a change in membrane curvature due to (i) molecular crowding of membrane-bending proteins, (ii) curve-introducing lipids (e.g. in the presence of non-bilayer lipids such as CL or lipids with small polar heads such as PE) or (iii) a local imbalance between the membrane tension and the osmotic pressure exerted by the aqueous phase of the vesicular lumen that forms, enriched in certain components; see Fig. 1 and Box 1 (Chamberlain et al. 2003; Chen, Roberts and Szostak 2004; Yeagle 2016). These events can be linked, or they can take place separately or all together, but eventually they lead to an imbalance between membrane tension and osmotic pressure that can become a driving force of energy for membrane fission (vesicle release) in an ATP/GTP-independent manner, thus preserving cellular and vesicular integrity (Fig. 1 and Box 1). The complexity of the Gram-negative cell wall is transferred onto the types of BMVs released by these bacteria. If the interface for vesiculation occurs between the periplasm and the OM, then the Gram-negative bacteria secrete outer membrane vesicles (OMVs), and if the interface involves the periphery of the cytosol, the IM, the periplasm and the OM, then bacteria can release outer-inner membrane vesicles (O-IMVs) (Kadurugamuwa and Beveridge 1995; Perez-Cruz et al. 2013; Sjostrom et al. 2015) as depicted in Fig. 1B and C. For clarity, even though most studies have been conducted with OMVs, we will refer to them collectively as BMVs throughout this review; if exceptions exist (e.g. a release of O-IMVs) we will point out which kinds of vesicles a particular bacterium releases.

The outcome of the single cell-based vesiculation is directly related to the maintenance of the integrity of the membrane dynamics. The BMVs can be released to counterbalance the excessive growth of the cell wall during normal bacterial growth, during protein and/or RNA restrictions or during the ingrowth of the division septum (Knox, Vesik and Work 1966; Burdett and Murray 1974; Hoekstra et al. 1976; Mug-Opstelten and Witholt 1978; Gamazo and Moriyon 1987; Cusnie, O’Driscoll and Lamb 2016). Bacteria can also employ the BMVs to maintain the asymmetry of their OM when their Mla system malfunctions or is downregulated, for example under iron restriction, leading to the incorporation of Fts in the outer leaflet of the OM (Roier et al. 2016; Zingl et al. 2020). The bacteria can also release misfolded proteins packed within BMVs as an envelope stress (McBroom and Kuehn 2007). Besides the benefits to the individual cell, the BMVs as a result of single cell-based vesiculation have been found to play a role in bacterial survival during environmental stress, lateral gene transfer (LGT), the regulation of microbial interactions within bacterial communities and bacteria-host interactions (Mayrand and Grenier 1989; Kulp and Kuehn 2010; Schwechheimer and Kuehn 2015).

The CD-mediated mechanisms are the most extreme case of vesiculation since these mechanisms are the result of cell lysis (Turnbull et al. 2016; Toyofuku et al. 2017) and depicted in Fig. 1D and E. Besides living in solitary conditions, 40–80% of the bacteria, as Flemming and Wurzert (2019) have calculated, also co-exist while organized into matrix-enclosed multicellular communities of differentiated cells, called biofilms (van Gestel, Vlamakis and Kolter 2015; Flemming and Wurzert 2019). Due to the matrix secreted by the bacteria, biofilms bring the cells in close proximity (thus allowing for social interaction), shield them from environmental dangers and make different concentrated nutrients, signaling molecules and LGT agents available to the community (van Gestel, Vlamakis and Kolter 2015; Flemming and Wurzert 2019). In such multicellular organizations, death becomes a common regulatory mechanism that ends certain bacterial lineages (for example, to abort the spread of phages while in their premature stages) as well as further supplying the biofilm with extracellular polymeric substances (DNA, proteins and exopolysaccharides), nutrients and lipids (Allocati et al. 2015). The CD-based vesiculation is based on the established relationship between the bacteria and some of their temperate bacteriophages existing as prophages (Turnbull et al. 2016; Toyofuku et al. 2017). This type of vesiculation involves the activity of the prophage-encoded holin–endolysin dyad (Turnbull et al. 2016; Toyofuku et al. 2017).

Holins are small hydrophobic pore-forming peptides that oligomerize within the CM and, upon pore formation, allow the endolysins (PG hydrolases) to escape and cleave the PG, followed by cell lysis (Catalao et al. 2013). This cell lysis delivers exogenous DNA and membrane fragments that spontaneously form BMVs used for the mechanical maintenance of the biofilm structure (Turnbull et al. 2016). The mechanism underlying this type of vesiculation is RecA-dependent and can be induced either spontaneously or by agents that trigger the SOS response (Turnbull et al. 2016). This kind of vesiculation is described as ‘explosive cell lysis’ (schematically present in Fig. 1D) in the case of Pseudomonas aeruginosa the genome of which carries P2- and λ-like prophages coding for the R2 and F2 bacteriocins (pyocins), respectively (Nakayama et al. 2000; Turnbull et al. 2016). Such kind of vesiculation involves the expression of the lys gene (which codes for an endolysin) from the prophage gene cluster (Fig. 1D, panel II), which in turn cleaves the PG, thus causing cell rounding that then leads to cell lysis in a subpopulation of bacteria (Fig. 1D, panel III) in interstitial biofilms of P. aeruginosa (Turnbull et al. 2016).

The other type of prophage-triggered vesiculation has been described in the case of B. subtilis (Toyofuku et al. 2017). It is represented schematically in Fig. 1E. It involves the expression of the XhIAB–XlyA (holin–endolysin) dyad encoded by the PBSX prophage (Toyofuku et al. 2017). Unlike the explosive cell lysis during which bacteria become spherical, the prophage-triggered vesiculation in B. subtilis does not lead to morphological changes of the bacteria, and the BMVs are released via vesiculation of the CM, the vesicles of which are released through the pores introduced in the PG by the XlyA activity (Turnbull et al. 2016; Toyofuku et al. 2017); for clarity, see Fig. 1D and E. The death of the cell is presumably caused by the disrupted membrane potential due to pores formed by the holin, which then leads to the loss of membrane integrity (Catalao et al. 2013; Toyofuku et al. 2017).

**THE ROLE OF BMVs IN BACTERIAL ECOLOGY**

In the previous section, we outlined the bacterial diversity—generated and preserved by various ecological, evolutionary and geological processes—that has resulted from successive selections in different extinct and extant environments. While we also introduced the dynamics of BMVs, in this one we will outline some of the specific roles of BMVs in bacterial ecology. We will also propose eco-evo feedback mechanisms in which BMVs may be active players.

As a general rule, the populations of every species have defined tolerances to, and requirements for, certain environmental parameters that together represent the ecological niche.
of the species (Begon, Townsend and Harper 2005). Being a resident in a particular habitat requires several factors: (i) a specific metabolism for a place in the food web, (ii) adaptation to the environmental physicochemical conditions and (iii) the means for competition or co-existence with other organisms (Kassen and Rainey 2004; Schlegel and Jannasch 2006; Zavarzin 2006). To achieve all of the above, bacterial populations, as is the case with individual bacteria per se, may develop a certain degree of individuality shaped by the expression of different systems for substrate utilization, by the production of extracellular organelles for attachment or motility and by the development of defensive mechanisms against environmental stress, predators and competitors (Kassen and Rainey 2004; Schlegel and Jannasch 2006). Ecosystems of strongly restrictive environmental characteristics, such as the Atacama lithic habitats, acid mines, black smokers and the Deccan traps, thermal springs, soda lakes and aquatic cold environments, are inhabited by a few bacterial species present in relatively large numbers, while ecosystems of fluctuating physicochemical and nutrient conditions, such as certain plant and animal hosts, soils and water ecosystems, among others, may be inhabited by a variety of bacterial species with individuals present in different numbers (Johnson and Hallberg 2003; Schlegel and Jannasch 2006; Ward 2006; Lozupone and Knight 2007; Mouchka, Hewson and Harvell 2010; Margesin and Miteva 2011; Johnson 2012; Huang et al. 2013; Sorokin et al. 2014; Jebbar et al. 2015; Gomez-Silva 2018; Cordovez et al. 2019; Dutta et al. 2019). The trend may be exemplified by a study that demonstrated the presence of 3560 operational taxonomic units (OTUs) in soil samples isolated from favorable conditions compared with the OTUs detected in sea ice isolates or acidic environments, which were found to range from 239 to 78 OTUs (Lozupone and Knight 2007). Other ecosystems exist with favorable environmental conditions and a constant flux of nutrients, such as the gastrointestinal ecosystems of different homeothermic animal hosts (Lozupone and Knight 2007; Oakley et al. 2014; Nishida and Ochman 2019).

‘Everything is everywhere, but the environment selects’ is also a good start for this part of the review. The idea can be interpreted from the perspective of the BMV release: every bacterium releases BMVs, but the environment may select the type, content and number of the BMVs (Mayrand and Grenier 1989; Beveridge et al. 1997; Kulp and Kuehn 2010; Manning and Kuehn 2013; Kulkarni and Jagannadham 2014; Schwechheimer and Kuehn 2015; Orench-Rivera and Kuehn 2016; Eberlein et al. 2018).

Figure 2 presents the relationships of the main bacterial species considered in this review, combined with their physiological properties and niche preferences. These species have a combination of adaptive phenotypes that provide a certain degree of individuality that stands out when we compare a species with the closely related species shown in Fig. 2 (for example, Vibrio cholerae bacteria are mesophilic, while the V. rumoiensis cells are psychrophilic). We can reason that this degree of individuality can be transferred to the BMVs, the different components of which may contribute to adaptations to different environmental factors, such as by a combinatorial result of the membrane structure and the luminal content. Thus, the secreted BMVs can serve as a multifunctional delivery system through which bacteria can respond efficiently and readily to different environmental changes (Mayrand and Grenier 1989; Beveridge et al. 1997; Kulp and Kuehn 2010; Manning and Kuehn 2013; Kulkarni and Jagannadham 2014; Schwechheimer and Kuehn 2015; Orench-Rivera and Kuehn 2016; Eberlein et al. 2018).

The production of BMVs also provides some case-study evidence that can lead to a better understanding of ecosystem engineering: a process that contributes to the modification of the physical environment (Wright and Jones 2006). Ecosystem/ecological ‘engineers’ are what Jones, Lawton and Shachak (1994) define as ‘organisms that directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials. In so doing, they modify, maintain or create habitats through a process known as ecosystem engineering (Jones, Lawton and Shachak 1994; Wright and Jones 2006). The BMVs are involved in the structure and maintenance of different biofilms, as reviewed in several previous works (Beveridge et al. 1997; Schooling and Beveridge 2006; Kulp and Kuehn 2010; Tashiro, Uchiyama and Nomura 2012; Manning and Kuehn 2013; Brown et al. 2015; Wang, Chanda and Zhong 2015), but it is the ecological context that will be considered in the following section that defines their dynamics in different biofilms.

In the following section, we will outline how BMVs stabilize different communities; we will also provide examples of how BMVs can serve as ecosystem engineers that can act as ‘detergents’ for hydrophobic environments, be mediators in biochemical cycles, sustain microenvironments and serve as aggregation bridges, among other functions.

**Strongly restrictive environments**

To accommodate the purpose of this review, any environment that offers conditions that vary from any of those found in environments that support relatively high biodiversity (i.e. habitats with pH 5–9, 1 atm pressure, salinity lower than 2 M NaCl, temperature between 15 and 60°C and abundant nutrients present in higher than 1–15 mg of C per liter) is considered ‘extreme’, and the inhabitants of such environments are called extremophiles (Morita 1975; Kuznetsov, Dubinina and Lapteva 1979; Rothschild and Mancinelli 2001; Schlegel and Jannasch 2006). Due to their physicochemical parameters, the extreme environments contain substrates either of low energy or of very low concentrations and such situation reflects on the generation time of the extremophiles that can range from several hours (as in some psychrophilic, acidophilic and halophilic bacteria) to a theoretically estimated thousands of years as in the bacteria inhabiting the deep-sea bed (Morita 1975; Kuznetsov, Dubinina and Lapteva 1979; Jorgensen and Boetius 2007; Johnson 2012; Mesbah and Wiegel 2012). The relatively long generation time compared with that of some of the better-studied non-extremophiles is one of many reasons that the biology of these exotic bacteria remains understudied—a lack that also includes the role of their BMVs. The BMVs of extremophiles are an extension of the key aspects of their adaptive biology, which creates a microenvironment that increases the chances for the bacterial population to exist. Learning the mechanism and function of the vesiculation of extremophiles compared with that of their mesophilic relatives should help to illuminate the organisms’ adaptive potential to challenging environmental factors.

**Acidic environments**

The biomineralization of the compounds of biologically significant chemical elements such as S and Fe, as well as the precipitation of toxic minerals containing chemical species of U and As, is often mediated by bacterial groups that are capable of either the oxidation or reduction of the ions of these aforementioned elements. The majority of these bacteria live in defined
environments in which the air-exposed minerals from ores produce iron- and sulfur-enriched water ecosystems with a pH that can range from 6.5 (e.g. the Storwartz mine in Norway) to 0.5 (the Iron Mountain in California) (Johnson and Hallberg 2003; Johnson 2012). Some mines may also contain, associated with the ore’s minerals, metalloids and actinides such as As and U present in different oxidized states (Johnson and Hallberg 2003; Mkandawire 2013).

Acidithiobacillus thiooxidans is a Gram-negative aerobic mesophilic chemolithoautotroph that gains energy from the oxidation of sulfides (S^{2−}), elemental sulfur (S^0), thiosulfates (S_2O_3^{2−}) and sulfites (SO_3^{2−}) for CO_2 fixation (Kelly and Wood 2004). It is a true acidophile that inhabits sulfur-enriched acid mine drainages with a pH of 2–3 (Kelly and Wood 2004). In order for these bacteria to gain energy from elemental sulfur, they first have to overcome the solid’s hydrophobicity (Yang et al. 2019). Acidithiobacillus thiooxidans has been shown to overcome this hydrophobic barrier via BMVs, which, due to their amphipathic nature, allow the bacteria to colonize and then dissolve the sulfur (Knickerbocker, Nordstrom and Southam 2000). Interestingly, the vesicle release has not been observed in sulfite-enriched cultures, which further supports the hypothesis that BMVs may shape a microenvironment that promotes colonization, but BMVs may also serve as a detergent that decreases the hydrophobicity of the elemental sulfur and solubilizes the sulfur so that bacteria can transport it to their periplasm and oxidize it to sulfuric acid with the help of oxygen (Knickerbocker, Nordstrom and Southam 2000).

Garcia-Meza et al. (2013) have shown that the electrooxidized chalcopyrite produces S_n^{2−}/S^{0}/CuS phases on which A. thiooxidans bacteria form a mat-like biofilm composed of different layers with a zonal hydrophobicity in the biofilm due to the synthesis of lipids and hydrophobic extracellular polymers (Garcia-Meza et al. 2013). One intriguing area of research would be to explore the role of A. thiooxidans BMVs in the formation of these hydrophobic zones and to determine whether the sulfur hydrophobicity serves as a trigger for their formation. The secretome of exponentially grown A. thiooxidans bacteria in media supplemented with 1% sulfur has been found to contain mainly Omp40-like proteins and a trimeric coiled-coil lipoprotein licanantase that increases the sulfur oxidation (Bobadilla Fazzini, Levican and Parada 2011; Abarca et al. 2014). As Bobadilla Fazzini, Levican and Parada (2011) briefly speculated, the sulfur-induced secretome of A. thiooxidans may partially be delivered via BMVs.

A further hypothesis is that the BMVs released by these bacteria may play an important role in biominalerization processes in extreme environments where heavy metals are present and where the released BMVs can serve as a nucleation center for the formation of novel biominerals (Benzzerara et al. 2008). This hypothesis is further supported by the finding that microbial polysaccharides can serve as a template for the production of unusual crystal structures (Chan et al. 2004), which adds a novel role of BMVs as mediators in the biogeochemical cycle of metals in nature. Bacteria, as mentioned above, most probably release these mineralized vesicles to let go of the precipitates encrusted on their cells (Fig. 3A).

Studies on the acid-mine-drainage microbial communities, found in Carnoules, France—the representatives of which belong to A. ferrooxidans, Gallionella ferruginea (both species capable of ferrous oxidation) and Thiomonas spp. [capable of oxidizing
Fe(II) and As(IV)—have revealed two different mineral precipitation patterns (Benzerara et al. 2008). Besides being associated with the bacterial cells, most of the mineral precipitates were found to be biomineralized vesicles (Benzerara et al. 2008). In addition to examining BMVs, another study on arsenic immobilization by a microbial community mainly composed of G. ferri

Researchers who have examined other bacteria capable of reducing U(VI) to U(IV), and thus accumulating uraninite crystals, have also proposed mineralized BMV release as a novel option for the secretion of insoluble compounds. For example, Shewanella oneidensis is a facultative anaerobic alteromonade capable of metal respiration. To prevent the accumulation of insoluble U(VI) on the cell wall, S. oneidensis bacteria produce exopoly saccharide (EPS), which moves the precipitate away from the cells; as a second strategy, they also secrete the encrusted uranates as part of their BMVs; see Fig. 3A (Shao, Comolli and Bernier-Latmani 2014).

BMV release as a metal-detox strategy is also exploited by another U(VI) reducer: Geobacter. Cologgi et al. (2011) have demonstrated that the reduction of U(VI) and the subsequent immobilization of U(IV) both take place on the pili of the bacteria, which they found prevented the precipitation of U(IV) in the periplasm. Furthermore, as Shao, Comolli and Bernier-Latmani (2014) have pointed out, the pili-deficient mutant exhibited increased vesiculation compared with the wild-type strain when the medium was supplemented with U(VI); in this way, the vesiculation is thought to serve as a back-up electron transfer mechanism (Cologgi et al. 2011; Shao, Comolli and Bernier-Latmani 2014).

Low-temperature environments

Membrane vesicles have been described as a common feature of some psychrophiles—bacteria whose optimal growth temperature is 15 °C or lower, while their maximal temperature for growth is ~20 °C (Ingraham and Stokes 1959; Morita 1975; Schlegel and Jannasch 2006). The ecological distribution of the psychrophiles is quite vast from Arctic and Antarctic ecosystems to human-generated cooling-system environments to the ocean waters, 90% of the volume of which is 5 °C or lower (Ingraham and Stokes 1959; Morita 1975; Schlegel and Jannasch 2006). A certain group of bacteria that grows at temperatures above 20 °C, but can also grow at lower temperatures, is referred to as being ‘psychrotolerant’; these bacteria are also known as facultative psychrophiles or psychrotrophs (Ingraham and
Stokes 1959; Morita 1975; Schlegel and Jannasch 2006). Also, very few studies on obtaining psychrophilic mutants from their mesophilic wild types have been recorded in the chronicles of microbiology—it has only been achieved with UV-irradiated mesophilic P. aeruginosa bacteria (Azuma, Newton and Witter 1962; Olsen and Metcalf 1968). In this review, we will collectively call the cold-loving bacteria ‘psychrophiles’. To survive in such environments, these bacteria have evolved capabilities that help them adapt to oxidative stress, low water-activity levels and physical disruption due to ice formation, among other adaptations (Scherer and Neuhaus 2006).

A common trend among some psychrophilic bacteria is to form biofilm composed of diverse EPS polymers and BMVs (Nevot et al. 2006b; Frias et al. 2010; Perez-Cruz et al. 2013; Yokoyama et al. 2017). Studies on Shewanella livingstonensis NF22, Shewanella vesiculosa M7, Pseudoalteromonas sp. M4.2, Pseudoalteromonas antarctica NF3, Psychrobacter fozzi NF23 and Marinobacter guineae M3B have confirmed that an extracellular matrix composed of EPS and BMVs may be a common feature among these cold-loving bacteria (Nevot et al. 2006a,b; Frias et al. 2010). The BMVs stability in the psychrophilic biofilm may be reinforced by the secreted EPS. In addition to its role in cryoprotection, osmoprotection, adhesion and nutrient scavenging (Nichols, Guezenec and Bowman 2005; Deming and Young 2017; Casillo et al. 2018; Lo Giudice et al. 2020), the EPS could also be involved in the stabilization of the released BMVs. Evidence for such kind of implication comes from studies on P. antarctica’s EPS that is predominantly glycoproteinaceous (de La Maza et al. 1999; Cocera et al. 2000; Cocera et al. 2001). The studies have demonstrated that the secreted EPS is able to protect PC-liposomes from the activity of different detergents such as SDS, dodecyl maltoside and octyl glucoside (de La Maza et al. 1999; Cocera et al. 2000; Cocera et al. 2001).

The psychrophilic BMV componentry, represented by putative TonB-dependent receptors, bifunctional UDP-sugar hydrolase, P-binding protein and phosphate-selective porins O and P, hints to involvement of the BMVs in nutrient acquisition (Nevot et al. 2006a,b; Frias et al. 2010) that could act in synergy with the produced EPS.

In a nutshell, the BMVs of the studied psychrophiles are part of a microenvironment in which the cold-loving bacteria can thrive under harsh conditions. Yet, the role(s) of the BMVs in cold adaptation remains per se unknown.

Be that as it may, we will also consider indirect studies that may hint to cryoprotection exerted by the psychrophilic BMVs. The first group of findings indicates that some bacteria may use membrane vesicles to deliver ice crystallization proteins (Phelps et al. 1986; Muryoi et al. 2003). Among other features, an important adaptation for life in thermal equilibrium with its environment of low temperatures is the regulation of different phase transitions (Lorv, Rose and Glick 2014; Collins and Margesin 2019). Studies on Pantoea agglomerans (Erwinia herbicola) and Pantoea ananatis have illustrated that these bacteria (even though not psychrophilic) can secrete ice-nucleating proteins (membrane proteins that trigger extracellular ice-formation; INPs) via BMVs under the form of extracellular ice-nucleating matter (EIM) (Phelps et al. 1986; Muryoi et al. 2003). Moreover, incorporation of INPs into PC-PE liposomes has increased the ice-nucleating activity of P. ananatis INPs (Muryoi et al. 2003). It is proposed that the secretion of INPs may prevent or reduce the cryodamage due to intracellular ice crystals (Lorv, Rose and Glick 2014; Collins and Margesin 2019). The second group of studies suggest that psychrophiles may use their vesicles for the delivery of PLs (such as PC, PE and others) as a part of the vesicular componentry that may act as biosurfactants. The biosurfactants have been proposed by Collins and Margesin (2019) and Perfumo, Banat and Marchant (2018) to contribute to ice growth inhibition, osmoprotection and decrease in surface tension. Finally, studies on cryopreservation with artificial vesicles,
which showed increased β-glucuronidase stability at low temperatures (4 °C and ~80 °C) when the enzyme was encapsulated into unsaturated fatty acid-esterified PC liposomes (Frank et al. 2018), hint to a putative cryoprotective role, i.e. the BMVs may also stabilize the proteins delivered by them.

The psychrophiles have also developed capabilities for homeoviscous maintenance of their membranes. To achieve the right membrane fluidity, they can increase the number of their unsaturated, branched or short fatty acids of the PLs (Scherer and Neuhaus 2006). Yokoyama et al. (2017) reported that membrane stress in S. livingstonensis Ac10 due to mutual deficiencies in eicosapentanoic acid (EPA) and branched-chain fatty acids causing altered lipid composition increased the vesicle production by up to 5-fold (Yokoyama et al. 2017). Based on previous studies on the importance of EPA for cellular morphogenesis and proper membrane protein folding (Kawamoto et al. 2009; Dai et al. 2012), Yokoyama et al. (2017) have proposed that the observed increase in the BMV release is a stress response of the bacteria resulting in the release of misfolded proteins similar to the BMV response to unfolded or misfolded periplasmic proteins (McBroom and Kuehn 2007). Interestingly, while the addition of unsaturated fatty acids in the growth medium could compensate for the cryosensitivity of some mesophilic mutant strains (for example, E. coli ΔfabA), the presence of EPA could not restore the normal growth of EPA-deficient S. livingstonensis at 4 °C (Caldcott 1996; Kawamoto et al. 2009). The extracellularly present EPA-PLs could do that (Kawamoto et al. 2009). This finding hints that the BMV-delivered PLs may also contribute to the maintenance of the homeoviscous adaptation.

Among the psychrophilic BMV producers studied, one species stands out: Shewanella vesiculosa, which is so named for its ability to release BMVs (Bozal et al. 2009). Indeed studies on S. vesiculosa M7T were what led to the discovery of the O-IMVs representing 0.1% of the total BMVs produced by these bacteria (Perez-Cruz et al. 2013). Pérez-Cruz and her colleagues also found that the O-IMVs carry short DNA molecules of ~600 nucleotides. Based on their EM experiments, the researchers proposed a mechanism for O-IMV biogenesis (illustrated in Fig. 1C) in which the cytoplasm is included in the vesicle biogenesis in a budding process that involves the CM and OM (Perez-Cruz et al. 2013).

Their model has also confirmed the Kadurugamuwa/Beveridge double-step hypothesis proposed to explain the presence of cytosolic components and DNA in P. aeruginosa’s BMVs (Kadurugamuwa and Beveridge 1995). The BMVs of another isolate of S. vesiculosa, strain HM13, have been shown to contain a single major transmembrane protein of unknown function called P49 (Chen et al. 2019). The authors demonstrated that the disruption of the P49 gene does not affect the BMV production and it was concluded that the loading of this protein takes place independently from the BMV biogenesis (Chen et al. 2019). From follow-up work, it has been reported that the secretion of P49 depends on the type 2 secretion system (for further information about the different secretion systems, see Fig. 5) and that the P49 association with BMVs requires the presence of a glycolipid, which, if not produced, results in BMV-independent P49 liberation in the extracellular environment (Kamasaka et al. 2020). Overall, further studies, on BMVs produced by S. vesiculosa HM13 may offer new possibilities to study the processes of the sorting and enrichment of the vesicle cargo, as suggested by Kamasaka et al. (2020).

**High-temperature environments**

Thermophiles, in contrast to the psychrophiles, are a group of bacteria adapted for life at high temperatures. Thermus spp., which are aerobic heterotrophic bacteria typically found in hydrothermal areas, have an optimal growth temperature of 70 °C (da Costa Allgayer, Nobre and Rainey 2001). Researchers have suggested that the LGT between the hyperthermophilic archaea and the representatives of Thermus played a significant role in the adaptation of the latter to high temperature (Averhoff 2009). Blesa and Berenguer (2015) have shown that different species of Thermus (e.g. T. aquaticus subsp. thermophilus and T. scotoductus) release a variety of vesicles in which extracellular DNA (eDNA) can be present and protected from the harsh environment (Blesa and Berenguer 2015). These thermophiles can be transformed at 100-fold higher efficiency with the eDNA present in the vesicles than with the free genomic DNA (Blesa and Berenguer 2015). In the case of Thermus spp., the BMVs not only serve as a possible source for genetic material but also play a role in preserving the DNA, and they function as a vehicle for an effective alternative route of transformation (Blesa and Berenguer 2015).

These findings should prompt further studies. One avenue of interest would be to conduct genetic experiments to test directly if exchange occurs between representatives of Thermus and hyperthermophilic archaea that involve BMV-mediated LGT. Another suggested direction for future study could be based on the comparison between the BMVs released by representatives of Thermus compared with those secreted by the group of psychrophilic bacteria mentioned above. The common theme for these future studies may be the content of DNA, the thermophiles can seemingly use their BMVs as a form of LGT (Blesa and Berenguer 2015), while the role of the short DNA pieces within the BMVs of psychrophiles in LGT has yet to be clarified (Perez-Cruz et al. 2013). The DNA within the psychrophiles’ BMVs, in combination with the EPS, can also be studied regarding the maintenance of water activity and decreased freezing temperatures due to the presence of these components in biofilm.

**Aquatic ecosystems**

The aquatic ecosystem, the largest ecosystem on earth, includes both marine and freshwater environments. Based on the nutritional availability, the aquatic ecosystem incorporates two types of habitats: coastal and oligotrophic (Kuznetsov, Dubinina and Lapteva 1979; Poindexter 1981; Koch 2001). The first, occupied by copiotrophs, provides favorable growth and nutritional conditions (for example, the carbon concentration of the available C sources is above 500 mg L−1), while the second is characterized by a scarcity of nutrients where the available C sources range between 1 and 15 mg L−1 (Kuznetsov, Dubinina and Lapteva 1979; Poindexter 1981). Examples of such oligotrophic environments (in addition to the cold ecosystems, and some soil-embedded and soil-embedding environments) include the open seas and oceans, which are typically inhabited by bacteria, called oligotrophs (Kuznetsov, Dubinina and Lapteva 1979; Poindexter 1981; Koch 2001; Torvsik and Øvreås 2008). (Also of note is that copiotrophs can still be found in oligotrophic systems, and oligotrophs can be present in coastal waters.) To survive in environments with depleted energy sources, the oligotrophs have diverse nutrient uptake systems with low substrate specificity and multiple substrate-biding sites (Kuznetsov, Dubinina and Lapteva 1979; Poindexter 1981; Koch 2001). The nutrient flux is further facilitated by the increased surface-to-volume (S/V) ratio that oligotrophs achieve either by having the morphology of small spheres or thin rods, or by producing membrane projections such as the stalk of caulobacteria and hyphomicrobia.
Figure 4. Comparison of the physically induced pearling with the ‘biopearling’ produced by oligotrophs. (A) Bar-Ziv–Moses’ diagram describing the pearling state of the cylindrical membrane due to competition between curvature and tension. The bottom line represents the instability line; above the second line, the pearling state of the membrane is expected to appear (modified from Bar-Ziv and Moses 1994). (B) Micrographs of strain Hel3_A1.48 cells in stationary growth phase. Membrane tubes (t) and thicker vesicles (assigned as O-IMVs) are indicated. Cells grown in HaHa, 100 V medium at 21°C to stationary phase were negatively stained with 1% uranyl acetate for transmission electron microscopy (TEM) (a–c). The cells were passively settled on a silica wafer, dehydrated by an ethanol series and preserved using critical point drying for scanning electron microscopy (SEM) (d–f). Bar corresponds to 100 nm (c), 500 nm (a, b, e and f) or 1 μm. (d) Applied and Environmental Microbiology, 2019, Vol. 84, page 3, DOI: 10.1128/AEM.00829-19, reproduced with permission from American Society for Microbiology. This image/content is not covered by the terms of the Creative Commons license of this publication. For permission to reuse, please contact the rights holder.

The marine members of Bacteroidetes are among the main representatives of the bacterioplankton (Pinhasi, Zweifel and Hagström 1997; Pinhasi and Hagström 2000; Giovannoni and Stingl 2005; Anne-Carlijn, Eva and Gerhard 2006; Pommier et al. 2007; Lindh et al. 2015; Bunse and Pinhasi 2017). One recently discovered member of Flavobacteriaceae, Formosa strain Hel3_A148, revealed another strategy for the optimization of nutrient uptake that involves BMV release (Fischer et al. 2019). As a result of a process called ‘biopearling’—the membranes of these bacteria protrude and form up to 2-μm-long tubular appendages and start to oscillate due to abiogenic physical forces resulting from the competition between membrane curvature and tension that together introduce instability in the tubular structure and produce a chain of linked with short neck vesicles or ‘pearls’; see Box 2 and Fig. 4B (Fischer et al. 2019). Proteomics studies of the BMVs of this strain have shown that the BMVs are enriched in porins (the OmpC-like type), hydrolytic enzymes (including endonucleases, peptidases and glycoside hydrolases), TonB-dependent proteins and gliding motility-associated proteins, while BMVs have also been found to exhibit laminarin-binding properties, which suggests a role in carbohydrate binding and utilization (Fischer et al. 2019).

Box 2.

Physical factors that induce pearling

Physics can also help to explain the dynamics of vesiculation. For example, Bar-Ziv and Moses (1994) have shown that optical tweezers can be used to induce sinusoidal perturbations in a cylindrical membrane vesicle (Bar-Ziv and Moses 1994) (Fig. 4A). In their experiment, this excitation transformed the membrane tube into vesicles linked by small necks in a way that the overall structure resembled ‘pearls on a string’—the same structure observed as a result of the biopearling described by Fischer et al. (2019) in the oligotrophic bacteria (Bar-Ziv and Moses 1994; Fischer et al. 2019); see Fig. 4A and B. Bar-Ziv and Moses have found that the laser-induced membrane tension competes with the membrane curvature, for example with \( E_k \), which forms the peristaltic mode of the system (Bar-Ziv and Moses 1994).
Mechanical stress and other factors, such as heating, electricity, and van der Waals forces, can also destabilize membrane cylinders (summarized by Bar-Ziv and Moses 1994). These kinds of oscillating systems, as exemplified by the cylindrical membrane vesicle with induced perturbations, increase the surface area while simultaneously keeping the volume unchanged, which may provide the oligotrophs (such as flavobacteria) with the ability to interact with their nutrient-poor environment in the least energetically costly manner (Fig. 4B).

This type of vesiculation has also been observed in not only other bacterial species that exhibit oligotrophic growth, such as S. livingstonensis, S. vesiculosa, S. oneidensis and Flavobacterium psychrophilum, but also some non-oligotrophic bacteria such as Neisseria meningitidis, Myxococcus xanthus and Francisella novicida (Devoe and Gilchrist 1973; Möller et al. 2005; McCaig, Koller and Thanassi 2013; Perez-Cruz et al. 2013; Remis et al. 2014; Yokoyama et al. 2017; Subramanian et al. 2018). Nevertheless, the BMV-mediated biopearling may be an adaptive mechanism used by oligotrophs because it leads to an increased S/V ratio in the least energetically costly manner (Box 2 and Fig. 4), as well as providing an adhesive mechanism coupled with the delivery of the hydrolytic enzymes (Fischer et al. 2019).

Notably, the physiological nature of bacteria sometimes blurs the borders between different trophic modes: some copiotrophs may thrive or persist in oligotrophic environments, while some oligotrophs can grow in nutrient-rich places (Kuznetsov, Dubinina and Lapteva 1979; Koch 2001; Fierer, Bradford and Jackson 2007; Torsvik and Øvreås 2008). Once they experience nutrient depletion, some copiotrophs trigger a program, similar to that of the oligotrophs, which involves upregulation of nutrient uptake systems and/or an increase of their S/V ratio (Koch 2001; Fierer, Bradford and Jackson 2007; Torsvik and Øvreås 2008). A part of this management can also result in the formation of membrane structures that may be a product of biopearling (McCaig, Koller and Thanassi 2013; Remis et al. 2014). In such cosmopolitan bacteria, the biopearling may have other functions than adaptation to oligotrophic conditions.

**Myxococcus xanthus** is a soil deltaproteobacterial species with a ‘biphasic’ life cycle defined by the presence or absence of available food sources (Dworkin and Bonner 1972; Zusman et al. 2007; Zhang et al. 2012). The bacteria of this species can also be isolated from different aquatic environments (Hook 1977; Reichenbach 1999; Li et al. 2002). If nutrients exist in sufficient quantities, then the bacteria can form ‘wolf packs’ to either prey on other bacteria or to acquire the amino acids and protein sources present in the environment. Using this lifestyle, also called vegetative, M. xanthus can target other bacteria via their BMVs the cargo of which is enriched with peptidases/proteases, chitinases, antibiotics and chelating agents (Kahnt et al. 2010; Evans et al. 2012; Berleman et al. 2014). In oligotrophic situations (i.e. any environment with unavailable or lacking amino acids and peptides), M. xanthus bacteria aggregate and initiate a developmental program that results in the production of fruiting bodies filled with myxospores (Dworkin and Bonner 1972; Zusman et al. 2007; Zhang et al. 2012). In the initial stage of development, the cellular aggregation (biofilm) is achieved by cell-to-cell and cell-to-surface interactions mediated via intercellular appendages, slime and BMVs, some of which are arranged in chains (Arnold and Shimkets 1988; Palsdottir et al. 2009; Remis et al. 2014). The fact that the vesicle chains have rarely been observed in the planktonic phase implies that these formations are typical for the biofilm stage of M. xanthus, during which the BMVs produced by the developmental cells (Remis et al. 2014). This may promote adhesion and nutrient scavenging, coupled with other
functions, due to the lifestyle of these bacteria, such as cell-to-cell bridging, and intercellular signaling (Palsson et al. 2009; Kahnt et al. 2010; Remis et al. 2014). A future avenue of study that piques our curiosity is to check whether the BMVs extracted from the two different lifestyles of *M. xanthus* can perform the extracellular complementation of the different mutant groups, each deficient in a certain stage of fruiting-body development (Shimkets 1999).

Another bacterial species that occurs in oligotrophic situations is Franciscella novicida (Margolis et al. 2009). The species was initially isolated from freshwater environments and it is considered as a waterborne intracellular human pathogen that can also be transmitted via arthropod vectors (Larson, Wicht and Jelison 1955; Hennebique, Boisset and Maurin 2019). When challenged in oligotrophic environments, *F. novicida* forms biofilms on abiotic surfaces and chitin and with the help of chitinases (ChiA and ChiB), the bacteria use *N*-acetyl-β-glucosamine as a carbon source (Margolis et al. 2009). McCaig, Koller and Thanassi (2013) have reported the production of OMVs and OM tubes (OMV/Ts) by *F. novicida*, as the tubes most probably are the released ‘horn-like’ (or stalk-like) structures previously described by Gil, Benach and Thanassi (2004) observed when the bacteria were incubated on solid Mueller–Hinton chocolate agar. The isolated OMV/Ts have been shown to be enriched with porins, to exhibit low cytotoxicity to the host cells (murine macrophages) and to trigger a proinflammatory response in a dose-dependent manner (McCaig, Koller and Thanassi 2013). McCaig, Koller and Thanassi (2013) have also noticed that the stalk-like structures are predominantly formed by the intracellular bacteria or by the exponentially growing bacteria in brain-heart infusion broth. One may speculate that the bacteria use biopearing to regulate their adhesion and host colonization by producing and releasing their stalks in the form of OMV/Ts depending on the environmental conditions defined by the habitat.

Various marine habitats, salt lakes, brines and other habitats are also inhabited by halophilic eubacteria that can thrive in high-salt concentrations. Although the actual contribution of BMVs in the bacterial adaptation to high-salt concentrations has yet to be elucidated, EM studies on the cellular morphology of certain halophilic bacteria and BMVs have revealed the potential role of BMV involvement (Vreeeland, Anderson and Murray 1984; Gauthier et al. 1992; Yun et al. 2017). The gammaproteobacterial heterotrophic species that belong to genus *Halomonas* are typically isolated from a variety of saline ecosystems, ranging from estuarine and open-ocean ecosystems to hypersaline lakes. *Halomonas elongata* can grow in environments with salt concentrations ranging from 0.05 to 3.4 M NaCl (Vreeeland et al. 1983). In their studies on the adaptive physiology of this organism, Vreeeland, Anderson and Murray (1984) showed that upon increased NaCl, the cell wall of *H. elongata* became more compact, with an increased quantity of negatively charged lipids, which overall resulted in structural maintenance and an increase in less-mobile ‘structured cell’ water that prevented the cell from dehydrating. Following this line of logic, one should expect decreased vesicle release by the halophilic bacteria: because of their structural integrity, their cell walls become more rigid. This scenario is also what Vreeeland, Anderson and Murray (1984) observed in their study: *H. elongata* cells incubated in 0.05 M NaCl produced blebs (released as BMVs), while the bacteria grown in 3.4 M NaCl did not. Further studies on the BMVs released by halophiles will be required to clarify how the observed decrease of BMV release might function as an adaptive strategy to high salt concentration.

Among primary inhabitants of different aquatic ecosystems are the oxygenic photosynthetic cyanobacteria. By combining data from culture and field studies, Biller et al. (2014) have shown that bacterial vesicles released by diverse bacterial species are abundant in marine ecosystems. The number of vesicles in coastal waters can reach ~6 × 10^8 ml^-1, and in the Sargasso Sea can reach ~3 × 10^8 ml^-1; both numbers are similar to the bacterial numbers found in these habitats (Biller et al. 2014). The bacterial vesicles also seem to be present in freshwater ecosystems produced by autotrophs (Silva et al. 2014; Gamalier et al. 2017).

Two interesting parallels may be suggested if we compare BMV distribution in aquatic ecosystems with their distribution in acid mines. For the first parallel, let us assume that vesicles released in the oceans can also serve as sites for nuleation, similar to the hypothesized role of acid-mine BMVs in biomineralization. If that is the case, then aquatic BMVs may have played an essential role in the formation of the stromatolites that are stratified rocks presumed to be lithified bacterial mats (Stal 2012). The bacterial mats are biofilms of vertically stratified layers of different physiological groups of bacteria that usually form on the bottom of different shallow aquatic environments (Stal 2012). These mats can precipitate insoluble inorganic salts, resulting in their mineralization (Stal 2012). As a result, the bacterial mats have been found to be calcified, silicified and sulfidized among other processes, which has presumably allowed the preservation of these biofilms in the form of laminated rocks (stromatolites) during different geological times (Wacey et al. 2011; Stal 2012; Baumgärtner et al. 2019). One interesting line of speculation is whether the BMVs released by the different bacterial guilds forming each layer of the mat—the top layer is represented by the cyanobacteria, the middle layer is composed of the representatives of the purple sulfur bacteria, followed by the green sulfur bacteria (Stal 2012)—may have played a role in this mineralization process, for example, by either serving as nucleation centers of pseudocrystals or bridging Ca^{2+}, SO_4^{2−}, S^{2−} or other ions that can be precipitated with the EPS to play a role as a matrix.

The second parallel is as follows: even though they are produced by very different bacterial groups, acid-mine BMVs may exhibit some of the same ecological trends of aquatic BMVs.

Among the primary BMV producers is Prochlorococcus, which is the dominant cyanobacterial species of the open ocean, an oligotrophic zone to which the bacterium has adapted via the reduction of its genome and cell size (Partensky and Garczarek 2013). In addition to Prochlorococcus, other cyanobacteria such as Synechococcus and Synechocystis also produce BMVs (Biller et al. 2014; Pardo et al. 2015). Prochlorococcus has been further demonstrated to produce large quantities of vesicles, in numbers ten times higher than those of the bacterial cells (Biller et al. 2014). The BMVs of *Prochlorococcus* are isolated from the environment with a rigid membrane made of the non-bilayer lipids monoglycosyl diacylglycerol and sulfolipid diacylglycerol esterified with saturated fatty acids, as well as certain unidentified glycolipids (Biller et al. 2014). The vesicles contain proteins, belonging to different functional groups (such as nutrient transporters, porins, hydrolases as well as many of unknown function), DNA (~3 kb) and RNA (Biller et al. 2014).

Based on calculations that *Prochlorococcus*-derived dissolved organic carbon delivered via BMVs represents 10^7 to 10^9 tonnes of organic carbon exported into the ocean per day, Biller et al. (2014) have demonstrated that these BMVs could serve as a carbon source for heterotrophs, especially for oligotrophs such as Alteromonas and Halomonas. To extend this concept, an examination of the extent to which BMVs can participate in the cross-feeding of substrates (i.e. syntrophy) among different
oligotrophic bacterial species would be an intriguing line of research.

Due to the natural competence of the unicellular cyanobacteria and the length of the DNA present in the BMVs (which covers half the bacterial chromosome, with sequence reads overrepresented at the terminus region) in the BMVs, a role can be suggested for BMVs in the LGT (Biller et al. 2014). This potential role of the cyanobacterial vesicles in LGT is even more interesting for several reasons. First, while it has been recently shown for the model cyanobacteria, such as S. elongatus, that the natural competence is determined by type 4 transformation machinery (Taton et al. 2020), many of the naturally competent cyanobacterial species do not code for the full set of genes of the transformation pilus (Wendt and Pakrasi 2019). An alternative mechanism such as the BMV-mediated transformation hence may be a good substitute for the lack of DNA-uptake machinery. The BMVs are also potentially useful LGT vehicles because of the protective role of the BMV envelope against certain DNA-degrading agents and because of the form in which the DNA is present in the vesicles. Previous studies have demonstrated that the DNA for transformation can be DNA/DNA or DNA/RNA complexes of homologous or heterologous DNA the uptake of which is dose-dependent and severely disrupted in the presence of DNases (Trehan and Sinha 1981; Porter 1986; Essich, Stevens and Porter 1990; Barten and Lií 2006; Almeida et al. 2017).

The occasional observations of bacteriophages attached to BMVs from environmental samples suggest that BMVs can also serve as a broad phage-decay mechanism (i.e. the bacteria titer away the bacteriophages via BMVs, which may carry the receptors for the adhesion of different phages) (Biller et al. 2014). This idea is further supported by two studies. First, Manning and Kuehn have aptly demonstrated that BMVs produced by E. coli can irreversibly bind T4 bacteriophages, which reduces the infection of E. coli by 90% (Fig. 3D) (Manning and Kuehn 2011). The second study, by Reyes-Robles et al. (2018), has demonstrated that BMVs released by V. cholerae can neutralize T4- and T7-like bacteriophages. The BMVs released by planktonic bacteria are a good example of an alternative eco-ego feedback. Eco-evo studies on E. coli-bacteriophage communities have demonstrated that if an ecological niche is structured, then the niche can offer a spatial escape to refugees that can hide from phages (Bohannan and Lenski 2000). Since the planktonic lifestyle cannot offer this kind of structured ecosystem per se, one can speculate that the bacteria may obtain a relatively phage-free environment with the help of the BMVs. One hypothetical scenario envisions the formation of spatial environments created by the bacteria and their BMVs due to the differences in their diffusion coefficient (D), which expresses how fast the particles of one species (atoms, ions, molecules and so on) can diffuse through another environment (higher values of D define faster diffusion). Based on experiments with artificial membrane vesicles, the D of the BMVs of 50 nm in diameter can be approximated to fall in the range of 10−8 cm2 s−1 and it can be spatially restricted by the ionic strength and the pH of the environment, as well as by the size and composition of the BMVs (Kyoung and Sheets 2008). Yet, the range of D for a bacterial population (E. coli) in liquid medium is between 10−6 and 10−5 cm2 s−1 suggesting that bacteria may diffuse faster than the BMVs (Licata et al. 2016). One fascinating finding is that the bacteria can regulate their D coefficient with the help of motility (Licata et al. 2016). In their particular study, Licata et al. (2016) showed that E. coli can decrease D via tumbling to such a degree that ‘hypertumbly’ cells are theoretically calculated to have D = 0. Based on these two approximations, one can envision that bacteria can regulate their D coefficient so they can diffuse faster, slower or together with the BMVs, which can bind and clear away the bacteriophages before the phages have the chance to encounter the cells. In addition, BMV release helps bacteria, avoid or temporarily delay, certain evolutionary trade-offs (at least until these trade-offs become a necessity) that can provide them with genetic resistance to phages but can also make them less competitive over phage-sensitive bacteria, for example via mutations in the lpcA and lpcB genes, whose products are important for LPS core formation and provide resistance against T4 phages but can also reduce the fitness of the resistant bacteria (Bohannan and Lenski 2000).

UV radiation, which is likely among the most ancient of dangers, existed before the Great Oxygenation Event (Beckers et al. 2004). Bacteria have developed different defense mechanisms to endure such radiation. Studies on a toxin-producing cyanobacterium, Cylindrospermopsis raciborskii, have demonstrated that this bacterium responds to UV treatment by increased vesiculation (Zarantonello et al. 2018). A triggering signal for the bacteria’s BMV release may be the exposure of phosphatidylserine (a very atypical bacterial phospholipid) on the OM of its cells (Zarantonello et al. 2018). Increased vesiculation due to UV irradiation has also been observed in other aquatic bacteria (Gamalier et al. 2017). We should point out that together with the increased vesiculation triggered by UV irradiation, Zarantonello et al. (2018) and Gamalier et al. (2017) have also observed a decrease in cell viability, which suggests that this type of vesiculation is due to the vesicle release accompanied by cell death. Whether the increased vesicle formation under UV stress (which is accompanied with cell death) is at the end protecting the population remains to be shown. In this line, we have previously shown that the occasional human marine pathogen V. cholerae increases BMV release upon UV exposure, and that the released BMVs do indeed protect the bacteria from UV irradiation (Song and Wai 2009). The molecular mechanism behind the defense mechanism of V. cholerae is σE mediated, which signals activation of the expression of the sRNA VraA, which in turn down-regulates the OmpA transcript (Song and Wai 2009). Thus, due to decreased OmpA protein in the OM, the process leads to increased vesicle production (Song and Wai 2009).

Host-provided ecosystems

Plant hosts

Compared with what appears to have been the first report on BMVs released by E. coli in 1966, the first analysis of BMV release by phytopathogenic bacteria (Xanthomonas campestris) was published only in 2008, and thus the exploration of the role of BMVs in the development of pathogenic or symbiotic relationships with plants has begun more recently (Knox, Veski and Work 1966; Sidhu et al. 2008). Although the human-associated bacteria have received the most attention, recent devastating outbreaks caused by certain bacterial phytopathogens in different agroecosystems have revealed the need for more in-depth studies on plant–bacteria interactions. The importance of a better understanding of the ecology of bacteria–plant associations has re-emerged as an urgent topic in the prevention of such outbreaks from happening again (Saponari et al. 2013; Sicard et al. 2018). In addition, understanding the symbiotic associations between plants and N2-fixing bacteria can further improve the primary production in various agroecosystems. In the following section, we summarize recent studies that have been conducted on the role of BMVs released by plant-associated bacteria.

Members of genus Xanthomonas are chemoorganotrophic, motile, aerobic gammaproteobacteria that cause diseases in 124
monocots and 268 dicots (Sadder and Bradbury 2005a; Jacques et al. 2016). Among the main characteristics to contribute to the pathogenicity of these bacteria is their ability to secrete enzymes that degrade cellulose, hemicellulose, pectin and proteins: the various components of the plant cell wall (Sadder and Bradbury 2005a; Jacques et al. 2016). A comparison between the BMVs produced by Xanthomonas campestris pv. campestris and those produced by pv. vesicatoria reveals several interesting features. In both pathovars, the BMVs are part of the secretome of these bacteria, in which different cellulolytic enzymes are sorted and concentrated in such a way that, when secreted, the BMVs can cause sequential degradation to the plant cell wall from a distance before the bacteria come in close proximity to the plant cells (Sidhu et al. 2008; Sole et al. 2015). The presence of different cellulolytic enzymes in the BMVs, produced by X. campestris pv. vesicatoria, has been demonstrated via immunogold EM, but enzymatic activity has yet to be shown in vitro (Sole et al. 2015).

The BMVs produced by X. campestris pv. campestris contain cellulase (Egl), xylan 1,4-beta-xilosidase (XynB) and beta-glucosidase (Bgl) (Sidhu et al. 2008; Sole et al. 2015) also demonstrated in X. campestris pv. vesicatoria that a protease and a lipase, in addition to the xylanases, could be exported either via the type 2 secretion system (T2SS) or via the BMVs (Fig. 5A and C). These studies suggest that BMVs can serve as an alternative secretion mechanism to the T2SS of X. campestris. The fact that Sidhu et al. (2008) also detected substrates of the type 3 secretion system (T3SS), such as AvrBs1, suggests that the BMV release can deliver a combination of T2SS and T3SS substrates that may cause greater damage to the plant cell in a contact-independent manner; see Fig. 5C (Sidhu et al. 2008; Sole et al. 2015). Finally, infection experiments conducted on the EPS-deficient X. campestris pv. vesicatoria bacteria have demonstrated that BMVs are also released in planta and have highlighted their presence in vivo (Sole et al. 2015).

Xylella fastidiosa is closely related to Xanthomonas. The current era is the second time this phytopathogen has been in the spotlight in the history of microbiology. For >80 years before the pathogen was recognized as an important and globally emerging bacterial agent that causes nercosis of the vascular tissue in broad-leaved trees and vines due to water stress, it was thought to be a virus until Davis, Purcell and Thomson (1978) successfully showed that the pathogen is a bacterium (Gardner and Hewitt 1974; Davis, Purcell and Thomson 1978; Hopkins and Purcell 2002; Chatterjee, Almeida and Lindow 2008a; Purcell 2013; Saponari et al. 2013; Martelli et al. 2016). Xylella fastidiosa can also be isolated as commensal from different plant species such as weeds (Taraxacum officinale, Vernonia sp. and Digitaria sp., among other plant species) or bushes (e.g. Rubus sp.), which can serve as natural reservoirs (Sadder and Bradbury 2005b). From there, they can be transmitted into susceptible hosts where the bacteria are unwellcome ‘guests’ (Sadder and Bradbury 2005b). This species circumscribes xylem-inhabiting, non-flagellated gammaproteobacteria (fam. Xanthomonadaceae) with de rigueur vector transmission by sap-feeding hemipters of the Cicadellidae family (Chatterjee, Almeida and Lindow 2008a; Sicard et al. 2018).

Interestingly, in a competition with other phytopathogens, only X. fastidiosa bacteria managed to adhere to insect wings that mimicked the foregut cuticle, thus highlighting how closely associated this bacterium has evolved to its vector (Killiny and Almeida 2009).

The biology of X. fastidiosa allows the bacteria to exist in two different habitats: in the xylem of its host and in the foregut of its vectors (Chatterjee, Almeida and Lindow 2008a; Sicard et al. 2018). In both habitats, the bacteria form structurally different biofilms on the dead tissue of the host and the vector; in planta, they co-aggregate and adhere to the xylem wall and secrete EPS, in which the bacteria remain embedded either tightly or loosely associated with each other (Purcell, Finlay and McLean 1979; Newman et al. 2004; Chatterjee, Wistrom and Lindow 2008b; Sun et al. 2013). In its vector, the bacteria colonize the oesophagus and the cibarium of the foregut in a polar manner, forming a mat-like biofilm (Purcell, Finlay and McLean 1979; Newman et al. 2004; Chatterjee, Wistrom and Lindow 2008b; Sun et al. 2013). Both biofilms must be strong enough to allow the bacteria to remain in the particular environment; at the same time, they must not be so strong that the bacteria can be disseminated or transmitted, which happens when a subpopulation of the bacteria in the biofilm enters its ‘exploratory phase’, as shown in Fig. 6 (Chatterjee, Almeida and Lindow 2008a).

This sophisticated lifestyle is tightly regulated by the RpfF–RpfC system, which is involved in quorum sensing via α,β-unsaturated fatty acid diffusible signal factor (DSF), in such a way that rpfF codes for an enoyl-CoA hydratase, and rpfC codes for a two-component hybrid system (Newman et al. 2004; Chatterjee, Wistrom and Lindow 2008b). The ΔrpfF mutants have been shown to be more virulent, but they fail to colonize and form biofilms in their vector, which determines their meagre transmission rate (Newman et al. 2004). But, ΔrpfC mutants over-express rpfF, leading to DSF overproduction and the formation of robust biofilm due to hyper-attachment, which overall results in reduced virulence and spreading in plants, as well as reduced vector transmission (Chatterjee, Wistrom and Lindow 2008b).

Xylella fastidiosa’s biology also provides its bacteria with the adaptive traits required for a host jump (i.e. infecting new plant species) and for persistence, both of which lead to recurrent infection (Hopkins and Purcell 2002; Chatterjee, Almeida and Lindow 2008a; Sicard et al. 2018). Genetic and experimental data have shown that X. fastidiosa does not code for T3SS, as shown in Fig. 5 (since its lifestyle does not involve direct contact with living host or vector cells), or its effector substrates, but X. fastidiosa secretes the extracellular enzymes through T2SS (Fig. 5), produces the typical EPS xanthan (with slight modifications) and can perform twitching motility with the help of type IV pili (Simpson et al. 2000; Chatterjee, Almeida and Lindow 2008a; Sicard et al. 2018). The adhesion and colonization of the host and the vector are achieved via the differential expression and localization of fimbrial and afimbrial adhesins (Simpson et al. 2000; Chatterjee, Almeida and Lindow 2008a; Sicard et al. 2018).

Xylella fastidiosa may regulate the different states of its lifestyles via BMV release (Fig. 6). Besides demonstrating the delivery of virulence factors (such as lipases/esterases, proteases and pectinases among others), proteomic and metabolomic experiments have also demonstrated that the BMVs produced by X. fastidiosa are rich in DSF, which suggests their involvement in the intercellular communication of these bacteria (Nascimento et al. 2016; Feitosa-Junior et al. 2019). Reports on different afimbrial adhesins, such as the hemagglutinins (HAS) HxfA and HxfB, the AT-1 autotransporters XadA1 and XatA, and the Omp PD1063, have also shown that the adhesins can be exported within BMVs and, together with the type 1 fimbriae, these adhesins contribute to the different stages of biofilm formation and cell-to-cell aggregation (Guilhabert and Kirkpatrick 2005; Feil, Feil and Lindow 2007; Voegel et al. 2010; Matsumoto et al. 2012; Ionescu et al. 2014; Pierce, Voegel and Kirkpatrick 2014). Based on mutational and phenotypic experiments, afimbrial PD1063 has been found to predominantly facilitate cell-to-cell contact, HxF and HxFB to initiate host colonization and XaA to participate in both
The life cycle of *Xylella fastidiosa*. Under the regulation of RpfF, the bacterium forms a biofilm in plants and in the bacterium’s vector. Both transmissions (from the vector into the plant and from the plant into the vector) require a loosening of the biofilm, which is mediated via BMVs, by serving as either an anti-adhesion factor or a secretion system of the afimbrial adhesins HfxA and HfxB. The black arrows represent the transmission to its vector, while the red arrows represent the processes that take place upon infection.

Studies on the BMV released by the Pierce’s disease-causing *X. fastidiosa* strain Temecula 1 have demonstrated that the final destination of HfxA and HfxB depends on the way they are secreted: they can be located on the bacterial OM, secreted independently in the extracellular milieu or released within BMVs; see Fig. 6 (Voegel et al. 2010). By random mutagenesis and infection experiments with Chardonnay grapevines, Guilhabert and Kirkpatrick (2005) demonstrated that ΔhfxA and ΔhfxB deletion mutants exhibit hypervirulent phenotypes. They proposed that HfxA and HfxB can serve as anti-virulence factors involved in the intercellular aggregation and attenuation of bacterial colonization (Guilhabert and Kirkpatrick 2005). If we combine their findings with those from the work of Voegel et al. (2010), we can propose that the BMV release of HAs can serve as a regulatory mechanism for the virulence of *X. fastidiosa*. When required, HfxA and HfxB are present on the cell envelope, but when the bacteria need to decrease their adhesiveness to be taken in by the vector, they secrete the HAs packed in BMVs, as shown in Fig. 6 (Voegel et al. 2010).

Killiny and Almeida’s (2009) experiments, however, demonstrated that HfxA and HfxB are upregulated by RpfF and that their importance for the early attachment and biofilm formation of *X. fastidiosa* bacteria in the vector also reflects their later transmission to plants. The transmission rates were found to be lower when the bacterial strains were inoculated separately in the vectors, most probably due to the reduced ability to remain in the vector: the bacteria showed a 36% transmission rate for the ΔhfxA bacteria, 46% for the ΔhfxB bacteria and 88% for the wild-type cells (Killiny and Almeida 2009). The presence of XatA further shows evidence of the involvement of the *X. fastidiosa’s* BMVs in biofilm formation (Matsumoto et al. 2012). XatA is an AT-1 autotransporter, previously shown to be involved in autoaggregation and biofilm formation; the symptoms caused by bacteria deficient in XatA are severely attenuated (Matsumoto et al. 2012). The fact that Matsumoto et al. detected XatA together with its passenger domain in the BMVs provides another layer of complexity: a secretion system within a secretion system that can be activated under the right conditions; see Fig. 5B.

In addition to the sessile and motile bacteria, a portion of *X. fastidiosa* cells (0.005% of the bacterial population) may endure extreme environmental hardships, such as the presence of copper and tetracycline, via entering growth arrest, associated with reduced metabolic activity: *X. fastidiosa* can form persisters (Muranaka et al. 2012). So far, studies have shown that MqsR is the main effector to not only positively regulate the biofilm formation but also increase the formation of persisters upon Cu stress (Lee et al. 2014; Merfa et al. 2016). Interestingly, mqsR expression also occurs under the regulation of the RpfF–RpfC system, since its gene expression is increased in the ΔrpfF mutant background (Wang, Li and Lindow 2012). Santiago et al. (2016) have provided evidence that the MqsR anti-toxin (YgiT) is secreted via BMVs. Although the mechanism of cytosolic YgiT anti-toxin secretion has yet to be discovered, one can envision that these bacteria might secrete the anti-toxin to shift the balance toward increased toxin activity, which eventually upregulates biofilm formation or activates the persister state (Santiago et al. 2016).

*Xylella fastidiosa’s* BMVs offer an interesting case for consideration—can they be regarded as bona fide virulence-associated factors, or are they present due to general mechanisms such as envelope stress, which has been shown to lead to increased vesiculation? Studies by Ionescu et al. (2014) offer interesting insights into the answer. The researchers have shown that *X. fastidiosa* uses the BMVs to control adhesion
to different surfaces, thus contributing to the transition from biofilm to the ‘exploratory’, planktonic phase (Fig. 6). In vivo and in vitro experiments demonstrated that the BMVs’ pre-treatment of insect wings led to a 3-fold decrease in bacterial attachment, and the presence of BMVs led to a 20-fold reduction in the bacterial adhesion to xylem 20-fold. Ionescu et al.’s study was one of the few to provide a direct link between BMV production and regulation by a dedicated virulence-regulatory system: the X. fastidiosa ΔrpfF hypervirulent mutant produced three times the number of BMVs, which, together with the other findings, suggests that these BMVs might have evolved into a mechanism to block bacterial adhesion.

Rhizobium etli is a soil-based, N₂-fixing alphaproteobacterium that enters symbiotic associations predominantly with the legumes Phaseolus vulgaris and Mimosa affinis (Poole, Ramachandran and Terpolilli 2018). Two recent studies on the BMVs produced by the plant endosymbiont R. etli demonstrated that these bacteria produce different BMV populations in a growth-phase-dependent manner and upon stimulation with naringenin (Taboada et al. 2019a,b). Taboada et al. (2019b) found that vesicles isolated from non-naringenin induced bacteria, mimicking the life of the bacteria in soil, contained different classes of proteins. The presence of the Rap and curlin autoaggregation/adherence proteins, and the PlyB-orthologous, PlyC and polysaccharidases in the vesicular content suggested involvement in biofilm formation and the presence of different ABC substrate binding transporters for substrate scavenging: a strategy used by oligotrophs. The BMVs were also shown to contain iron(III), hemin and hemichrome acquisition proteins, as well as Cu oxidase and KatG (catalase), which provide a cross-resistant adaptive mechanism linked to iron supply and defense against Cu- and oxidative stress (Taboada et al. 2019a,b). The authors also directly demonstrated that the rhizobial BMVs inhibited the growth of another bacterial soil competitor, B. subtilis (Taboada et al. 2019b). In contrast, the BMVs isolated from bacteria treated with naringenin contained increased levels of proteins that were found to participate in carbohydrate and nitrogen utilization (Taboada et al. 2019a). Ultimately, the authors showed that the BMVs did indeed carry the Nod factors (chitoooligosaccharides, which serve as a signal for nodulation), and they demonstrated that the BMVs from naringenin-treated bacteria could induce root-hair curling, which is a physiological response for establishing a symbiotic relationship (Taboada et al. 2019a).

Animal hosts

The majority of previous research on BMVs has focused on their mechanism and release by certain human pathogens and their interactions with hosts. Bacterial BMVs secreted by pathogenic bacteria can deliver a broad range of virulence factors to the host cells, including small molecules, endotoxins, exotoxins, lipids, DNA, RNA and microRNAs. BMVs thus enable both intracellular and extracellular bacteria to communicate with the host cell and to deliver their virulence factors to the intracellular compartments important for the modulation of various signaling pathways.

We have earlier demonstrated the role of the BMV-mediated transport of E. coli pore-forming toxin, cytolysin A (ClyA) (Wai et al. 2003). Based on our findings, we proposed that the BMV release can serve as a secretion mechanism for proteins that do not have a dedicated signal sequence for one of the secretion machineries. Furthermore, the BMV-associated ClyA has found to have considerably higher cytotoxicity toward mammalian cells compared with purified toxin due to the favorable redox environment offered by the vesicles (Wai et al. 2003). Several other studies have shown the importance of BMVs in the delivery of other virulence factors during host infection, including the
delivery of type 1-secreted α-hemolysin (Balsalobre et al. 2006) and cytotoxic necrotizing factor-1 (CNF1) (Kouokam et al. 2006). Similarly, Bielaszewska et al. (2013) have shown that Enterohemorrhagic E. coli (EHEC) delivers pore-forming toxins via BMVs that induce apoptosis in the host cells. In addition to delivering pore-forming toxin hemolysin, EHEC BMVs act as a potent delivery system for the transport of several other virulence factors to different cellular compartments of host cells. These factors include Shiga toxin 2a (Stx2a), flagellin and cytolethal distending toxin V (CdtV) (Bielaszewska et al. 2017).

Endotoxin (lipid A) is reportedly responsible for immune modulation during pathogenic invasions. Vanaja et al. (2016) revealed that BMVs produced by Gram-negative bacteria may act as vehicles for the delivery of LPS into the host cytosol and therefore lead to atypical inflammesome activation in vitro and in vivo. Several toxins bound to BMVs have been reported among Vibrio species, including the choler toxin and other secreted toxins and enzymes that are important to V. cholerae pathogenesis, such as the metalloprotease of Vibrio (PrtV) (Vaitkevicius et al. 2006; Rompikuntal et al. 2015), Zn-dependent HA protease (HAP) (Hase and Finkelstein 1991; Ghosh et al. 2006), Vibrio cytolysin (VCC) (Olivier et al. 2007), trypsin-like serine protease (VesC) (Snygkon et al. 2010) and accessory choler enterotoxin (Ace) (Kaper, Morris and Levine 1995).

BMVs produced by Helicobacter pylori play an important role in the bacteria’s pathogenesis by inducing local and systemic host inflammatory and immune responses. BMVs isolated from H. pylori are enriched in several lipid species including PE, lysophosphatidylethanolamine (LPE), phosphatidylethanolamine (PC) and lysophosphatidylcholine (LPC) (Olofsson et al. 2010). LPS anchored by lipid A in the OM is another important virulence component of H. pylori BMVs. In addition to lipid species, several proteins essential to effective H. pylori virulence have been found to be associated with BMVs, including blood-group antigen-binding adhesin (BabA), outer membrane protein A (OmpA), vacuolating cytotoxin (VacA), CagA, H. pylori neutrophil-activating protein (HP-NAP), adherence-associated lipoprotein (AlpA), urease and sialic acid-binding adhesin (SabA) (Olofsson et al. 2010; Lekmeechai et al. 2018). The free-soluble toxin VacA secreted by H. pylori is highly toxic to the epithelial cells, as it induces vacuolization in the host cells; BMV-bound VacA, however, mostly remains inactive (Ricci et al. 2005). The shedding of H. pylori OM has been detected by EM not only during the late stationary growth phase but also in gastric biopsies of infected individuals (Keenan et al. 2000; Yonezawa et al. 2011; Grande et al. 2015). BMVs are generally considered to be an effective way for extracellular bacterial pathogens to translocate the bacterial effector molecules through the mucosa to immune cells in tissues or even to the blood-circulation system.

Ecospeciation

At the end of this section on the role of BMVs in bacterial ecology, we would like to speculate how the development of a new vesiculation pattern, defined by the size, amount and content of the released BMVs, can contribute to certain ecospeciation, i.e. adaptive divergence of bacterial populations inhabiting different ecological environments (Begon, Townsend and Harper 2005; Nosil 2012; Lassalle, Muller and Nesme 2015). For ecological speciation to come into play, a bacterial subpopulation has to respond to a new environmental factor with a change in the pattern of their BMVs—a change that is considered adaptive. Consequently, this adaptive change should have a genetic basis that allows divergence between the given subpopulation and the rest of the individuals of the species. But if a pattern for bacterial vesiculation can develop, and be selected for due to external environmental stimuli, could it become genetically maintained? Even though the question regarding genetic control mechanism(s) of the degree of vesiculation has yet to be settled, we may consider findings pointing toward the environments where a hypothetical vesiculation pattern could be selected for and upon which genes natural selection could presumably act.

A body of evidence illustrates that different bacterial species can respond to challenging environments or the presence of nutritional substrate(s) with increased vesiculation (Dutson et al. 1971; Marvin, ter Beest and Witholt 1989; McBroom and Kuehn 2007; Manning and Kuehn 2011; Baumgarten et al. 2012; Mare-dia et al. 2012; Bager et al. 2013; Macdonald and Kuehn 2013; Yamaoka et al. 2014; Orench-Rivera and Kuehn 2016; Eberlein et al. 2018; Gerritzen et al. 2018). For example, Mycobacterium tuberculosis, H. pylori, Haemophilus influenzae, V. cholerae and E. coli increase their formation of BMVs upon iron limitation as summarized in Orench-Rivera and Kuehn (2016). Besides iron limitation, stresses due to oxygen radicals, temperature and osmotic changes, detergents and antibiotics have also been shown to enhance BMV production (McBroom and Kuehn 2007; Song et al. 2008; Manning and Kuehn 2011; Baumgarten et al. 2012; Mare-dia et al. 2012; Macdonald and Kuehn 2013; Orench-Rivera and Kuehn 2016; Gerritzen et al. 2018). In addition to the changes in different conditions, the presence of certain nutrients can also trigger BMV release as is the case with Pseudomonas fragi, the protosels of which are exported via BMVs when their bacteria are inoculated on muscle tissue, or with Meioterms ruber whose bacteria increase their vesiculation in the presence of feather keratin (Dutson et al. 1971; Tarrant et al. 1973; Yamaoka et al. 2014).

Furthermore, an increase in the BMV production, due to environmental stimuli, should also contribute to the bacterial fitness, i.e. the BMVs would be an adaptive trait. Such contributions may be part of a compensatory mechanism for the loss of envelope stress regulation, as exemplified by the BMV production by hypervesiculating E. coli strains, for example with a deletion in the yieM gene, when incubated in the presence of ethanol, polymixin B or colistin (McBroom and Kuehn 2007; Manning and Kuehn 2011). The BMV-mediated adaptive traits may also be linked to the increase in hydrophobicity resulting in biofilm formation by Pseudomonas putida when subjected to osmotic stress and heat shock (Baumgarten et al. 2012).

While the aforementioned examples introduce the putative selective conditions for vesiculation, other evidence resulting from genetic screens hints to the potential genetic pool of candidate genes for such natural selection (McBroom and Kuehn 2007; Song et al. 2008; Macdonald and Kuehn 2013; Kulp et al. 2015). A genome-wide screen revealed that mutations that result in defects in the enterobacterial common antigen and in changes of the LPS chain are leading to increased BMV release by E. coli (Kulp et al. 2015). Other evidence from genetic studies with E. coli, Salmonella, P. aeruginosa and V. cholerae suggests that mutations in genes that code for the Tol-Pal and OmpA proteins can change the degree of vesiculation and that is also an outcome of the overexpression of σE, the alternative sigma factor for envelope stress response (McBroom and Kuehn 2007; Song et al. 2008; Macdonald and Kuehn 2013; Kulp et al. 2015).

Some of the genes in the pool subjected to adaptive mutagensis may also be linked to certain trade-offs, as exemplified from earlier studies on cryoprotection (Bennett, Seaver and Calcott 1981; Calcott 1986). Experiments with S. Typhimurium strains that carry different defects in their LPS structure, such as ArfG and ArfA, demonstrated that these strains are more sensitive...
to low temperatures, detergents and antibiotics (Bennett, Seaver and Calcott 1981; Calcott 1986). Even though their corresponding E. coli deletion mutants are shown to be hypervesculating (Kulp et al. 2015), it is unlikely for such kind of bacterial mutants to be selected as prolific vesicle producers.

On the other hand, some genes may undergo selection that can improve bacterial fitness at low temperature correlating with enhanced vesicle production (Calcott 1986; McBroom and Kuehn 2007). In the context of cryopreservation, Calcott (1986) showed how viability of different E. coli mutants deficient in outer membrane proteins was altered when bacteria were subjected to freeze-thawing. One of the mutants lacking the EnvZ-OmpR two-component system exhibited 1.4-fold increased viability compared with the wild type (Calcott 1986). Interestingly, deficiency in the ompR gene has been shown by McBroom and Kuehn (2007) to also trigger hypervesculation and to improve bacterial fitness. From the findings of these two different studies, we suggest that the ompR gene would be a right candidate in the search for natural selection that can trigger ecospeciation under osmotic and low-temperature stress. Furthermore, we note that the ompR gene in fact has been shown to be a subject of adaptive mutagenesis as demonstrated by E. coli mutants deficient in the EnvZ–OmpR system that have already been isolated from the mouse gut after the bacteria successfully had become adapted to the intestinal environment (Giraud et al. 2008).

Another interesting aspect concerns the BMV release on supraspecies level. Again, when exactly the pattern of BMVs released by bacterial species within a genus (for example, compare Fig. 3B to C), and the regulation of this BMV release can be considered a new adaptive capability is still a matter of discussion. Let us assume for now that if an innovation in the pattern of vesiculation appears, the species can adapt to particular environmental conditions such as low or high temperatures, thus leading to ecospeciation.

One notable example is the gammaproteobacterial genus Vibrio, the representatives of which (some pathogenic) inhabit marine and estuarine ecosystems and can be associated with the surface or intestinal content of marine animals. All the vibrios examined to date produce BMVs, including V. anguillarum, V. cholerae, V. tasmanianis, V. shilomii, V. vulnificus and Aliivibrio fischeri (Kondo, Takade and Amako 1993; Song et al. 2008; Hong et al. 2009; Vanhove et al. 2015; Aschtgen et al. 2016; Li et al. 2016; Hampton et al. 2017) as some examples are shown in Fig. 3B and C. The logical question that follows is whether the natural vesiculation typical of vibrios can contribute to the adaptation of a Vibrio species to the different conditions under which BMV production appears to be adaptive.

Adaptation to low temperatures is one such condition, and Yamoto et al. (1999) isolated one such Vibrio species (V. rumoiensis) from an H2O2-treated drainage pool. Vibrio rumoiensis is a facultatively psychrophilic bacterium with a growth range between 2 and 34 °C; for an illustration, compare V. rumoiensis with V. cholerae, shown in Fig. 2; and Fig 3B and C. Even before the more intensive research on Vibrio’s BMVs started, Yamoto et al. (1999) had noticed that V. rumoiensis cells produce many blebs, i.e. BMVs (Fig. 3B). Besides being psychophilic, V. rumoiensis also has a high tolerance to oxidative stress due to the VktA catalase activity of its bacteria (Yamoto et al. 2000; Ichise et al. 2008). Since there is no N-terminal secretion signal revealing a likely secretion route, the development of the new BMV pattern may be considered as a potential mechanism for the extracellular delivery that contributes to the peroxide resistance (Yamoto et al. 2000; Ichise et al. 2008).

We can ask then whether the cold temperature de facto triggers the bacteria’s vesicle production (as is evident from the explored BMV-producing cold-loving γ-proteobacteria shown in Fig. 2) that later becomes a genetic trait. If that is the case, then we may consider that there must be a facultative psychrophile, which is profuse in vesiculation within a mesophilic genus of a species that is not. A new species was described in a study about isolates from the Norwegian tundra soil and denoted as the ‘sea ice’ bacterium Pedobacter arcticus (Zhou et al. 2012). Pedobacter arcticus is the only species from the soil inhabitants of Pedobacter (Sphingobacteriaceae, Bacteroidetes; compare P. arcticus to P. agri in Fig. 2) the bacteria of which were reported to both be psychrophilic and exhibit budding (Zhou et al. 2012).

CONCLUSIONS AND FUTURE DIRECTIONS

The guiding theme of this review has been the involvement of bacterial membrane vesicles in cyclic processes, collectively called eco-evolutionary (or ‘eco-evo’) dynamics, in which an environmental change induces an evolutionary change, and vice versa. While most of the studies from the literature examined for this review have considered the BMVs’ role in ecosystem engineering, their role in influencing evolutionary feedbacks—for example as feedbacks that result in speciation—has yet to be discovered.

To conclude this review, we would like to propose possible approaches and examples for future study. The time might be right to consider that the more we understand BMVs, the more feasible they can become as real, naturally occurring models in combination with artificial models for studies of how vesicles might play a role in the origin of life. The use of the tools of physics for direct manipulation, as well as the use of force spectroscopy techniques such as optical tweezers, may be beneficial for the field of BMVs, because such tools can provide answers to questions that are linked to not only vesicle dynamics and related shape transformations but also which forces may trigger these processes and which changes in a particular habitat can lead to their induction. In general, a comparison of the pattern of vesiculation of extremophiles with that of their closely related non-extremophiles may help to illustrate the adaptive potential provided by vesicles, because we may assume that the results of extremophile homeostasis could likely be translated to a non-extremophile lifestyle. Another potentially intriguing avenue of research would be to examine the role of the vesiculation of the alkaliphilic bacteria (optimum pH 10–11.5) because of the fact that their membranes may function well at high pH, but around neutral pH, they may become too fluid.

As is often the case with scientific quests, searches lead to new searches, and research on bacterial membrane vesicles in particular can offer an abundance of research avenues.

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REFERENCES

Abarca F, Gutierrez-Maldonado SE, Parada P et al. Insights on the structure and stability of Licanantase: a trimeric acid-stable coiled-coil lipoprotein from Acidithiobacillus thiooxidans. PeerJ 2014;2:e457.

Alegado RA, Brown IW, Cao S et al. A bacterial sulfolipid triggers multicellular development in the closest living relatives of animals. eLife 2012;1:e00013.

Allocati N, Masulli M, Di Ilio C et al. Die for the community: an overview of programmed cell death in bacteria. Cell Death Dis 2015;6:e1609.

Almeida DV, Martens SBB, Lanes CFC et al. Improved genetic transformation of Synechococcus elongatus PCC 7942 using linear DNA fragments in association with a DNase inhibitor. Biotechnol Res Innov 2017;1:123–8.

Andrade-Dominguez A, Salazar E, Vargas-Lagunas Mdel C et al. Eco-evolutionary feedbacks drive species interactions. ISME J 2014;8:1041–54.

Anne-Charllin A, Eva S, Gerhard JH. Abundance and activity of major groups of prokaryotic plankton in the coastal North Sea during spring and summer. Aquat Microb Ecol 2006;45:237–46.

Arnold JW, Shimkets LJ. Inhibition of cell–cell interactions in Myxococcus xanthus by congo red. J Bacteriol 1988;170:5765–70.

Aschtgen MS, Lynch JB, Koch E et al. Rotation of Vibrio fischeri flagella produces outer membrane vesicles that induce host development. J Bacteriol 2016;198:2156–66.

Averhoff B. Shuffling genes around in hot environments: the Thermusthermophilusflagella produces outer membrane vesicles that induce host development. J Bacteriol 2016;198:2156–66.

Azuma Y, Newton SB, Witter LD. Production of psychrophilic mutants from mesophilic bacteria by ultraviolet irradiation. J Dairy Sci 1962;45:1529–30.

Bager RJ, Persson G, Nesta B et al. Outer membrane vesicles reflect environmental cues in Gallibacterium anatis. Vet Microbiol 2013;167:565–72.

Balsalobre C, Silvan JM, Berglund S et al. Release of the type I secreted alpha-haemolysin via outer membrane vesicles from Escherichia coli. Mol Microbiol 2006;59:99–112.

Bar-Ziv R, Moses E. Instability and “pearling” states produced in tubular membranes by competition of curvature and tension. Phys Rev Lett 1994;73:1392–5.

Baross J, Hoffman SE. Submarine hydrothermal vents and associated gradient environments as sites for the origin and evolution of life. Origins Life Evol B 1985;15:327–45.

Barten R, Lill H. DNA-uptake in the naturally competent cyanobacterium, Synechocystis sp. PCC 6803. FEMS Microbiol Lett 2006;129:83–7.

Battistuzzi FU, Feijao A, Hedges SB. A genomic timescale of prokaryote evolution: insights into the origin of methanogenesis, phototrophy, and the colonization of land. BMC Evol Biol 2004;4:44.

Baumgarten T, Sperling S, Seifert J et al. Membrane vesicle formation as a multiple-stress response mechanism enhances Pseudomonas putida DOT-T1E cell surface hydrophobicity and biofilm formation. Appl Environ Microbiol 2012;78:6217–24.

Baumgartner RJ, Van Kranendonk MJ, Wacey D et al. Nanoporous pyrite and organic matter in 3.5-billion-year-old stromatolites record primordial life. Geology 2019;47:1039–1043.

Bayer ME, Bayer MH. Effects of bacteriophage fd infection on Escherichia coli HB11 envelope: a morphological and biochemical study. J Virol 1986;57:258–66.

Bayer ME. Areas of adhesion between wall and membrane of Escherichia coli. J Gen Microbiol 1968;53:395–404.

Bayer ME. Zones of membrane adhesion in the cryofixed envelope of Escherichia coli. J Struct Biol 1991;107:268–80.

Beatty JT, Overmann J, Linke MT et al. An obligately photosynthetic bacterial anaerobe from a deep-sea hydrothermal vent. Proc Natl Acad Sci U S A 2005;102:9306–10.

Becking B. Baas Becking’s: Geobiology or Introduction to Environmental Science. Chapter II The Environment. The Hague, The Netherlands: John Wiley & Sons, Ltd[ W. P. Van Stockum & Zoon, 1934], 1–16.

Begon M, Townsend CR, Harper JH. Resources. In: Ecology from Individuals to Ecosystems, USA, UK, Australia: Blackwell Publishing, 2006, 58–88.

Bekker A, Holland HD, Wang PL et al. Dating the rise of atmospheric oxygen. Nature 2004;427:117–20.

Bell EA, Boehnke P, Harrison TM et al. Potentially biogenic carbon preserved in a 4.1 billion-year-old zircon. Proc Natl Acad Sci U S A 2015;112:14518–24.

Bennett GM, Seaver A, Calcott PH. Effect of defined lipopolysaccharide core defects on resistance of Salmonella typhimurium to freezing and thawing and other stresses. Appl Environ Microbiol 1981;42:843–9.

Benzerara K, Morin G, Yoon TH et al. Nanoscale study of As biomineralization in an acid mine drainage system. Geochim Cosmochim Acta 2008;2008:3949–63.

Berczal N, Muller M, Walde P et al. Growth and transformation of vesicles studied by ferritin labeling and cryotransmission electron microscopy. J Phys Chem B 2001;105:1056–64.

Berleman JE, Allen S, Danielewicz MA et al. The lethal cargo of Myxococcus xanthus outer membrane vesicles. Front Microbiol 2014;5:474.

Beveridge TJ, Makin SA, Kadurugamuwa JL et al. Interactions between biofilms and the environment. FEMS Microbiol Rev 1997;20:291–303.

Beveridge TJ. Ultrastructure, chemistry, and function of the bacterial wall. Int Rev Cytol 1981;72:229–317.

Bielaszewska M, Rüter C, Bauwens A et al. Host cell interactions of outer membrane vesicle-associated virulence factors of enterohemorrhagic Escherichia coli O157: intracellular delivery, trafficking and mechanisms of cell injury. PLoS Pathog 2017;13:e1006159.

Bielaszewska M, Rüter C, Kunsmann L et al. Enterohemorrhagic Escherichia coli hemolysin employs outer membrane vesicles to target mitochondria and cause endothelial and epithelial apoptosis. PLoS Pathog 2013;9:e1003797.

Biller SJ, Schubotz F, Roggensack SE et al. Bacterial vesicles in marine ecosystems. Science 2014;343:183–6.

Blesa A, Berenguer J. Contribution of vesicle-protected extracellular DNA to horizontal gene transfer in Thermus spp. Int Microbiol 2015;18:177–87.

Bobadilla Fazzini RA, Leigvan C, Parada P. Acidithiobacillus thiooxidans secretome containing a newly described lipoprotein
Licant nantase enhances chalcopyrite bioleaching rate. Appl Microbiol Biotechnol 2011;89:771–80.

Bocian-Ostryzka KM, Grzesczuk MJ, Banaś AM et al. Bacterial thiol oxidoreductases - from basic research to new antibacterial strategies. Appl Microbiol Biotechnol 2017;101:3977–89.

Bohannan BJM, Lenski RE. Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. Ecol Lett 2000;3:362–77.

Bozal N, Montes MJ, Miñana-Galbis D et al. Sheuannaella vesiculos sp. nov., a psychrotolerant bacterium isolated from an Antarctic coastal area. Int J Syst Evol Microbiol 2009;59:336–40.

Braun V, Sieglin U. The covalent murein-lipoprotein structure of the Escherichia coli cell wall. Eur J Biochem 1970;13:336–46.

Brown L, Wolf JM, Prados-Rosales R et al. Through the wall: extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. Nat Rev Microbiol 2015;13:620–30.

Bunce C, Pinhasi J. Marine bacterioplankton seasonal succession dynamics. Trends Microbiol 2017;25:494–505.

Burde ID, Murray RG. Electron microscope study of septum formation in Escherichia coli strains B and B-r during synchronous growth. J Bacteriol 1974;119:1039–56.

Calcott PH. Cryopreservation of microorganisms. Crit Rev Biotechnol 1986;4:279–97.

Cascales E, Lloubes R. Deletion analyses of the peptidoglycan biosynthesis systems: bacteriophages show the way. FEMS Microbiol Rev 2013;37:554–71.

Cavalier-Smith T. The neomuran origin of archaeabacteria, the negibacterial root of the universal tree and bacterial mega-classification. Int J Syst Evol Microbiol 2002;52:7–76.

Chamberlain AK, Faham S, Yohannon S et al. Construction of helix-bundle membrane proteins. Adv Protein Chem 2003;63:19–46.

Chan CS, De Stasio G, Welch SA et al. Microbial polysaccharides template assembly of nanocrystal fibers. Science 2004;303:1656–8.

Chatterjee S, Almeida RP, Lindow S. Living in two worlds: the plant and insect lifestyles of Xylella fastidiosa. Annu Rev Phytopathol 2008a;46:243–71.

Chatterjee S, Wistrom C, Lindow SE. A cell–cell signaling sensor is required for virulence and insect transmission of Xylella fastidiosa. Proc Natl Acad Sci U S A 2008b;105:2670–5.

Chen C, Kawamoto J, Kawai S et al. Isolation of a novel bacterial strain capable of producing abundant extracellular membrane vesicles carrying a single major cargo protein and analysis of its transport mechanism. Front Microbiol 2019;10:3001.

Cheng S, Thomas JK, Kulpa CF. Dynamics of pyrene fluorescence in Escherichia coli membrane vesicles. Biochemistry 1974;13:1135–9.

Cheng ZL, Luisi PL. Coexistence and mutual competition of vesicles with different size distributions. J Phys Chem B 2003;107:10940–5.

Chen IA, Roberts RW, Szostak JW. The emergence of competition between model protocells. Science 2004;305:1474–6.

Chen IA, Szostak JW. A kinetic study of the growth of fatty acid vesicles. Biophys J 2004;87:988–98.

Clavel T, Germon P, Vianney A et al. TolB protein of Escherichia coli K-12 interacts with the outer membrane peptidoglycan-associated proteins Pal, Lpp and OmpA. Mol Microbiol 1998;29:359–67.

Cocera M, Lopez O, Codereh L et al. Partitioning of SDS in liposomes coated by the exopolymer excreted by Pseudoalteromonas antarctica NF3 as a measure of vesicle protection against this surfactant. J Biomater Sci Polym Ed 2001;12:255–66.

Cocera M, Lopez O, Parra JL et al. Protective effect caused by the exopolymer excreted by Pseudoalteromonas antarctica NF3 on liposomes against the action of octyl glucoside. Int J Pharm 2000;207:39–47.

Collins T, Margesin R. Psychrophilic lifestyles: mechanisms of adaptation and biotechnological tools. Appl Microbiol Biotechnol 2019;103:2857–71.

Cologgi DL, Lampa-Pastirk S, Speers AM et al. Extracellular reduction of uranium via Geobacter conductive pili as a protective cellular mechanism. Proc Natl Acad Sci U S A 2011;108:15248–52.

Cordovez V, Dini-Andreote F, Carrion VJ et al. Ecology and evolution of plant microbiomes. Annu Rev Microbiol 2019;73:69–88.

Crowlesmith I, Schindler M, Osborn MJ. Bacteriophage P22 is not a likely probe for zones of adhesion between the inner and outer membranes of Salmonella typhimurium. J Bacteriol 1978;135:259–69.

Cushnie TP, O’Driscoll NH, Lamb AJ. Morphological and ultrastructural changes in bacterial cells as an indicator of antibacterial mechanism of action. Cell Mol Life Sci 2016;73:4471–92.

da Costa Allgayer M, Nobre M, Rainey F. Thermus. In: Margesin R (ed). Classification. Int J Syst Evol Microbiol 2013;63:19–46.

Davis MJ, Purcell AH, Thomson SV. Pierce’s disease of Pierce’s disease of reclaim pecans. Reclaim pecans. In: Davis MJ, Purcell AH, Thomson SV. Pierce’s disease of Xylella fastidiosa. Proc Natl Acad Sci U S A 2008b;105:2670–5.

Deming JW, Young JN. The role of exopolysaccharides in microbial adaptation to cold habitats. In: Margesin R (ed). Psychrophiles: From Biodiversity to Biotechnology. Cham: Springer International Publishing, 2017, 259–84.

De Mot R, Vanderleyden J. The C-terminal sequence conservation between OmpA-related outer membrane proteins and MotB suggests a common function in both Gram-positive and Gram-negative bacteria, possibly in the interaction of these domains with peptidoglycan. Mol Microbiol 1994;12:333–4.

Devoe IW, Gilchrist JE. Release of endotoxin in the form of cell wall blebs during in vitro growth of Neisseria meningitidis. J Exp Med 1973;138:1156–67.

Dutson TR, Pearson AM, Price JF et al. Observations by electron microscopy on pig muscle inoculated and incubated with Pseudomonas fragi. Appl Microbiol 1971;22:1152–8.
Dutta A, Peoples LM, Gupta A et al. Exploring the piezotolerant/piezophilic microbial community and genomic basis of piezotolerance within the deep subsurface Deccan traps. Extremophiles 2019;23:421–33.

Dworkin M, Bonner JT. The myxobacteria: new directions in studies of procaryotic development. CRC Crit Rev Microbiol 1972;1:435–52.

Eberlein C, Baumgarten T, Starke S et al. Immediate response mechanisms of Gram-negative solvent-tolerant bacteria to cope with environmental stress: cis–trans isomerization of unsaturated fatty acids and outer membrane vesicle secretion. Appl Microbiol Biotechnol 2018;102:2583–93.

Eme L, Spang A, Lombard J et al. Archaea and the origin of eukaryotes. Nat Rev Microbiol 2018;16:120.

Essich E, Stevens SE, Jr, Porter RD Chromosomal transformation in the cyanobacterium *A. quadruplicatum*. J Bacteriol 1990;172:1916–22.

Evans AGL, Davey HM, Cookson A et al. Predatory activity of Myxococcus xanthus outer-membrane vesicles and properties of their hydrodase cargo. Microbiology 2012;158:2742–52.

Feil H, Feil WS, Lindow SE. Contribution of fimbrial and afimbrial adhesins of *Xylella fastidiosa* to attachment to surfaces and virulence to grape. Phytopathology 2007;97:318–24.

Feitosa-Junior OR, Stefanello E, Zaini PA et al. Proteomic and metabolomic analyses of *Xylella fastidiosa* OMV-enriched fractions reveal association with virulence factors and signaling molecules of the DSF family. Phytopathology 2019;109:1344–53.

Feng DF, Cho G, Doolittle RF. Determining divergence times with a protein clock: update and reevaluation. Proc Natl Acad Sci U S A 1997;94:13028–33.

Fierer N, Bradford MA, Jackson RB. Toward an ecological classification of soil bacteria. FEMS Microbiology Reviews 2007;31:541–58.

Gardner MW, Hewitt WB. Pierce’s Disease of the Grapevine: The Anaheim Disease and the California Vine Disease: a Historical Account of the Long Search and Final Solution on the Cause and Nature of a Destructive Disease of the Grapevine. Berkeley: University of California, 1974.

Gauthier MJ, Lafay B, Christen R et al. Marinobacter hydrocarbon-oclasticus gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. Int J Syst Bacteriol 1992;42:568–76.

Gerritzen MJH, Maas RHW, van den Ijssel J et al. High dissolved oxygen tension triggers outer membrane vesicle formation by *Neisseria meningitidis*. Microb Cell Fact 2018;17:157.

Gilbert SF, Bosch TC, Ledon-Rettig C. Eco-Evo-Devo: developmental symbiosis and developmental plasticity as evolutionary agents. Nat Rev Genet 2015;16:611–22.

Gil H, Benach JL, Thanassi DG. Presence of pili on the surface of *Francisella tularensis*. Infect Immun 2004;72:3042–7.

Gill S, Catchpole R, Forterre P. Extracellular membrane vesicles in the three domains of life and beyond. FEMS Microbiol Rev 2019;43:273–303.

Giovannoni SJ, Stingl U. Molecular diversity and ecology of microbial plankton. Nature 2005;437:343–8.

Giraud A, Arous S, De Paepe M et al. Dissecting the genetic components of adaptation of *Escherichia coli* to the mouse gut. PLoS Genet 2008;4:e2.

Gomez-Silva B. Lithobiontic life: “Atacama rocks are well and alive”. Antonie Van Leeuwenhoek 2018;111:1333–43.

Gould SB, Garg SG, Martin WF. Bacterial vesicle secretion and the evolutionary origin of the eukaryotic endomembrane system. Trends Microbiol 2016;24:525–34.

Grande R, Di Marcantonio MC, Robuffo I et al. Helicobacter pylori ATCC 43629/NCTC 11639 outer membrane vesicles (OMVs) from biofilm and planktonic phase associated with extracellular DNA (eDNA). Front Microbiol 2015;6:1369.

Guilhabert MR, Kirkpatrick BC. Identification of *Xylella fastidiosa* antivirulence genes: hemagglutinin adhesins contribute to biofilm maturation to X. fastidiosa and colonization and attenuate virulence. Mol Plant Microbe Interact 2005;18:856–68.

Hampton CM, Guerrero-Ferreira RC, Storms RE et al. The opportunistic pathogen *Vibrio vulnificus* produces outer membrane vesicles in a spatially distinct manner related to capsular polysaccharide. Front Microbiol 2017;8:2177.

Hanczyc MM, Fujikawa SM, Szostak JW. Experimental models of primitive cellular compartments: encapsulation, growth, division. Science 2003;302:618–22.

Hansen SK, Rainey PB, Haagensen JA et al. Evolution of species interactions in a biofilm community. Nature 2007;445:533–6.

Hase CC, Finkelstein RA. Cloning and nucleotide sequence of the Vibrio cholerae hemagglutinin/protease (HA/protease) gene and construction of an HA/protease-negative strain. J Bacteriol 1991;173:3311–7.

Hedges SB, Chen H, Kumar S et al. A genomic timescale for the origin of eukaryotes. BMC Evol Biol 2001;1:4.

Hellmann S, Sneppen K, Krishna S. Sustainability of virulence in a phage-bacterial ecosystem. J Virol 2010;84:3016–22.

Henderson JC, Zimmerman SM, Crofts AA et al. The power of asymmetry: architecture and assembly of the Gram-negative outer membrane lipid bilayer. Annu Rev Microbiol 2016;70:255–78.

Hennebique A, Boisset S, Maurin M. Tularemia as a waterborne disease: a review. Emerg Microbes Infect 2019;8:1027–42.
Hoekstra D, van der Laan JW, de Leij L et al. Release of outer membrane fragments from normally growing Escherichia coli. Biochim Biophys Acta 1976;455:889–99.

Hong GE, Kim DC, Park EM et al. Identification of Vibrio anguillarum outer membrane vesicles related to immunostimulation in the Japanese flounder, Paralichthys olivaceus. Biosci Biotechnol Biochem 2009;73:437–9.

Hook LA. Distribution of myxobacteria in aquatic habitats of an alkaline bog. Appl Environ Microbiol 1977;34:333–5.

Hopkins DL, Purcell AH. Xylella fastidiosa: cause of Pierce’s disease of grapevine and other emergent diseases. Plant Dis 2002;86:1056–66.

Huang Q, Jiang H, Briggs BR et al. Archaeal and bacterial diversity in acidic to circumneutral hot springs in the Philippines. FEMS Microbiol Ecol 2013;85:452–64.

Iichise N, Hirota K, Ichihashi D et al. H2O2 tolerance of Vibrio rumoiensis S-1(T) is attributable to the cellular catalase activity. J Biosci Bioeng 2008;106:39–45.

Ingraham JL, Stokes JL. Psychrophilic bacteria. Bacteriol Rev 1959;23:97–108.

Ionescu M, Zaini PA, Baccari C et al. Xylella fastidiosa outer membrane vesicles modulate plant colonization by blocking assembly and functional implication of the gene cluster for selection and analyses of outer membrane vesicles. Natl Acad Sci U S A 2014;111:E3910–8.

Jacques MA, Arelat M, Boulander A et al. Using ecology, physiology, and genomics to understand host specificity in Xanthomonas. Annu Rev Phytopathol 2016;54:163–87.

Jarić M, Seifert U, Wintz W et al. Vesicular instabilities: the prolate-to-oblate transition and other shape instabilities of fluid bilayer membranes. Phys Rev E 1995;52:6623–34.

Jebbar M, Franzetti B, Girard E et al. Microbial diversity and adaptation to high hydrostatic pressure in deep-sea hydrothermal vents prokaryotes. Extremophiles 2015;19:721–40.

Johnson DB, Hallberg KB. The microbiology of acidic mine waters. Res Microbiol 2003;154:466–73.

Johnson DB. Geomicrobiology of extremely acidic subsurface environments. FEMS Microbiol Ecol 2012;81:2–12.

Jones CG, Lawton JH, Shackah M. Organisms as ecosystem engineers. Oikos 1994;69:373–86.

Jordan SF, Rammu H, Zheludev IN et al. Promotion of protocell self-assembly from mixed amphiphiles at the origin of life. Nat Ecol Evol 2019;3:1705–14.

Jorgensen BB, Boetius A. Feast and famine: microbial life in the deep-sea bed. Nat Rev Microbiol 2007;5:770–81.

Kadurugamuwa JL, Beveridge TJ. 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 1995;177:3998–4008.

Kahnt J, Aguiluz K, Koch J et al. Profiling the outer membrane proteome during growth and development of the social bacterium Myxococcus xanthus s by selective biotinylation and analyses of outer membrane vesicles. J Proteome Res 2010;9:5197–208.

Kalynchyn S, Morona R, Cygler M. Progress in understanding the assembly process of bacterial O-antigen. FEMS Microbiol Rev 2014;38:1048–65.

Kamasaka K, Kawamoto J, Chen C et al. Genetic characterization and functional implications of the gene cluster for selective protein transport to extracellular membrane vesicles of Sheuanaella vesiculosa HM13. Biochem Biophys Res Commun 2020;526:525–31.

Kaper JB, Morris JG, Jr, Levine MM. Cholera. Clin Microbiol Rev 1995;8:48–86.

Kassen R, Rainey PB. The ecology and genetics of microbial diversity. Annu Rev Microbiol 2004;58:207–31.

Kawamoto J, Kurihara T, Yamamoto K et al. Ecosapentaenoic acid plays a beneficial role in membrane organization and cell division of a cold-adapted bacterium, Sheuanaella livingstonensis Ac10. J Bacteriol 2009;191:632–40.

Keenan J, Day T, Neal S et al. A role for the bacterial outer membrane in the pathogenesis of Helicobacter pylori infection. FEMS Microbiol Lett 2000;182:259–64.

Kellenberger E. The ‘Bayer bridges’ confronted with results from improved electron microscopy methods. Mol Microbiol 1990;4:697–705.

Kelly D, Wood A. Genus Acidithiobacillus. In Garrity G ed. Bergey’s Manual of Systematic Bacteriology, Springer, 2004, 60–2.

Killiny N, Almeida RP. Xylella fastidiosa afmbrial adhesins mediate cell transmission to plants by leafhopper vectors. Appl Environ Microbiol 2009;75:521–8.

Kluyver AJ. The changing appraisal of the microbe. Leeuwenhoek Lect 1953;141:147–61.

Knickerbocker C, Nordstrom DK, Southam G. The role of “blebbing” in overcoming the hydrophobic barrier during biooxidation of elemental sulfur by Thiobacillus thiooxidans. Chem Biol 2000;169:425–33.

Knox KW, Ves M, Work E. Relation between excreted lipopolysaccharide complexes and surface structures of a lysine-limited culture of Escherichia coli. J Bacteriol 1966;92:1206–17.

Koch AL. Oligotrophs versus copiotrophs. Bioessays 2001;23:657–61.

Kondo K, Takada A, Amako K. Release of the outer membrane vesicles from Vibrio cholerae and Vibrio parahaemolyticus. Microbiol Immunol 1993;37:149–52.

Kouokam JC, Wai SN, Fallman M et al. Active cytotoxic necrotizing factor 1 associated with outer membrane vesicles from uropathogenic Escherichia coli. Infect Immun 2006;74:222–30.

Kulkarni HM, Jagannadham MV. Biogenesis and multifaceted roles of outer membrane vesicles from Gram-negative bacteria. Microbiology 2014;160:2109–21.

Kulp A, Kuehn MJ. Biological functions and biogenesis of secreted bacterial outer membrane vesicles. Annu Rev Microbiol 2010;64:163–84.

Kulp AJ, Sun B, Ai T et al. Genome-wide assessment of outer membrane vesicle production in Escherichia coli. PloS One 2015;10:e0139200.

Kumar S, Stecher G, Suleski M et al. TimeTree: a resource for timelines, timetrees, and divergence times. Mol Biol Evol 2017;34:1812–9.

Kuznetsov SI, Dubinina GA, Lapteva NA. Biology of oligotrophic bacteria. Annu Rev Microbiol 1979;33:377–87.

Kyoung M, Sheets ED. Vesicle diffusion close to a membrane: intermembrane interactions measured with fluorescence correlation spectroscopy. Biophys J 2008;95:5789–97.

Larson CL, Wicht W, Jellison WL. A new organism resembling P. tularensis isolated from water. Public Health Rep 1955;70:253–8.

Lassalle F, Muller D, Nesme X. Ecological speciation in bacteria: reverse ecology approaches reveal the adaptive part of bacterial cladogenesis. Res Microbiol 2015;166:729–41.

Leduc M, Frehel C. Characterization of adhesion zones in P. aeruginosa isolated from water. Annu Rev Microbiol 2003;57:466–73.

Lee MW, Tan CC, Rogers EE et al. Bacteriol Rev 2020;85:452–64.

Leeuwenhoek Manual of Systematic Bacteriology. Springer, 2004, 60–2.
Leive L. Domains involving nonrandom distribution of lipopolysaccharide in the outer membrane of Escherichia coli. Proc Natl Acad Sci U S A 1977;74:5065–8.

Lekmeechai S, Su YC, Brant M et al. Helicobacter pylori outer membrane vesicles protect the pathogen from reactive oxygen species of the respiratory burst. Front Microbiol 2018;9:1837.

Lewis K. Persister cells. Annu Rev Microbiol 2010;64:357–72.

Licata NA, Mohari B, Fuqua C et al. Diffusion of bacterial cells in porous media. Biophys J 2016;110:247–57.

Li J, Azam F, Zhang S. Outer membrane vesicles containing signalling molecules and active hydrolytic enzymes released by a coral pathogen Vibrio shilonii AK1. Environ Microbiol 2016;18:3850–66.

Lindh MV, Sjöstedt J, Andersson AF et al. Disentangling seasonal bacterioplankton population dynamics by high-frequency sampling. Environ Microbiol 2015;17:2459–76.

Li Y-Z, Hu W, Zhang Y-Q et al. A simple method to isolate salt-tolerant myxobacteria from marine samples. J Microbiol Methods 2002;50:205–9.

Lo Giudice A, Poli A, Finore I et al. Peculiarities of extracellular polymeric substances produced by Antarctic bacteria and their possible applications. Appl Microbiol Biotechnol 2020;104:2923–34.

Lopez J, Webster RE. Assembly site of bacteriophages f1 corresponds to adhesion zones between the inner and outer membranes of the host cell. J Bacteriol 1985;163:1270–4.

Lorv JS, Rose DR, Glick BR. Bacterial ice crystal controlling proteins. Sciencia (Cairo) 2014;2014:976895.

Lozupone CA, Knight R. Global patterns in bacterial diversity. Proc Natl Acad Sci U S A 2007;104:11436–40.

Luigi PL, Stano P, Rasi S et al. A possible route to prebiotic vesicle formation and vesicle formation systems in Archaea. Nat Rev Microbiol 2004;2:731–41.

Macdonald IA, Kuehn MJ. Stress-induced outer membrane vesicle production by Pseudomonas aeruginosa. J Bacteriol 2013;195:2971–81.

Makarova KS, Yutin N, Bell SD et al. Evolution of diverse cell division and vesicle formation systems in Archaea. Nat Rev Microbiol 2010;8:731–41.

Malinverni JC, Silhavy TJ. An ABC transport system that maintains lipid asymmetry in the Gram-negative outer membrane. Proc Natl Acad Sci U S A 2009;106:8009–14.

Manning AJ, Kuehn MJ. Contribution of bacterial outer membrane vesicles to innate bacterial defense. BMC Microbiol 2011;11:258.

Manning AJ, Kuehn MJ. Functional advantages conferred by extracellular prokaryotic membrane vesicles. J Mol Microbiol Biotechnol 2013;23:131–41.

Maredia R, Devineni N, Lentz P et al. Vesiculation from Pseudomonas aeruginosa under SOS. ScientificWorldJournal2012;2012:402919.

Margolin J, El-Etr S, Joubert L-M et al. Contributions of Francisella tularensis subsp. novicida chitinases and Sec secretion system to biofilm formation on chitin. Appl Environ Microbiol 2009;75:596–608.

Martelli GP, Boscia D, Porcelli F et al. The olive quick decline syndrome in south–east Italy: a threatening phytosanitary emergency. Eur J Plant Pathol 2016;144:235–43.

Martin W, Baross J, Kelley D et al. Hydrothermal vents and the origin of life. Nat Rev Microbiol 2008;6:805–14.

Marvin HJ, ter Beest MB, Witholt B. Release of outer membrane fragments from wild-type Escherichia coli and from several E. coli lipopolysaccharide mutants by EDTA and heat shock treatments. J Bacteriol 1989;171:5262–7.

Matsumoto A, Huston SL, Killiny N et al. XatA, an AT-1 autotransporter important for the virulence of Xylella fastidiosa Temecula1. Microbiologyopen 2012;1:33–45.

Mayrand D, Grenier D. Biological activities of outer membrane vesicles. Can J Microbiol 1989;35:607–13.

McBroom AJ, Kuehn MJ. Release of outer membrane vesicles by Gram-negative bacteria is a novel envelope stress response. Mol Microbiol 2007;63:545–58.

McCaig WD, Koller A, Thanassi DG. Production of outer membrane vesicles and outer membrane tubes by Francisella novicida. J Bacteriol 2013;195:1120–32.

Merfa MV, Niza B, Takita MA et al. The MgsR toxin-antitoxin system from Xylella fastidiosa plays a key role in bacterial fitness, pathogenicity, and persister cell formation. Front Microbiol 2016;7:904.

Mesbah NM, Wiegel J. Life under multiple extreme conditions: diversity and physiology of the halophilic alkalithermophiles. Appl Environ Microbiol 2012;78:4074–82.

Mkandawire M. Biogeochemical behaviour and bioremediation of uranium in waters of abandoned mines. Environ Sci Pollut Res Int 2013;20:7740–67.

Moller JD, Barnes AC, Dalsgaard I et al. Characterisation of surface blebbing and membrane vesicles produced by Flavobacterium psychrophilum. Dis Aquat Organ 2005;64:201–9.

Morein S, Andersson A, Rilfors L et al. Wild-type Escherichia coli cells regulate the membrane lipid composition in a “window” between gel and non-lamellar structures. J Biol Chem 1996;271:6801–9.

Morita RY. Psychrophilic bacteria. Bacteriol Rev 1975;39:144–67.

Mouchka ME, Hewson I, Harvell CD. Coral-associated bacterial assemblies: current knowledge and the potential for climate-driven impacts. Integr Comp Biol 2010;50:662–74.

Mug-Opstelten D, Witholt B. Preferential release of new outer membrane fragments by exponentially growing Escherichia coli. Biochim Biophys Acta 1978;508:287–95.

Muranaka LS, Takita MA, Olivato JC et al. Global expression profile of biofilm resistance to antimicrobial compounds in the plant-pathogenic bacterium Xylella fastidiosa reveals evidence of persister cells. J Bacteriol 2012;194:4561–9.

Muryoi N, Matsukawa K, Yamade K et al. Purification and properties of an ice-nucleating protein from an ice-nucleating bacterium, Pantoaea ananatis KUIN-3. J Biosci Bioeng 2003;95:157–63.

Nakayama K, Takashima K, Ishihara H et al. The R-type pyocin of Pseudomonas aeruginosa is related to P2 phage, and the F-type is related to lambda phage. Mol Microbiol 2000;38:213–31.

Namani T, Deamer DW. Stability of model membranes in extreme environments. Org Life Evol Biosph 2008;38:329–41.

Nascimento R, Gouran H, Chakraborty S et al. The type II secreted lipase/esterase LesA is a key virulence factor required for Xylella fastidiosa pathogenesis in grapevines. Sci Rep 2016;6:18598.

Nevot M, Deroncele V, Lopez-Iglesias C et al. Ultrastructural analysis of the extracellular matter secreted by the psychrotolerant bacterium Pseudoalteromonas antarctica NF3. Microb Ecol 2006a;51:501–7.

Nevot M, Deroncele V, Messner P et al. Characterization of outer membrane vesicles released by the psychrotolerant bacterium Pseudoalteromonas antarctica NF3. Environ Microbiol 2006b;8:1523–33.
Newman KL, Almeida RF, Purcell AH et al. Cell–cell signaling controls Xylella fastidiosa interactions with both insects and plants. Proc Natl Acad Sci U S A 2004;101:1737–42.

Nichols CA, Guzennec J, Bowman JP. Bacterial exopolysaccharides from extreme marine environments with special consideration of the southern ocean, sea ice, and deep-sea hydrothermal vents: a review. Mar Biotechnol (NY) 2005;7:253–71.

Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 2003;67:593–656.

Nishida AH, Ochman H. A great-ape view of the gut microbiome. In: Nishida AH, Ochman H. A great-ape view of the gut microbiome. Science 2016;352:524–527.

Nosil PI. What is ecological speciation? In: Ecological Speciation, Oxford: OUP, 2012, 3–21.

Nichols CA, Guezennec J, Bowman JP. Bacterial exopolysaccharides from extreme marine environments with special consideration of the southern ocean, sea ice, and deep-sea hydrothermal vents: a review. Mar Biotechnol (NY) 2005;7:253–71.

Parsons LM, Lin F, Orban J. Peptidoglycan recognition by Pal, an outer membrane lipoprotein. J Bacteriol 2005;187:267–70.

Newman KL, Almeida RF, Purcell AH et al. Cell–cell signaling controls Xylella fastidiosa interactions with both insects and plants. Proc Natl Acad Sci U S A 2004;101:1737–42.

Nichols CA, Guzennec J, Bowman JP. Bacterial exopolysaccharides from extreme marine environments with special consideration of the southern ocean, sea ice, and deep-sea hydrothermal vents: a review. Mar Biotechnol (NY) 2005;7:253–71.

Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 2003;67:593–656.

Nishida AH, Ochman H. A great-ape view of the gut microbiome. In: Nishida AH, Ochman H. A great-ape view of the gut microbiome. Science 2016;352:524–527.

Nosil PI. What is ecological speciation? In: Ecological Speciation, Oxford: OUP, 2012, 3–21.

Nichols CA, Guezennec J, Bowman JP. Bacterial exopolysaccharides from extreme marine environments with special consideration of the southern ocean, sea ice, and deep-sea hydrothermal vents: a review. Mar Biotechnol (NY) 2005;7:253–71.

Parsons LM, Lin F, Orban J. Peptidoglycan recognition by Pal, an outer membrane lipoprotein. J Bacteriol 2005;187:267–70.

Newman KL, Almeida RF, Purcell AH et al. Cell–cell signaling controls Xylella fastidiosa interactions with both insects and plants. Proc Natl Acad Sci U S A 2004;101:1737–42.

Nichols CA, Guzennec J, Bowman JP. Bacterial exopolysaccharides from extreme marine environments with special consideration of the southern ocean, sea ice, and deep-sea hydrothermal vents: a review. Mar Biotechnol (NY) 2005;7:253–71.

Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 2003;67:593–656.

Nishida AH, Ochman H. A great-ape view of the gut microbiome. In: Nishida AH, Ochman H. A great-ape view of the gut microbiome. Science 2016;352:524–527.

Nosil PI. What is ecological speciation? In: Ecological Speciation, Oxford: OUP, 2012, 3–21.

Nichols CA, Guezennec J, Bowman JP. Bacterial exopolysaccharides from extreme marine environments with special consideration of the southern ocean, sea ice, and deep-sea hydrothermal vents: a review. Mar Biotechnol (NY) 2005;7:253–71.

Parsons LM, Lin F, Orban J. Peptidoglycan recognition by Pal, an outer membrane lipoprotein. J Bacteriol 2005;187:267–70.

Newman KL, Almeida RF, Purcell AH et al. Cell–cell signaling controls Xylella fastidiosa interactions with both insects and plants. Proc Natl Acad Sci U S A 2004;101:1737–42.

Nichols CA, Guzennec J, Bowman JP. Bacterial exopolysaccharides from extreme marine environments with special consideration of the southern ocean, sea ice, and deep-sea hydrothermal vents: a review. Mar Biotechnol (NY) 2005;7:253–71.

Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 2003;67:593–656.

Nishida AH, Ochman H. A great-ape view of the gut microbiome. In: Nishida AH, Ochman H. A great-ape view of the gut microbiome. Science 2016;352:524–527.

Nosil PI. What is ecological speciation? In: Ecological Speciation, Oxford: OUP, 2012, 3–21.
