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Highlight

Promising biotechnological applications of antibiofilm exopolysaccharides

Patricia Bernal and María A. Llamas*
Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas (CSIC), C/ Prof. Albareda, 1, E-18008 Granada, Spain.

Bacterial exopolysaccharides

Polysaccharides are polymers of carbohydrates with an enormous structural diversity, from long linear repetition of the same monomer to highly branched structures of different sugars. This high structural diversity reflects the functional diversity of these molecules. There are two types of polysaccharides, storage polysaccharides (i.e. glycogen) and structural polysaccharides, which are normally secreted by the cell and form different cell structures (i.e. cellulose, chitin). Extracellular polysaccharides or exopolysaccharides belong to this last group.

Exopolysaccharides are produced not only by microorganisms, but also by algae, plants and animals (Sutherland, 2005). Bacterial exopolysaccharides are a major component of the extracellular polymeric substance (EPS) or matrix of biofilms, and mediate most of the cell-to-cell and cell-to-surface interactions required for biofilm formation and stabilization (Flemming and Wingender, 2010). The matrices of biofilms from natural environments, such as marine and fresh water, soil, or chronic infections, contain a ubiquitous composition of polysaccharides. More than 30 different matrix polysaccharides have been characterized so far. Several are homopolysaccharides (i.e. glucans, fructans, cellulose), but most of these are heteropolysaccharides consisting on a mixture of sugar residues. Exopolysaccharides can even differ between strains of single species, as exemplified by strains of *Pseudomonas aeruginosa*, which can produce one, two or three different exopolysaccharides (alginate, Pel and Psl) (Ryder *et al.*, 2007). Since most mutants deficient in the synthesis of exopolysaccharides are impaired for biofilm formation it was assumed that bacterial exopolysaccharides play only a structural role in biofilms (Sutherland, 2001). However, in recent years an unexpected function for these molecules as inhibitors of biofilm formation has been described. This new function has been thoroughly reviewed by Rendueles and colleagues in a recent issue of *Environmental Microbiology* (2012).

Polysaccharides with the ability to inhibit and/or destabilize biofilm formation are referred to as antibiofilm polysaccharides, and their production appears to be a well-conserved ability among living organisms. Interestingly, antibiofilm polysaccharides do not have biocidal activity, a property that could increase the technological applications of these molecules as antibiofilm agents in industry and medicine by diminishing the emergence of resistance by natural selection.

Antibiofilm polysaccharides

The first bacterial polysaccharide with antibiofilm activity was identified in an uropathogenic *Escherichia coli* strain. This bacterium contains a group II capsular polysaccharide able to inhibit biofilm formation by several bacteria, including commensal *E. coli* strains, *P. aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus* and *Enterococcus* (Valle *et al.*, 2006). Since then, an increasing number of antibiofilm polysaccharides from several bacterial species encountered in many different environments have been discovered (Rendueles *et al.,* 2012). Most of these antibiofilm agents are able to inhibit the biofilm formation of a broad range of bacteria, suggesting that they may play an essential role in microbial competition and niche exclusion. However, several antibiofilm exopolysaccharides also inhibit the biofilm formation of the producer strain in an auto-regulatory process to control biofilm architecture (Bendaoud *et al.*, 2011). Of special interest are the Psl and Pel exopolysaccharides of *P. aeruginosa* that are capable of carrying out antagonistic functions. These molecules inhibit biofilm formation of the competitors (i.e. *Staphylococcus epidermidis*) (Qin *et al.*, 2009), while at the same time boosting that of the producer strain (Colvin *et al.*, 2011).

Polysaccharides with antibiofilm activity are also produced by eukaryotes (Rendueles *et al.*, 2012). The first one described was furonan, a polysaccharide produced

*For correspondence. E-mail marian.llamas@eez.csic.es; Tel. (+34) 958181600 Ext. 309; Fax (+34) 958129600.

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by the seaweed *Gloipeltis furcata* that inhibits biofilm formation of several oral bacteria and is able to prevent plaque formation when administrated as chewing gum (Sato *et al*., 1998). Several polysaccharides isolated from plants (i.e. okra fruit, *Aloe vera*, licorice root, ginseng, blackcurrant) have been shown to be able to inhibit the binding of *Helicobacter pylori*, among other bacteria, to human gastric cells (Rendueles *et al*., 2012).

Although the mode of action of antibiofilm polysaccharides is not completely known yet, none of the compounds identified to date exhibits biocidal activity. This indicates that the antibiofilm activity of these polysaccharides is mediated through different mechanisms. Most evidence suggests that these molecules act by modifying the physical properties of both abiotic and biotic (i.e. bacterial cell) surfaces (Rendueles *et al.,* 2012). For example, *E. coli* exopolysaccharides can increase the hydrophilicity of glass surfaces, and can also inhibit the autoaggregation via adhesins of bacterial cells (Valle *et al*., 2006; Rendueles *et al*., 2011). Polysaccharides, as sugar polymers, have also the capacity to act as lectin inhibitors. Lectins are proteins that specifically recognize and bind sugars without modifying these molecules. In bacteria, the primary function of lectins is to facilitate attachment or adherence of bacteria to host cells. These proteins play an important role in biofilm formation, and are essential for bacterial colonization and infection. Lectins are mainly located on the surface of bacteria cells where they can access and bind to the glycan substrates present on the surface of host cells (Esko and Sharon, 2009). By competing for the sugar binding domain of lectins, polysaccharides can inhibit lectin-dependent adhesion of pathogens and biofilm formation. In fact, several plant, microbial and milk polysaccharides have been shown to block various lectins from human pathogenic bacteria by competitive inhibition (Zinger-Yosovich and Gilboa-Garber, 2009; Zinger-Yosovich *et al*., 2010).

Nevertheless, some studies also suggest that exopolysaccharides can act as signalling molecules to regulate gene expression. Microarray analyses have shown that the *Lactobacillus acidophilus* A4 polysaccharide down-regulated the expression of *E. coli* genes involved in biofilm formation (Kim *et al*., 2009). Interestingly, the synthesis of the extracellular cellulosome complex of *Clostridium thermocellum*, which is required for hydrolysis of the plant cell wall by the bacterium, is regulated by the presence of exopolysaccharides via alternative sigma factors (Nataf *et al*., 2010). Exopolysaccharides are recognized by the extracellular carbohydrate-active module of a transmembrane anti-sigma factor. Binding of the exopolysaccharide to the anti-sigma factor results in the activation of its cognate alternative sigma factor, which then interacts with the RNA polymerase and initiates transcription of the cellulosomal synthetic operon. Identification and characterization of more of such regulatory proteins containing both polysaccharide binding and regulatory domains will help to unravel the molecular mechanism behind the antibiofilm activity of exopolysaccharides.

Biotechnological applications

Biofilm formation represents the prevailing microbial lifestyle in natural environments and occurs on all surface types, including places where humans do not want them. Since biofilms interfere with the action of several antibiotics and bacterial drugs, biofilm-forming bacteria are more difficult to eradicate (Costerton *et al*., 1999). In hospitals, an environment markedly susceptible to contamination by bacterial pathogens, biofilms are a particularly important problem. Medical devices, such as catheters, heart valves, prostheses, surgical pins, etc., can be colonized by bacterial biofilms and become sources of infections (Davies, 2003). Moreover, many chronic infections, like diabetic ulcers or lung infections in patients with cystic fibrosis, result from bacteria growing as biofilms (Costerton *et al*., 1999). Bacterial biofilms are also very problematic in industrial settings. The presence of biofilms is common in for example the food industry and, because several foodborne bacteria are human pathogens, it is considered a serious public health risk. Moreover, biofilms cause several other problems in industry, like mechanical blockages and impedance of heat transfer processes, shortening the lifetimes of modules in fermentors, and increase the corrosion rate of surfaces (Kumar and Anand, 1998). Consequently, the use of bacterial mutant strains that present a deficiency in biofilm formation have been shown to enhance biotechnological processes (Sung *et al*., 2006). However, there is still a clear unmet need for controlling bacterial biofilms. Antibiofilm polysaccharides represent a promising alternative to battle biofilm formation and bacterial infections. These molecules combine two important characteristics: they are able to block biofilm formation and do not have biocidal activity, which may prevent the emergence of resistance to a great extent. Furthermore, antibiofilm polysaccharides have other important features, such as biocompatibility, biodegradability and non-toxicity, which increase their potential to be used in medical and industrial applications. Additionally, exopolysaccaharides act on a wide range of bacterial strains, so can be useful for the treatment of multispecies infection, and some of them are able to disrupt preformed biofilms. As described by Rendueles and colleagues in their recent review (2012), antibiofilm polysaccharides could be co-administered with common antibiotics, as they would disaggregate the biofilm facilitating the access of the antibiotics. Moreover, exopolysaccharides are surfactant molecules that alter the physico-chemical properties of surfaces and could be therefore used as

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anti-adhesive coating for medical devices. Additionally, since some polysaccharides and oligosaccharides inhibit attachment of bacteria to human gastric cells, they could be used as probiotics. In fact, plant and microbial polysaccharides are already being widely used in the food industry as emulsifiers and stabilizers (i.e. the food additive guar gum also known as E412).

Nowadays, an estimated 20% of all bacterial exopolysaccharides are known (Rendueles *et al.,* 2012). However, the ongoing development of new and potent high-throughput technologies will allow the discovery of a large number of new compounds, including new antibiofilm exopolysaccharides. For instance the recently developed nanoDESI platform allows the direct sampling of extracellular compounds from almost any surface, including bacterial colonies, plants, and animals (Watrous *et al.*, 2012). This new approach applies rapid and highly sensitive mass spectrometry (MS) to directly monitor and characterize secreted molecules produced from live cells. This powerful technology is able to detect and characterize compounds that could not be identified by traditional biochemical approaches. The use of this technology opens an important new field of research that will allow the discovery of many new natural products, which in turn will have important biotechnological implications. Glycan arrays are another example of a new high-throughput technology that will lead to the discovery of new antibiofilm compounds. This technology enables a high-sensitivity and high-throughput analysis of carbohydrate–protein interactions. Several platforms have been created in which hundreds of glycan molecules (including polysaccharides) are covalently or non-covalently immobilized to a surface (i.e. glass, polystyrene, aluminium, silicon) (Liang and Wu, 2009). Using fluorescence-based measurements (among others), polysaccharides capable of attaching to for example bacterial adhesins (i.e. lectins) can be directly detected. Once identified, the carbohydrate-based inhibitor can be used to prevent lectin binding to its receptor. Glycoarrays are in fact becoming a priceless tool for the discovery of new biofilm inhibitors that target bacterial adhesins (Blanchard *et al.,* 2008).

Antibiofilm agents have been considered a promising strategy for the development of novel therapeutics for the control of bacterial proliferation for a long time. However, despite the increasing knowledge on biofilms, no antibiofilm products are on the market yet. As recently discussed by Romero and Kolter (2011), the main reasons for this may be economic rather than scientific. Nevertheless, continued research efforts in this area lead to the identification of new strategies that may be translated into products in the future. Antibiofilm exopolysaccharides are one of these newly identified strategies that, given the years of successful use of other polysaccharides (Sutherland, 1998), may have a promising future in the market.

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