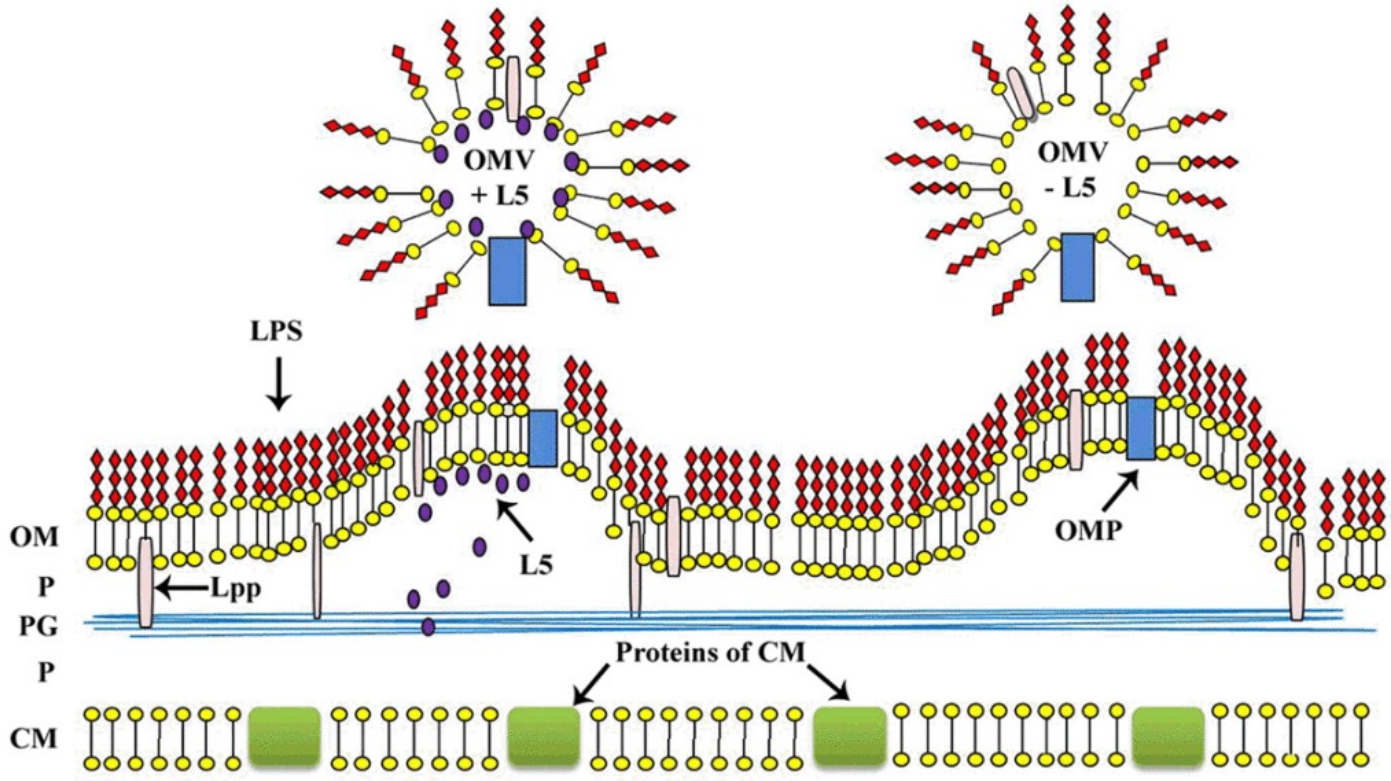
Participation of secreted protein L5 in formation of outer membrane vesicles produced by Lysobacter sp. XL1

Formation of outer membrane vesicles is a physiological feature of Gram-negative bacteria. Vesicles formation is result of splitting off the evagination of bacterial outer membrane. Its size varies from 20 to 300 nm. During formation of vesicles, the capture of some compounds of periplasm take place. This results in enrichment of vesicle content with toxic metabolites, diverse hydrolytic enzymes, and, in some cases, with toxins and factors of virulence. All these factors are important for survival of bacterial population providing the competitive power, nutrition, resistance for stress conditions, virulence for pathogenic bacteria, etc.

During last decade a number of experimental data devoted to vesicules of bacteria from different systematic groups were obtained. However the process of vesicles formation is still left unclear. One of the models of vesicles formation suggests the participation of the accumulated wrongly folded proteins and peptidoglycan fragments in periplasm that create inside pressure, provoking the start of vesicles formation.



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 from the inner side of the outer membrane. It is in those loci that vesicles containing it are formed. 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; OMP, outer membrane proteins; OMV + L5, vesicles containing protein L5; OMV – L5, vesicles containing no protein L5.

The object of research in Laboratory of microbial cell surface biochemistry of IBPM RAS is the Gram-negative bacterium *Lysobacter* sp. XL1 which secretes into extracellular space bacteriolytic enzymes (L1 - L5). These enzymes destroy the peptidoglycan, the main element in structure of cell walls of competitive bacteria. Based on this bacteriolytic enzymes the antimicrobial drug “lysoamidase” with unusually wide spectrum of activity was created. One of lytic enzymes of this bacterium – the L5 protein – penetrates into the extracellular space by means of outer membrane vesicles. In conditions favorable for secretion of this protein, the *Lysobacter* sp. XL1 cells were forming a number of vesicules with different sizes. When the protein secretion were suppressed a very few vesicles with the same diameter were found. This fact has become a reason of our assumption about the participation of secreted protein L5 in the process of vesicles formation. During experimental trials it was found that during topogenesis L5 protein gets into periplasm space and concentrates at definite loci close to internal layer of outer membrane. This, probably, may create some pressure on the membrane from inside of periplasm space, what could promote to vesicules formation at these loci (see figure). This mechanism of vesicles formation in *Lysobacter* sp. XL1 cells could be discussed in the frame of the model described. On the other hand, for this bacterium in known that the vesicles are the natural transportation vehicle for L5 protein, which itself is a factor initiating the process of vesicles formation. Along with that we have found that the *Lysobacter* XL1 cells are forming the vesicles do not containing the L5 protein. This may be result of participation in vesicles formation of another factors also, what can explain the formation of heterogenic vesicles by one bacterial cell (see figure). It seems that in future a new data about influence of secreted proteins on vesicles formation as well as about participation of other factors in this process will enrich the existing models or became a base for a new ones.

It should be noted that secretion of L5 protein by means of outer membrane vesicles remarkably increased antimicrobial efficacy of *Lysobacter* sp. XL1. Compare to homogeneous L5 protein, the vesicles containing this protein were effective against of much more wide list of Gram-positive and Gram-negative bacteria, including multiply resistant to antibiotics. The vesicles containing L5 protein could become a model for development of modified or artificial containers delivery of medicinal drugs.

Irina V. Kudryakova, Natalia E. Suzina, Natalia V. Vasilyeva

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