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Commentary

The Genomics-Driven Discovery of Novel Surface Polysaccharide Modification Pathways in Bacteria: Prospects for Future Antimicrobial Drug Discovery

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DESCRIPTION

Bacterial surface-associated polysaccharides represent the first line of defense towards the outside environment. Hence, the ability of bacteria to quickly respond and adapt to external conditions directly impacts their overall fitness and survival. Throughout evolution, bacteria have evolved multiple mechanisms that help them survive in the often harsh and extreme conditions of their host immune systems or other natural habitats. Among them, alteration of the cell surface-associated components such as polysaccharides and/or lipids and transition to a metabolically dormant, biofilm-forming lifestyle are some of the most widely employed adaptation strategies.

Alteration of the cell surface structures by the addition of charged substituents effectively shields bacteria against antibiotics produced by the competing microbes and human defensins. The addition of D-alanine to teichoic acid-producing Gram-positive bacteria and phosphoethanolamine, L-4-aminoarabinose, or glycine to the lipopolysaccharides of Gram-negative bacteria contribute to antimicrobial resistance and pathogenicity of *Staphyloccocus aureus*, group *B Streptococci*, enterobacteria (*E. coli and Salmonella*) and El Tor biotype of pandemic *Vibrio cholerae* [1]. Similarly, the introduction of alkyl substituents, e.g. acetyl groups, during alginate production by *Pseudomonas aeruginosa* enhances its pathogenicity and virulence by providing several levels of protection against the host glycanases and immune cells [2].

In the newly published paper titled 'Genomic and molecular evidence reveals novel pathways associated with cell surface polysaccharides in bacteria,' we report the genomics-informed discovery of a conserved putative pathway that is linked with gene clusters that direct biosynthesis of surface-associated polysaccharides in both Gram-negative and Gram-positive bacteria [3]. This pathway employs a noncanonical acyl carrier protein-dependent Amino Acid Ligase (AAL) related to archeal seryl tRNA synthetases, a flavin-dependent dehydrogenase, a protein of an unknown function that shares structure-function similarity with cysteine acyltransferases and a member of the family glycoside hydrolase family 2, we named the AAL cassette.

By performing systematic gene co-localization calculations, we found that the AAL cassette was highly conserved in certain lineages of *α-* and *β-Proteobacteria*, including the genera *Burkholderia*, *Cupriavidus* and *Rhizobium* as well as mycolic *Actinobacteria* of the *Nocardiceae* family that gather genera *Rhodococcus* and *Williamsia*. High conservation of the AAL cassette among the sequenced genomes of the above species implied the importance of the AAL cassette in the physiology and ecology of these organisms. Particularly intriguing was the observation that in the β-proteobacterial species of the genera of *Burkholderia* and *Cupriavidus*, AAL cassette was invariably associated with polysaccharide gene cluster in >85% of all sequenced genomes. Colocalization of AAL cassette and PS gene clusters was less conserved in *Rhodoccocus* and *Rhizobium*, yet, we could show that in these genera PS genes were contained on remote loci, suggesting a case of evolutionary fragmentation of PS gene clusters, possibly as a result of recombination events.

Based on the *in vitro* biochemical evidence that AAL and Acp act in tandem to activate amino acid(s), we speculated that AAL cassette may constitute a novel surface polysaccharide modification route that introduces hydroxylated aminoacyl moieties into nascent polysaccharide chains. Indeed, by thoroughly searching the literature and transcriptomic data, we came across several published studies that supported our hypothesis. AAL-cassette-associated polysaccharide gene clusters of both *Burkholderia cepacia* and *Burkholderia pseudomallei* were shown to direct assembly of an EPS that was a major constituent of *Burkholderia*'s biofilm [4,5]. Furthermore, individual deletion of genes within the AAL cassette in *B. pseudomallei* had a similar impact on EPS production and biofilm formation as did mutations in the polysaccharide structural genes [5]. Also, transcriptomic studies on several clinical isolates of *B. pseudomallei* from melioidosis-infected patients revealed induced co-expression of AAL cassette and the surrounding PS genes,

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and levels of their expression were positively correlated with a hypermucoid phenotype [6,7]. Curiously, none of the above authors recognized the potential function of the AAL cassette in the context of PS biogenesis. Nevertheless, although the exact structural evidence for this pathway is still missing, we believe that the insights presented in this study will entice research groups to provide the structural proof.

Potential implications for future antimicrobial drug discovery to treat *Burkholderia*-related diseases

Highly conserved cell surface-associated pathways present valuable targets for the development of novel classes of antibiotics. In addition to planktonic, novel classes of antibiotics should be able to effectively target persister cells and biofilm-growing pathogens by exerting novel mechanisms of action. Development of murepavadin, an LPS inhibitor of *P. aeruginosa*, synthetic derivatives of arylomycin that target membrane-associated type I signal protease, and inhibitors of the Bam outer membrane complex are some of the recent examples of such antimicrobials [8]. Importantly, this approach to antimicrobial drug discovery eliminates the problems associated with antibiotics that target intracellular processes such as protein and nucleic acid pathways in Gram-negative bacteria due to their expulsion by the highly efficient efflux pumps.

B. cepacia and *B. pseudomallei* are Gram-negative bacteria that cause life-threatening lung infections in immunocompromised patients and melioidosis, respectively, the latter of which is associated with high mortality rates [9]. Given the high conservation of the AAL pathway in both groups of pathogens and the relevant role of the AAL pathway in biofilm formation, the AAL pathway holds the potential of becoming an attractive target for the development of novel, *Burkholderia*-specific antimicrobials. As we move towards the age of personalized medicine, the development of precision antimicrobial therapeutics is urgently needed in the light of an alarming rise of antimicrobial resistance [10].

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