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Analysis of the amino acid sequences aminoacyl-phosphatidylglycerol synthases of gram-positive bacteria

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Motivation and Aim: Aminoacyl-phosphatidylglycerol synthases (aaPGSs) serve as defense mechanism in bacterial cells. These enzymes catalyze the transfer of amino acids from aminoacyl-tRNAs to phosphatidylglycerol (PG) and transport the resulting product into the outer layer of the bacterial membrane. Through the aminoacylation of PG, bacteria are able to alter the overall negative charge of their cell envelope, decreasing its affinity for charged substances and adapting to different environmental conditions. This process is crucial for the pathogenicity of certain microbes, providing them with protection against antimicrobial peptides from host organisms and therapeutic antibiotics [1].

An integral membrane protein aaPGSs known as Multiple Peptide Resistance Factor (MprF) exists in the form of homodi- and tetramers. Changes in the structure of this protein can sometimes restore sensitivity to antibacterial compounds in resistant microorganisms [2]. Assessing the prevalence of this defense mechanism among the two largest bacterial phyla with a gram-positive phenotype, *Bacillota* and *Actinomycetota* will enhance our understanding of its role in adaptive processes and help identify strategies to overcome it [3].

Methods and Algorithms: The bioinformatic analysis was performed by the method of multiple alignment of amino acid sequences of the MprF protein using the BLAST algorithm blastp (the database RefSeq Select proteins). The search for proteins was carried out among the reference sequences of the phyla *Bacillota* and *Actinomycetota* bacteria available in the NCBI database (https://www.ncbi.nlm.nih.gov). Sequences were retrieved by BLAST [4] using the sequence Lysyl-PGS from *Bacillus subtilis* (YP_003097695.1) as a template for *Bacillota* and the LysylX-PGS *Mycobacterium tuberculosis* (NP_216156.1) as a template for *Actinomycetota*. AaPGSs sequences displaying an E value < 10E-6.

Results: To date, putative aaPGS shave been identified in 921 distinct species of the 129 genera of the *Bacillota* and 1,453 distinct species of the 208 genera of the *Actinomycetota*. Approximately 250 genera are considered to be within the *Bacillota* phylum and more 400 genera – the *Actinomycetota* phylum. However, the protein reference database continues to grow, and a gap in the number of species does not necessarily mean that they lack aaPGSs.

AaPGSs of the *Bacillota* consist of two separate domains to synthesize and translocate aminoacyl phospholipids to the outer leaflets of bacterial membranes. Both domains vary in size and in sequence. AaPGSs found in *Actinomycetota* (*Actinomycetales*, *Micrococcales*, *Mycobacteriales* and others), are fused with an additional Lys-RS

domain that exhibits all of the features associated with tRNA lysylation such as a tRNA anticodon binding domain along with all the conserved positions in Lys-RSs necessary for substrate binding (Lys, ATP, and tRNA) [2]. However, the analysis of the peptide sequences extracted from the database showed there are aaPGSs of *Bifidobacteriales* and *Kineosporiales* (both orders belong to class *Actinomycetia*, phylum *Actinomycetota*), which is characterized by the absence of an additional Lys-RS domain.

Conclusion: The study of aminoacylphosphatidylglycerol synthases (aaPGSs) and their role as defense mechanisms in bacterial cells, sheds light on the adaptive processes of bacteria in response to environmental challenges. The bioinformatic analysis conducted to assess the prevalence of aaPGSs among the *Bacillota* and *Actinomycetota* phyla has revealed significant insights into the structural variations and functional diversity of these enzymes. The identification of aaPGSs in a wide range of bacterial species underscores the importance of these enzymes in providing protection against antimicrobial agents. Further research in this area will not only enhance our understanding of bacterial adaptation but also contribute to the development of strategies to overcome bacterial resistance mechanisms.

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