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MINI-REVIEW

Outer membrane vesicles of *Lysobacter* sp. XL1: biogenesis, functions, and applied prospects

Irina V. Kudryakova[1] · **Nina A. Shishkova**[2] · **Natalia V. Vasilyeva**[1]

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Abstract Outer membrane vesicles (OMVs) produced by Gram-negative bacteria have been intensively investigated in recent times. Vesicle formation models have been proposed, some factors affecting the process were established, and important roles vesicles play in vital activities of their producing cells were determined. Studies of pathogenic bacterial vesicles contribute to understanding the causes of acute infection and developing drugs on their basis. Despite intensive research, issues associated with the understanding of vesicle biogenesis, the mechanisms of bacterium–bacterium and pathogen–host interactions with participation of vesicles, still remain unresolved. This review discusses some results obtained in the research into OMVs of *Lysobacter* sp. XL1 VKM B-1576. This bacterium secretes into the environment a spectrum of bacteriolytic enzymes that hydrolyze peptidoglycan of competing bacteria, thus leading to their lysis. One of these enzymes, lytic endopeptidase L5, has been shown not only to be secreted by means of vesicles but also to be involved in their formation. As part of vesicles, the antimicrobial potential of L5 enzyme has been found to be considerably expanded. Vesicles have been shown to have a therapeutic effect in respect of anthrax infection and staphylococcal sepsis modelled in mice. The scientific basis for constructing liposomal antimicrobial preparations from vesicle phospholipids and recombinant bacteriolytic enzyme L5 has been formed.

Keywords Outer membrane vesicles · Vesicles biogenesis · *Lysobacter* sp. XL1 · Bacteriolytic enzymes · Antimicrobial agents

✉ Natalia V. Vasilyeva
vasilyevanv@rambler.ru

[1] Laboratory of Microbial Cell Surface Biochemistry, G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, 5 Prospect Nauki, Pushchino, Moscow Region, Russia

[2] Laboratory of the Microbiology of Anthrax, State Research Center for Applied Microbiology and Biotechnology, Obolensk, Moscow Region, Russia

Introduction

Formation of OMVs is a widespread process among Gram-negative bacteria (Kadurugamuwa and Beveridge 1997; Beveridge 1999; Kuehn and Kesty 2005; Balsalobre et al. 2006; Vasilyeva et al. 2008, 2009; Olofsson et al. 2010; Moon et al. 2012). Vesicles are represented by spherical structures 20–300 nm in size. The first publication on vesicles dealt with their formation in *Vibrio cholerae* by splitting off the outer membrane of the bacterium (Chatterjee and Das 1967). In 1989, after a research into their structure, they began to be called outer membrane vesicles (Mayrand and Grenier 1989). Apart from outer membrane components (proteins, lipopolysaccharide, phospholipids), vesicles comprise components of the periplasm (periplasmic proteins, including autolytic enzymes; cell wall fragments) and, in pathogenic bacteria, virulence factors (Kadurugamuwa and Beveridge 1995; Horstman and Kuehn 2000; Kato et al. 2002; Lee et al. 2008; Olofsson et al. 2010; Roier et al. 2015). An increasing number of recent works indicate that vesicles include components of the cytoplasm (including DNA and RNA) and cytoplasmic membrane (Lee et al. 2008; Olofsson et al. 2010; Pérez-Cruz et al. 2013; Zielke et al. 2014; Pérez-Cruz et al. 2015). Their small size and specific composition enable vesicles to perform important functions in bacterial vital activities: secretion of proteins, utilization of toxic metabolites, acquisition of nutrients, and expansion of the ecological niche (Li et al. 1996, 1998; Kadurugamuwa and

Beveridge 1997; Kobayashi et al. 2000; Kuehn and Kesty 2005; Haurat et al. 2011; Evans et al. 2012; Vasilyeva et al. 2014; Olsen and Amano 2015; Xie 2015; Wang et al. 2015). Comprehension of the importance of vesicular studies by the scientific community contributed to the intensive development of two trends in their research, the studies of the formation mechanism (biogenesis) and of the functional significance. The trends were founded by American scientists Jagath L. Kadurugamuwa and Terry J. Beveridge. The more information started to appear about vesicles, about their structure, performed functions and biogenesis, the more questions began to emerge about how they form, which factors are involved in their formation, how vesicles perform their functions. We will try to partially answer these questions, as exemplified by one bacterium, *Lysobacter* sp. XL1.

The genus *Lysobacter* belongs to the family *Xanthomonadaceae* and was first described in 1978 (Christensen and Cook 1978). Initially, the genus included four species: *Lysobacter antibioticus*, *Lysobacter brunescent*, *Lysobacter enzymogenes*, and *Lysobacter gummosus* (de Bruijn et al. 2015). To date, approximately 25 species have been described. The genus owes its name to a high lytic activity manifested by its representatives with respect to Gram-positive and Gram-negative bacteria, fungi, nematodes, and unicellular algae (Christensen and Cook 1978). A broad spectrum of *Lysobacter* spp. lytic action is due to production of a range of biologically active compounds: extracellular enzymes (proteases, peptidoglycan hydrolases, glucanases, lipases, chitinases), short peptides (e.g., cyclo(L-Pro-L-Tyr)), antibiotics (cyclodepsipeptides, cyclic lipodepsipeptides, etc.) (Bone et al. 1989; Kato et al. 1998; Ahmed et al. 2003; Palumbo et al. 2003, 2005; Ogura et al. 2006; Ko et al. 2009; Xie et al. 2012; Cimmino et al. 2014; Pidot et al. 2014; Puopolo et al. 2014). *Lysobacter* spp. are Gram-negative rod-like bacteria (Reichenbach 2006). Their representatives inhabit soils and freshwater reservoirs (Christensen and Cook 1978; Reichenbach 2006; de Bruijn et al. 2015). *Lysobacter* spp. are anaerobic organisms, though they can survive at low (10 %) concentrations of oxygen; optimum pH varies from 7 up to 9; the temperature optimum, in the range of 30 °C, though it varies depending on the species (Reichenbach 2006). Before our research, nothing was known about the ability of *Lysobacter* representatives to form vesicles.

Our laboratory investigates the bacterium *Lysobacter* sp. XL1. This bacterium was isolated from waters of the Oka River near Pushchino. The bacterium produces a complex of bacteriolytic enzymes L1–L5, based on which an efficient antimicrobial drug, lysoamidase, was developed (Kulaev et al. 2006). Lysoamidase is efficient against Gram-positive pathogenic bacteria multiply resistant to antimicrobial preparations and is permitted for external use. Bacteriolytic enzymes, constituents of the preparation, exhibit various substrate specificities with respect to peptidoglycan of pathogenic bacteria. Enzymes L1, L4, and L5 are endopeptidases and break the peptide bond of the peptide subunit in the interpeptide bridge of peptidoglycan; L1 and L2 are amidases and destroy the bond between the first amino acid of the peptide subunit and *N*-acetylglucosamine; L3 is muramidase that cleaves the bond between *N*-acetylglucosamine and *N*-acetylmuramic acid (Stepnaya et al. 1996, 2005; Begunova et al. 2003; Vasilyeva et al. 2014). Each lysoamidase protein is a potential base for developing new-generation antimicrobial drugs for internal use, which could be used for treatment of infections caused by strains resistant to antibiotics. For this reason, our interest in research into the topogenesis of these proteins is evident. Thus, studies of the secretion of L5 from cells of *Lysobacter* sp. XL1 into the environment found this bacterium to be capable of forming vesicles (Vasilyeva et al. 2008). The secretion proved to be performed by means of these vesicles. The antimicrobial action spectrum of protein L5 within vesicles was also found to be significantly broader than that of its soluble form (Vasilyeva et al. 2014). Those results contributed to the development of three trends of research: studies of vesicle biogenesis, investigation of the antimicrobial potential and curative action of vesicles, and construction of liposomal antimicrobial preparations based on particular lysoamidase lytic enzymes and phospholipids of *Lysobacter* sp. XL1 vesicles. This will be discussed in the review.

Vesicle biogenesis of Gram-negative bacteria

The vesicle formation mechanism (biogenesis) is at present the most debatable topic of investigations in this field. All accumulated information about this process was reduced by Mashburn-Warren and Whiteley (2006) to three models describing the vesicle formation; the models were then supplemented by other investigators (Kulp and Kuehn 2010; Schwechheimer et al. 2013, 2014; Schwechheimer and Kuehn 2015). The first model combines the data that the biogenesis of vesicles occurs in sites of a temporary rupture of bonds between the inner leaflet of the outer membrane and peptidoglycan (e.g., the breakdown of the lipoprotein–peptidoglycan bond) (Hoekstra et al. 1976; Wensink and Witholt 1981; Schwechheimer et al. 2013; Schwechheimer et al. 2014). The second model is based on the results supporting the formation of vesicles in sites of periplasmic components' pressure on the inner side of the outer membrane (these can be peptidoglycan fragments and misfolded proteins) (Zhou et al. 1998; Hayashi et al. 2002; McBroom and Kuehn 2007; Tashiro et al. 2009). The basis of the third model are the data on the involvement of cell envelope components, that form sites of outer membrane destabilization due to their biochemical structure, in the vesicle formation process (e.g., B-type lipopolysaccharide, Pseudomonas quinolone signal (PQS)

molecules) (Kadurugamuwa and Beveridge 1995; Sabra et al. 2003; Mashburn-Warren and Whiteley 2006; Schertzer and Whiteley 2012).

Thus, formation of vesicles is performed by one mechanism, through bulging and pinching off of the outer membrane. The factors that determine the process can be different but all of them are either components of the cell envelope or are functionally coupled with it (e.g., periplasmic proteins). As the result of the effect of these factors, there occurs a locus disturbance of outer membrane rigidity, which is accompanied by the formation of vesicles. However, it is not to be ruled out that the process is affected by both several factors simultaneously and each of them separately, which can lead to the formation of heterogeneous vesicles within one cell. Heterogeneous vesicles have already been found to be formed by *Aggregatibacter actinomycetemcomitans*, *E. coli*, *Helicobacter pylori* (Balsalobre et al. 2006; Olofsson et al. 2010; Rompikuntal et al. 2012). Formation of these vesicles by one bacterial taxon can be determined by specific features of their biogenesis, in particular, by the action of several factors on the bacterial outer membrane. Our research has in part been aimed to confirm this.

Vesicle biogenesis of *Lysobacter* sp. XL1

The ability of cells of *Lysobacter* sp. XL1 to form vesicles was established in studies of extracellular bacteriolytic enzymes it secretes. The bacteriolytic enzymes of this bacterium efficiently hydrolyze peptidoglycan, the main structural component of cell walls in competitive bacteria. One of the most investigated enzymes is lytic protease L5, which is 56 % homologous to α-lytic protease of *Lysobacter enzymogenes* (Granovsky et al. 2010, 2011; Lapteva et al. 2012). Proceeding from the homology to α-lytic protease (Silen et al. 1989; Fujishige et al. 1992), it could have been assumed that, being synthesized as prepro proteins, L5 are secreted into the environment in two stages: first via the cytoplasmic membrane into the periplasm, which is accompanied with the processing of the pre moiety, and then via the outer membrane, presumably, by means of a type II secretory mechanism, which is accompanied with the splitting-off of the pro moiety and the appearance of mature protein in the extracellular medium. However, L5 was found to be secreted into the environment by means of OMVs, which proved to form cells of *Lysobacter* sp. XL1 (Fig. 1a) (Vasilyeva et al. 2008). *Lysobacter* sp. XL1 vesicles are 30 up to 160 nm in diameter and have a protein composition similar to, but not identical to, outer membranes (Vasilyeva et al. 2009). It was also found that vesicles of smaller, about 20 nm, but homogeneous size (Fig. 1b) form under conditions blocking the secretion of lytic enzymes. The totality of the data prompted several ideas: first, that vesicles of *Lysobacter* sp. XL1 are heterogeneous not only by size but also by composition and, possibly, by performed functions; and second, that one of the factors that determine the vesicle formation process can be secreted protein L5 itself.

To prove the heterogeneity, a preparation of *Lysobacter* sp. XL1 vesicles was fractionated in a sucrose density gradient (Kudryakova et al. 2015). Two subpopulations of vesicles were isolated: of a lighter fraction, 30–65 nm in diameter and containing protein L5; and of a heavier fraction, 65–100 nm in diameter, containing no protein L5 in their composition. A comparative electrophoregram of vesicular proteins in the fractions revealed an additional difference in their protein composition. Thus, it was established that *Lysobacter* sp. XL1 did form vesicles heterogeneous by size, density, and protein composition. The same experiments confirmed that protein L5 could play a role in the formation of special secretory vesicles. The role of protein L5 in vesicle biogenesis was also studied by the electron microscopic immunocytochemistry of ultrathin sections of *Lysobacter* sp. XL1 cells (Fig. 2). It is seen in the figure that in the process of the topogenesis protein L5 is translocated to the periplasmic space and concentrates in certain periplasm loci adjacent to the inner leaflet of the outer membrane; subsequently, vesicles are formed out of those loci. Probably, there is some affinity of protein L5 to the outer membrane that contributes to its concentration in certain loci; this leads to a pressure on it and a disturbance of rigidity. Additional confirmation of the involvement of L5 in vesicle biogenesis was obtained in studies of its secretion in recombinant strain *P. fluorescens* Q2–87/B (Vasilyeva et al. 2013). Recombinant protein L5 was found not only to be secreted by means of vesicles of strain Q2–87/B but also to affect the vesicle formation process. Using electron microscopy, strain Q2–87/B expressing recombinant protein L5 was found to form a greater number of vesicles of a more heterogeneous diameter as compared with vesicles of parent strain Q2–87.

On the whole, the involvement of *Lysobacter* sp. XL1 secreted protein L5 can be considered within the framework of the second model of vesicle biogenesis. However, in this case, the biogenesis proceeds with the participation of protein, which is functionally significant for the cell and makes use of vesicles as a transport means, not protein debris as described for this model. Besides, the totality of all the data indicates that protein L5 has some functional features that determine its ability to affect the vesicle formation process. Future research (including structure studies) will, possibly, clarify this issue. It is not to be ruled out that data on the involvement of secreted proteins in the vesicle biogenesis of Gram-negative bacteria will subsequently be accumulated, which would allow considering this process within the framework of a new model.

Thus, we have found that *Lysobacter* sp. XL1 forms heterogeneous vesicles. And one of the factors that determine this process is secreted protein L5. But the process may also involve other factors: phospholipids, lipopolysaccharide, and lipoprotein. Unlike the latter two, the involvement of

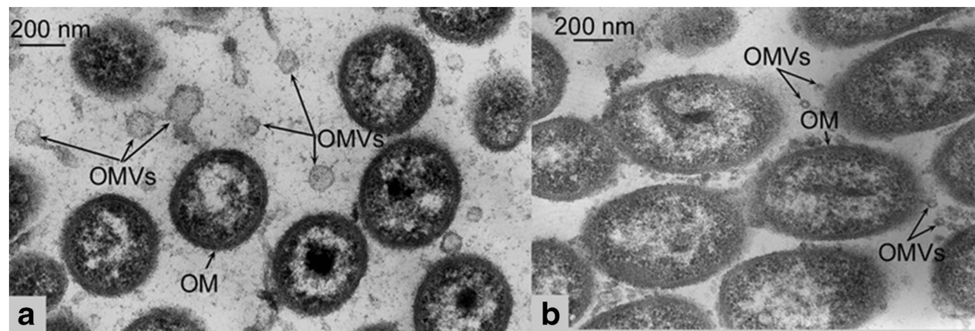

Fig. 1 Ultrathin sections of *Lysobacter* sp. XL1 cells. **a** Under secretion conditions, *Lysobacter* sp. XL1 forms vesicles 30–160 nm in size. **b** Under blocked secretion conditions, formed vesicles are 20 nm in diameter. *OM* outer membrane, *OMVs* outer membrane vesicles

phospholipids has not yet been attributed to any of the considered vesicle biogenesis models, because experimental data are still scarce. But phospholipids together with lipopolysaccharide are the main components of outer membranes. Namely, phospholipids determine the spherical shape of vesicles. It would be logical to assume their important role in the formation of these structures. To date, there are only some data on the role of phospholipids in vesicle formation. Thus, *P. syringae* Lz4W vesicles' phospholipids were found to be enriched with unsaturated branched fatty acids (Chowdhury and Jagannadham 2013). An assumption was made that an increased membrane flexibility of segments enriched with phospholipids with these fatty acids might contribute to vesicle biogenesis. On the contrary, for *P. aeruginosa* vesicles it was shown that vesicle phospholipids consisted predominantly of elongated and saturated fatty acids, which was indicative of the vesicle formation exclusively from the more rigid segments of the outer membrane (Tashiro et al. 2011). We felt it topical to continue our research from establishing the participation of phospholipids in the biogenesis of *Lysobacter* sp. XL1 vesicles.

We carried out a comparative analysis of phospholipids of *Lysobacter* sp. XL1 outer membranes and vesicles by two-dimensional thin-layer chromatography (unpublished data) (Fig. 3). In the figure, it is seen that outer membranes contain a range of phospholipids: the major ones among them are cardiolipin, phosphatidylethanolamine, and unidentified phospholipid; phosphatidylglycerol, phosphatidylmonomethylethanolamine, and a group of unidentified phospholipids occur in minor amounts. In contrast, vesicles reveal a small diversity of phospholipids: the major phospholipid is cardiolipin, and phosphatidylglycerol and an unidentified phospholipid occur in minor amounts. Evidently, vesicles form predominantly out of outer membrane segments enriched with cardiolipin. As the hydrophilic head of cardiolipin carries two negative charges, the rigidity of the outer membrane can possibly be disturbed due to their intermolecular repulsion in segments enriched with this phospholipid. Thus, we established one more factor determining the formation of vesicles in *Lysobacter* sp. XL1.

Based on the literature data and our own results, we can supplement the model of *Lysobacter* sp. XL1 vesicle biogenesis published earlier (Kudryakova et al. 2015). We feel that the vesicle formation mechanism is the same for all Gram-negative bacteria: the outer membrane evaginates due to a disturbance of its rigidity, and the evagination is completed by the formation of a vesicle. But the factors that determine the process can be different. For *Lysobacter* sp. XL1, two such factors have been established: secreted protein L5 and acid phospholipid cardiolipin (Fig. 4). It is not to be ruled out that both factors act simultaneously. Subsequently, our plans are to establish the action of other factors, too, which will not only

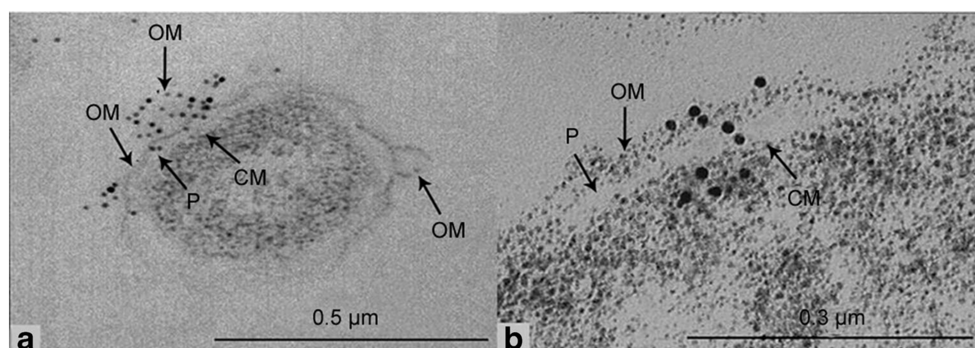

Fig. 2 Electron microscopic immunocytochemistry of *Lysobacter* sp. XL1 cells. **a** Heterogeneous vesicles form within one bacterial cell. **b** Protein L5 concentrates in certain periplasmic loci adjacent to the inner leaflet of the outer membrane. *OM* outer membrane, *P* periplasm, *CM* cytoplasmic membrane

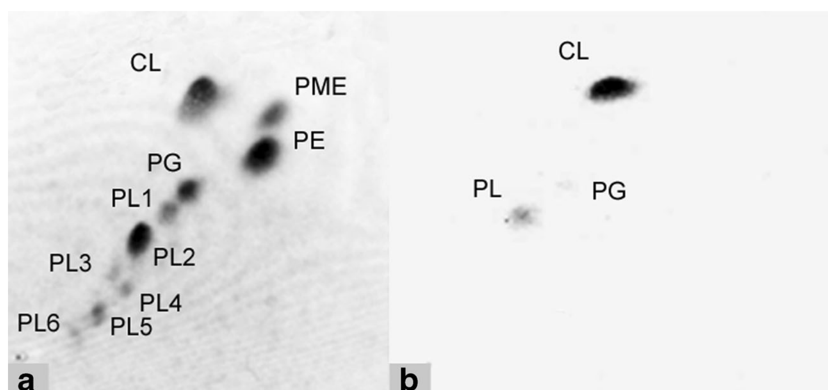

Fig. 3 Two-dimensional thin-layer chromatography of *Lysobacter* sp. XL1 outer membranes and vesicles. **a** Spectrum of outer membrane phospholipids. **b** Spectrum of vesicle phospholipids. *CL* cardiolipin, *PME* phosphatidylmonomethylethanolamine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PL–PL6* unidentified phospholipids

expand the views of this process in *Lysobacter* sp. XL1 but will also contribute to the understanding of vesicle biogenesis in Gram-negative bacteria on the whole.

Functional significance and applied prospects of vesicles

OMVs play an enormous role in bacterial cell activity. They have been the topic of numerous studies. To date, vesicles have been proven to be involved in protection of bacteria from stress factors (Kadurugamuwa and Beveridge 1995, 1996, 1997; Kobayashi et al. 2000). Vesicles mediate the formation of biofilms, including due to the co-aggregation with other bacteria, which also determines a better survivability of microorganisms in the environment (Kuehn and Kesty 2005; Olsen and Amano 2015; Xie 2015; Wang et al. 2015). It has been found that β-lactamase is secreted into the environment by means of vesicles, which enables protection of the bacterium from the group of β-lactam antibiotics, deeper layers of the biofilm including (Ciofu et al. 2000). Vesicles are a convenient means for exchanging advantageous material (plasmids, DNA fragments) between bacteria, which contributes to increasing their survivability (Yaron et al. 2000; Renelli et al. 2004; Biller et al. 2014). Vesicles of *P. aeruginosa* PAO1 contain autolysin lyzing other bacteria, which enables it to acquire nutrients and occupy a

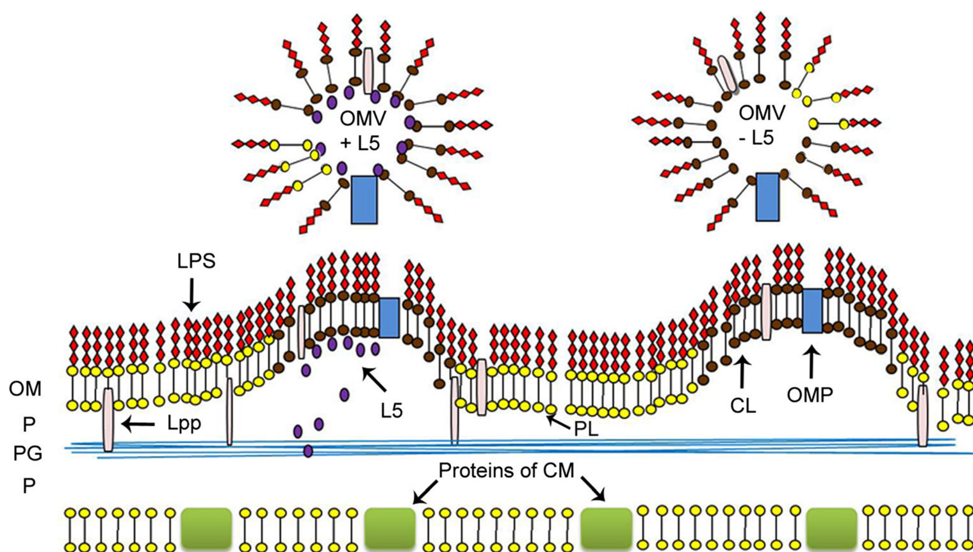

Fig. 4 A model of the biogenesis of *Lysobacter* sp. XL1 vesicles. As *Lysobacter* sp. XL1 vesicles are heterogeneous, they form under the influence of various factors. One of the factors is secreted protein L5, which concentrates in certain loci of the periplasm on the inner leaflet of the outer membrane. It is in those loci that vesicles containing it are formed. Another factor, presumably, due to its biochemical structure, is acid phospholipid cardiolipin: as the hydrophilic head of cardiolipin carries two negative charges, the rigidity of the outer membrane can be disturbed due to their intermolecular repulsion. The influence of other factors on the biogenesis of *Lysobacter* sp. XL1 vesicles is yet to be established. *PG* peptidoglycan, *P* periplasm, *OM* outer membrane, *CM* cytoplasmic membrane, *LPS* lipopolysaccharide, *Lpp* lipoprotein, *CL* cardiolipin, *PL* phospholipids, *OMP* outer membrane proteins, *OMV + L5* vesicles containing protein L5, *OMV−L5* vesicles containing no protein L5

certain ecological niche (Li et al. 1996). Because of such an aggression with respect to competing bacteria, the authors called them predatory vesicles. *Myxococcus xanthus* vesicles, secreting alkaline phosphatase into the extracellular space, under starvation conditions also procure phosphates required to cells by predatory activities (Evans et al. 2012). Very intensive research is under way into vesicles produced by pathogenic bacteria; they are used to release virulence factors, which makes them dangerous participants of the pathogenesis (Kato et al. 2002; Kuehn and Kesty 2005; Mashburn-Warren et al. 2008; Amano et al. 2010; Ellis and Kuehn 2010; Tashiro et al. 2012; Kulkarni et al. 2014; Avila-Calderón et al. 2015; Olsen and Amano 2015; Xie 2015). To date, some mechanisms of how vesicles of pathogenic bacteria penetrate into host tissues have been established (Amano et al. 2010; Olofsson et al. 2014; Kaparakis-Liaskos and Ferrero 2015; Olsen and Amano 2015). It is evident that research into the significance of vesicles in both bacterium–bacterium and pathogen–host interactions is of enormous importance for microbial ecology and medicine.

Antimicrobial potential of *Lysobacter* sp. XL1 vesicles

An important part of our studies is the research into bacteriolytic enzymes of *Lysobacter* sp. XL1. The occurrence of bacteriolytic enzyme L5 in *Lysobacter* sp. XL1 vesicles, certainly, contributed to studies of their antimicrobial and therapeutic potential.

We used the spot test method to investigate the antimicrobial action of *Lysobacter* sp. XL1 vesicles as compared with homogeneous protein L5. First, we chose opportunistic pathogenic strains from the laboratory collection, including Gram-positive and Gram-negative bacteria, yeasts and mycelial fungi. The lytic effect was determined by the zones of lysis in spots where preparations were applied. The lytic action spectrum of protein L5 within vesicles proved to be significantly extended as compared with its homogeneous form (Table 1) (Vasilyeva et al. 2014). Gram-positive bacteria were lysed by vesicles more intensively than Gram-negative ones. This can be due to particular features of their cell envelope structure and, correspondingly, by the different mechanisms of vesicular action on them. Mechanisms of the lytic action of autolysin-containing vesicles have already been studied earlier (Kadurugamuwa and Beveridge 1996). By analogy with the proposed schemes, during the interaction with the surface of Gram-positive bacteria vesicles presumably open, and protein L5, due to its high concentration, rather intensively cleaves the peptidoglycan in the adhesion zone. Gram-negative bacteria have an outer membrane, so, probably, vesicles fuse with it due to a high similarity of their structures. As the result of fusion, enzyme L5 goes out into the periplasmic space and freely diffuses in it; herewith, its concentration may decrease, which can be a cause of a weaker lytic effect.

Jointly with the State Research Center for Applied Microbiology and Biotechnology, we also investigated the lytic action of the vesicle preparation on pathogenic bacteria, including on clinical isolates multiply resistant to antimicrobial preparations (Vasilyeva et al. 2014). Vesicles lyzed excellently all chosen Gram-positive test objects, except lacto- and bifidobacteria (which is of interest), and absolutely failed to lyze Gram-negative test objects. Noteworthy is the efficient lysis of multiply resistant *S. aureus* (MRSA) strains, clinical isolates of the most widespread hospital-acquired infections.

Curative potential of *Lysobacter* sp. XL1 vesicles

Recent years have witnessed a significant worldwide increase in the resistance of infection agents to antibiotics. The World Health Organization considers antimicrobial resistance as one of the highest priority problems. In this connection, alternative ways of controlling pathogenic bacteria have to be searched for. Lytic bacterial enzymes capable of dissolving microbial cells are one of such ways. We have begun research into the curative effect of vesicles containing lytic enzyme L5. Vesicles in this case are a model, which in future can be used to develop liposomal antimicrobial preparations.

Jointly with the State Research Center for Applied Microbiology and Biotechnology, we investigated the curative action of vesicles on the anthrax infection, lethal for white outbred mice, induced by *Bacillus anthracis* vaccine strain 71/12 (Shishkova et al. 2013). Anthrax was modelled using pregerminated spores of *B. anthracis* 71/12. Mice were treated with vesicles and with doxycycline, an antibiotic traditionally used for these purposes. The treatment with doxycycline was started 3 h after the infection in the first day and was continued once a day for 5 days. The treatment with vesicles was by single dosing 3 h after the infection. The animals were observed for 14 days. As the result, the experimental animals treated with vesicles were found to be totally cured. The animals treated with doxycycline died in the next 10 days after the cancellation of the treatment. In the control group, all animals died in the first 2 days. For prophylaxis of the disease, the preparation of vesicles was introduced 3 h before the infection. As the result, the preparation ensured a 100 % prophylactic protection: not a single animal died. Thus, the efficiency of the vesicle preparation against lethal infection was established.

The next task was to establish the curative action of vesicles on staphylococcal infection caused by methicillin-resistant *S. aureus* strain 55. *S. aureus* is one of the prevailing pathogens in hospitals; what is more, as compared with other Gram-positive bacteria, namely strains of this bacterium determine a high mortality rate (Gostev et al. 2015; Yin et al. 2015). To study the therapeutic effect of the vesicle preparation with respect to the systemic staphylococcal sepsis, outbred white mice were infected with a fresh culture of MRSA

Table 1 Lytic action spectrum of the *Lysobacter* sp. XL1 vesicle and protein L5 preparations.

Microorganisms	Lytic effect of OMVs	Lytic effect of protein L5
	Gram-positive bacteria	
Bacillus subtilis W23	++[a]	+
Bacillus subtilis 168	++	+
Bacillus subtilis var. niger	++	+
Bacillus cereus 217	++	−
Bacillus cereus 504	++	−
Bacillus cereus 164	++	−
Bacillus megaterium 1433	++	Not detected
Bacillus thuringiensis 1373	++	Not detected
Bacillus thuringiensis EG 7566	++	Not detected
Bacillus mesentericus	++	Not detected
Bacillus brevis 1409	++	Not detected
Bacillus polymyxa 1396	++	Not detected
Bacillus anthracis 71/12	++	−
Bacillus anthracis M71	++	−
Bacillus anthracis STI	++	−
Bacillus anthracis STI PR	++	−
Bacillus anthracis STI pBC16	++	−
Bacillus anthracis STI 5	++	−
Micrococcus roseus B1236	++	+
Micrococcus luteus B1819	++	+
Corynebacterium xerosis	++	−
Staphylococcus aureus 209P	++	−
Rathayibacter tritici	++	−
Serratia marcescens 570	++	Not detected
Listeria monocytogenes L	++	−
Listeria monocytogenes R	++	−
	Gram-negative bacteria	
Pseudomonas fluorescens 1472	+b	−
Pseudomonas putida	+	+
Proteus vulgaris H-19	+	+
Proteus mirabilis N2	+	+
Escherichia coli K12	++	++
Erwinia carotovora B15	++	−
Alcaligenes faecalis	++	++
	Yeasts	
Torulaspora delbrueckii VKM Y-706	–c	−
Candida utilis VKM Y-74	+	+
Candida boidinii VKM Y-34	+	+
Candida guilliermondii VKM Y-41	+	+
Saccharomyces cerevisiae M660	−	−
Pseudozyma fusiformata VKM Y-2821	+	−
	Filamentous fungi	
Sclerotinium sclerotiorum	−	Not detected
Fusarium sporotrichiella	++	Not detected

The results were confirmed in three independent experiments

[a] Very good lytic effect

[b] Lytic effect

[c] No lytic effect

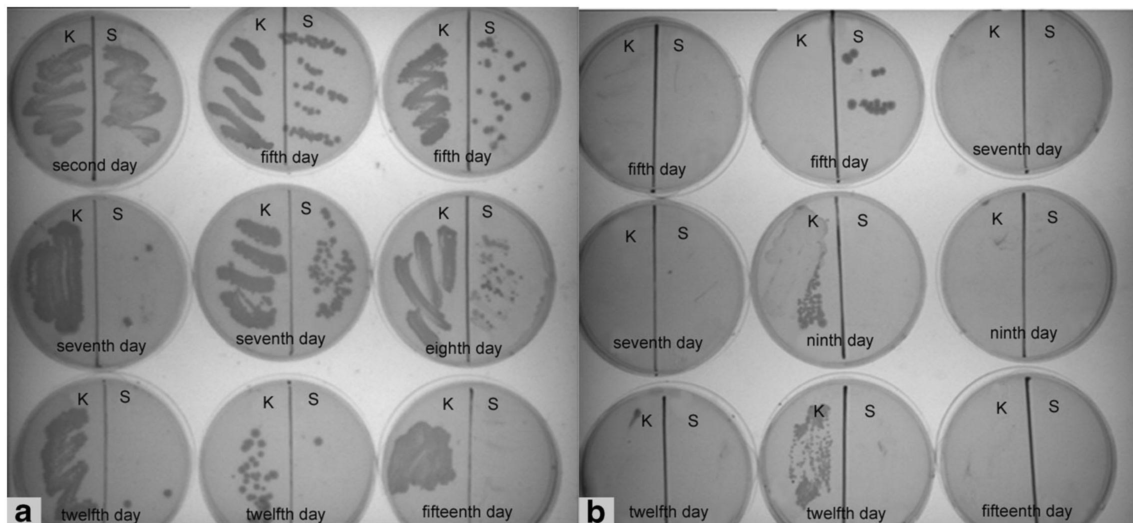

Fig. 5 Impression smears of internal organs on dishes with nutrient medium after infection with MRSA *S. aureus* 55. **a** Impression smears of internal organs of non-treated animals. The animals were dissected on days 2, 5, 7, 8, 12, and 15. **b** Impression smears of internal organs of experimental animals, to which the vesicle preparation was injected 3 h after infection. The animals were dissected on days 5, 7, 9, 12, and 15. *K* kidneys, *S* spleen

strain *S. aureus* 55 into the retro-orbital sinus (Shishkova et al. 2013). The group of experimental animals was injected with the vesicle preparation 3 h after infection; the control group was injected with no preparations. On days 5, 7, 9, 12, and 15, two animals each day were narcotized, dissected, and impression smears of their internal organs were made onto dishes with nutrient medium (Fig. 5). In this infection technique, the culture is isolated from the kidneys and spleen. It is seen in the figure that, in the control group, MRSA *S. aureus* 55 is seeded from the kidneys during the entire experiment; from the spleen, in 90 % of the cases (Fig. 5a). During the treatment with the vesicle preparation, the internal organs are cleared of the pathogenic bacterium. In one case only, the culture was seeded from the spleen and in two cases from the kidneys (Fig. 5b). Possibly, at an increase of introduced vesicles' dose the cure will be complete. Thus, the vesicle preparation containing *Lysobacter* sp. XL1 protein L5 possesses a high curative effect with respect to the chosen model infections. It is evident that this preparation cannot be used in medicine due to its multi-component composition, which can cause an acute allergic reaction. In view of this, the prospects of developing new-generation liposomal antimicrobial drugs based on particular lytic enzymes of *Lysobacter* sp. XL1 are apparent.

Future prospects

We plan to continue the research into *Lysobacter* sp. XL1 vesicles. The mapping of the bacterium's genome will enable us to establish the proteome of vesicles and, possibly, to find additional factors determining the process of their formation.

We would especially like to note the prospects of developing efficient new-generation liposomal antimicrobial drugs that cause no habituation in microorganisms. Modern biotechnological techniques make it possible to develop medicinal products based on liposomes (Gregoriadis 2007). The use of liposomes for delivery of biologically active substances can lead to a decrease of the toxicity of a drug, an increase of its bioavailability and, on the whole, result in the increased efficiency of the therapeutic effect. We have already started such studies.

Based on phospholipids obtained from *Lysobacter* sp. XL1 vesicles and recombinant bacteriolytic enzyme L5, we have produced a liposomal preparation. Its antimicrobial action on Gram-positive, including multiply resistant, bacteria was studied. The preparation also proved to lyze test objects as efficiently as vesicles and lysoamidase. Our future research will be aimed at further studies of the preparation and its preclinical tests.

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Compliance with ethical standards All applicable institutional guidelines for the care and use of animals were followed. Experiments with animals were approved by the bioethics commission of the State Research Center for Applied Microbiology and Biotechnology. Works with animals were conducted in accordance with Russian Federation legislation and the Directive of the European Parliament and the Council of the European Union on Protection of Animals used for Scientific Purposes.

Conflict of interest The authors declare that they have no competing interests.

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