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## Minireview

## Antibiofilm polysaccharides

Olaya Rendueles,<sup>1</sup> Jeffrey B. Kaplan<sup>2</sup> and Jean-Marc Ghigo<sup>1\*</sup>

<sup>1</sup>Institut Pasteur, Unité de Génétique des Biofilms, Département de Microbiologie, 25-28 rue du Dr Roux, F-75015 Paris, France.

<sup>2</sup>Department of Oral Biology, New Jersey Dental School, Newark, NJ 07103, USA.

## Summary

**Bacterial extracellular polysaccharides have been shown to mediate many of the cell-to-cell and cell-to-surface interactions that are required for the formation, cohesion and stabilization of bacterial biofilms. However, recent studies have identified several bacterial polysaccharides that inhibit biofilm formation by a wide spectrum of bacteria and fungi both *in vitro* and *in vivo*. This review discusses the composition, modes of action and potential biological roles of antibiofilm polysaccharides recently identified in bacteria and eukarya. Some of these molecules may have technological applications as antibiofilm agents in industry and medicine.**

## Introduction

Biofilm is the predominant mode of growth for bacteria in most natural, industrial and clinical environments. Biofilms typically consist of densely packed, multispecies populations of cells, encased in a self-synthesized polymeric matrix, and attached to a tissue or surface (Costerton *et al.*, 1987; Stoodley *et al.*, 2002). The biofilm lifestyle is associated with a high tolerance to exogenous stress, and treatment of biofilms with antibiotics or other biocides is usually ineffective at eradicating them (Hall-Stoodley and Stoodley, 2009). Biofilm formation is therefore a major problem in many fields, ranging from industrial corrosion and biofouling (Lopez *et al.*, 2010) to chronic and nosocomial infections (Francolini and Donelli, 2010; Hoiby *et al.*, 2011).

Received 29 March, 2012; revised 12 May, 2012; accepted 15 May, 2012. \*For correspondence. E-mail jmgghigo@pasteur.fr; Tel. (+33) 01 40 61 34 18; Fax (+33) 01 45 68 88 36.

One of the hallmarks of biofilm formation is the production of an extracellular matrix composed of 90% water and 10% extracellular polymeric substances (EPS) (Flemming and Wingender, 2010). Extracellular polymeric substance components mediate most of the cell-to-cell and cell-to-surface interactions that are necessary for the formation and stabilization of biofilm colonies. Structural components of the EPS matrix include cell-surface proteins, proteinaceous pili, DNA, RNA, lipids and polysaccharides (Flemming and Wingender, 2010). Among these, polysaccharides, often identical to cell-bound capsular polysaccharides produced by free-floating or 'planktonic' bacteria, constitute a major component of the biofilm matrix in many bacteria (Sutherland, 2001; Bazaka *et al.*, 2011). More than 30 different biofilm matrix polysaccharides have been characterized so far (Flemming and Wingender, 2010). Well-known examples include alginate, Pel and Psl produced by *Pseudomonas aeruginosa* (Davies and Geesey, 1995; Ramsey and Wozniak, 2005; Qin *et al.*, 2009); poly-*N*-acetylglucosamine (PNAG) produced by *Escherichia coli* (Wang *et al.*, 2004), *Staphylococcus aureus* (O'Gara, 2007), *Staphylococcus epidermidis* (Gerke *et al.*, 1998), *Acinetobacter baumannii* (Choi *et al.*, 2009), *Burkholderia* spp. (Yakandawala *et al.*, 2011) and *Bordetella* spp. (Parise *et al.*, 2007); cellulose secreted by *E. coli* (Zogaj *et al.*, 2001), *Pseudomonas fluorescens* (Spiers *et al.*, 2003) and *Salmonella* spp. (Solano *et al.*, 2002); and glucans produced by *Streptococcus mutans* (Bowen and Koo, 2011). Mutant strains unable to synthesize or export these exopolysaccharides usually exhibit decreased adherence, decreased biofilm formation, and increased sensitivity to killing by biocides and host defences. These results highlight the importance of exopolysaccharides in maintaining the integrity of the biofilm and in mediating the pathogenic potential of the biofilm lifestyle.

Bacterial exopolysaccharides exhibit highly variable structures and it is likely that they also perform additional functions besides their implied function in matrix stabilization and energy storage (Flemming and Wingender, 2010; Bazaka *et al.*, 2011). In fact, several studies showed that certain bacterial mutants deficient in capsular polysaccharide production exhibit increased biofilm formation. Examples include *E. coli* (Valle *et al.*, 2006),

**Table 1.** Bacterial antibiofilm polysaccharides.

| Polysaccharide | Molecular weight (kDa) | Components  | Species and strain          | Reference                      |
|----------------|------------------------|---|-----------------------------|--------------------------------|
| A101           | > 500                  | Galacturonic acid<br>Glucuronic acid<br>Rhamnose<br>Glucosamine | <i>Vibrio</i> sp. QY101     | Jiang <i>et al.</i> (2011)     |
| Ec111p         | > 500                  | Mannose<br>Glucose<br>Galactose<br>Glucuronic acid              | <i>E. coli</i> Ec111        | Rendueles <i>et al.</i> (2011) |
| Ec300p         | > 500                  | Mannose<br>Glucose<br>Galactose<br>Glucuronic acid              | <i>E. coli</i> Ec300        | Rendueles <i>et al.</i> (2011) |
| K2             | 500                    | Galactose<br>Glycerol<br>Phosphate<br>Acetate                   | <i>E. coli</i> CFT073       | Valle <i>et al.</i> (2006)     |
| PAM            | > 300                  | Galactofuranose   | <i>K. kingae</i> PYKK081    | Bendaoud <i>et al.</i> (2011)  |
| Pel            | Unknown                | Glucose-rich  | <i>P. aeruginosa</i> PAO1   | Qin <i>et al.</i> (2009)       |
| PI80 EPS       | 280                    | Arabinose   | <i>S. phocae</i> PI80       | Kanmani <i>et al.</i> (2011)   |
| Psl            | 4–6                    | Mannose<br>Glucose<br>Rhamnose                                  | <i>P. aeruginosa</i> PAO1   | Qin <i>et al.</i> (2009)       |
| r-EPS          | Unknown                | Unknown   | <i>L. acidophilus</i> A4    | Kim <i>et al.</i> (2009)       |
| SP1 EPS        | 1800                   | Galactose<br>Glycerol<br>Phosphate                              | <i>B. licheniformis</i> SP1 | Sayem <i>et al.</i> (2011)     |

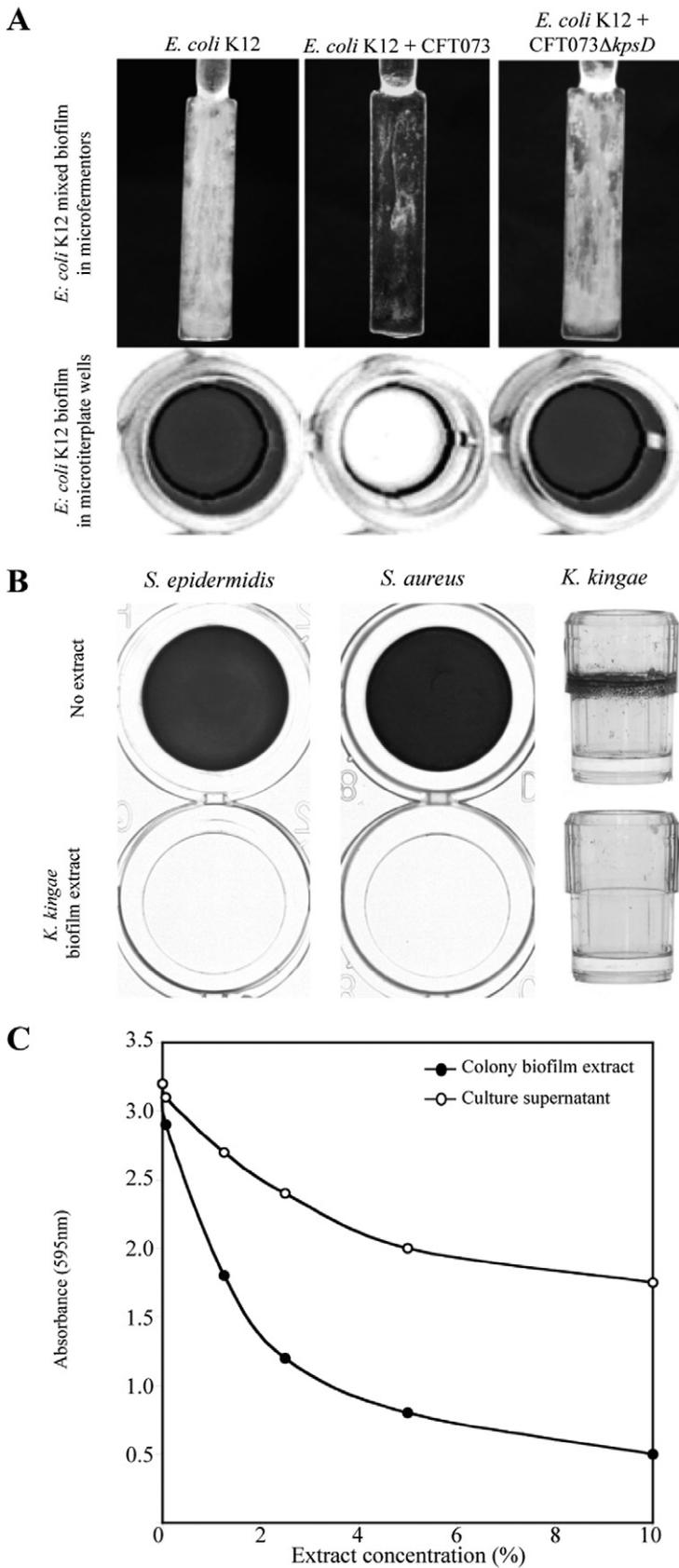
*Streptococcus pneumoniae* (Moscoso *et al.*, 2006; Domech *et al.*, 2009), *Staphylococcus haemolyticus* (Flahaut *et al.*, 2008), *Vibrio vulnificus* (Joseph and Wright, 2004), *Shewanella oneidensis* (Kouzuma *et al.*, 2010), *Tannerella forsythia* (Honma *et al.*, 2007) and *Porphyromonas gingivalis* (Davey and Duncan, 2006). These observations suggest that some bacterial exopolysaccharides can perform functions that inhibit or destabilize the biofilm. This review focuses on a series of recent studies (Table 1) that investigated the physical and biological characteristics of non-biocidal bacterial antibiofilm polysaccharides, and their role in interspecies interactions in mixed, multi-species biofilms.

### Bacterial antibiofilm polysaccharides

The first antibiofilm polysaccharide was discovered while studying interactions between uropathogenic and commensal strains of *E. coli* in mixed *in vitro* biofilms. Valle and colleagues (2006) found that the biomass of biofilms produced by the commensal MG1655 strain was reduced in the presence of uropathogenic CFT073 strain (Fig. 1A). The biofilm reducing activity was present in CFT073 culture supernatant, and was shown to be due to group II capsular polysaccharide commonly produced by extraintestinal *E. coli* of phylogenetic group B2 or D. A screen of culture supernatants derived from 110 *E. coli* strains of diverse origin revealed that only supernatants from 39

strains carrying group II capsule genes exhibited biofilm inhibition activity, indicating that the anti-adhesion property was a general characteristic of group II capsules (Valle *et al.*, 2006). Consistently, culture supernatants from group II capsular polysaccharide mutant strains lost their ability to inhibit biofilm formation (Fig. 1A). Finally, CFT073 group II capsular polysaccharide (serotype K2) displayed a broad spectrum of activity, as it inhibited biofilm formation by *E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae*, *S. aureus*, *S. epidermidis* and *Enterococcus faecalis* in both static and flow reactors without inhibiting growth (Valle *et al.*, 2006).

Subsequently, two additional antibiofilm polysaccharides were identified in a similar study on the interaction between the opportunistic, nosocomial pathogens *P. aeruginosa* and *S. epidermidis*, which may coexist on the surfaces of contact lenses or other medical devices. Qin and colleagues (2009) found that preformed *S. epidermidis* strain 1457 biofilms cultured in microtiter plates or on glass coverslips were dispersed but not killed by the addition of cell-free culture supernatant from *P. aeruginosa* strain PAO1. In contrast, *S. epidermidis* culture supernatant did not disperse preformed *P. aeruginosa* biofilms. Culture supernatants from single or double mutant *P. aeruginosa* strains deficient in the production of Pel or Psl biofilm matrix polysaccharides exhibited decreased biofilm dispersal activity against *S. epidermidis*. Crude polysaccharide isolated from wild-type PAO1



**Fig. 1.** Properties of antibiofilm polysaccharides.

A. Biofilm formation by *E. coli* K12 MG1655 F+ on glass spatulas in a continuous flow biofilm microfermentor (top) or in microtiter plate wells (bottom) in the presence of fresh media (control), *E. coli* CFT073 supernatant or K2 group II capsule mutant ( $\Delta kpsD$ ) supernatant.

B. Biofilm formation by *S. epidermidis*, *S. aureus* and *K. kingae* in microtiter plate wells in the absence or presence of *K. kingae* colony biofilm extract.

C. *S. aureus* biofilm formation in the presence of *K. kingae* colony biofilm extract or broth culture supernatant shows that PAM galactan is preferentially released within biofilms.

supernatants, but not Pel or Psl mutant supernatants, also dispersed preformed *S. epidermidis* biofilms, confirming the polysaccharide nature of the antibiofilm activity. Pel and Psl polysaccharides were also able to disrupt *S. aureus* biofilms. A subsequent study showed that the presence of *P. aeruginosa* cells reduced biofilm formation by *S. epidermidis* in dual-species biofilms *in vitro*. However, some *S. epidermidis* clinical isolates were resistant to the biofilm dispersal effects of *P. aeruginosa* cells and supernatants (Pihl *et al.*, 2010). Thus, Pel and Psl, along with alginate, not only mediate the adhesive and cohesive properties that allow *P. aeruginosa* cells to form pellicles, microcolonies and biofilms (Ma *et al.*, 2006; Colvin *et al.*, 2011), but also have a clear antibiofilm role against other species.

Moreover, culture supernatants derived from several marine bacteria have been shown to exhibit antibiofilm activity (You *et al.*, 2007; Thenmozhi *et al.*, 2009; Bakkiyaraj and Pandian, 2010; Dheilly *et al.*, 2010; Nithya and Pandian, 2010; Nithya *et al.*, 2010; 2011; Nithyanand *et al.*, 2010; Klein *et al.*, 2011). Sayem and colleagues (2011) screened cell-free culture supernatants from 10 different bacteria associated with the marine sponge *Spongia officinalis* and found that two supernatants significantly inhibited *E. coli* biofilm formation in a microtiter plate assay. One of the active supernatants (designated SP1) was from a bacterium identified as *Bacillus licheniformis* (Sayem *et al.*, 2011). In addition to inhibiting *E. coli* biofilm formation, SP1 supernatant also inhibited biofilm formation by *Acinetobacter* sp., *S. aureus*, *Salmonella typhimurium*, *Shigella sonnei*, *Listeria monocytogenes*, *Bacillus cereus*, *Bacillus amyloliquefaciens*, *Bacillus pumilus* and *Bacillus subtilis* without inhibiting growth. The biofilm inhibition activity was shown to be due to a high-molecular-weight polysaccharide termed SP1 EPS. Similarly, Jiang and colleagues (2011) found that culture supernatants of *Vibrio* sp. QY101, a species isolated from a decaying thallus of a brown alga *Laminaria*, inhibited biofilm formation by *E. coli*, *P. aeruginosa*, *Aggregatibacter actinomycetemcomitans*, *S. aureus* and *S. epidermidis* in static and flow cell assays. The biofilm inhibiting activity was shown to be due to a polysaccharide named A101, which was purified by ion exchange and gel filtration chromatography and shown to be non-biocidal.

Finally, antibiofilm polysaccharides have also been isolated from culture supernatants of two different lactic acid bacteria. Crude polysaccharide (referred to as released polysaccharide or r-EPS) isolated from culture supernatants of a *Lactobacillus acidophilus* strain A4 inhibited biofilm formation by *E. coli*, *Salmonella* sp., *Yersinia enterocolitica*, *P. aeruginosa*, *L. monocytogenes* and *B. cereus* in static and flow cell reactors (Kim *et al.*, 2009). *Lactobacillus acidophilus* is a common inhabitant of the human and animal gastrointestinal tract, mouth and

vagina. Similarly, Kanmani and colleagues (2011) found that exopolysaccharide purified from culture supernatants of *Streptococcus phocae* strain PI80, which is a pathogen of marine mammals and fish, inhibited biofilm formation by *L. monocytogenes*, *Salmonella typhi*, *P. aeruginosa*, *B. cereus* and *S. aureus* in polystyrene microtiter plates without inhibiting growth.

#### *Antibiofilm polysaccharides isolated from biofilms*

Specific environmental conditions prevailing within biofilms may induce profound genetic and metabolic rewiring of the biofilm-dwelling bacteria (Beloin and Ghigo, 2005). This could potentially lead to production of biofilm-specific metabolites or polymers, some of which may also exhibit an antagonist effect over competing microorganisms. Consistent with this hypothesis, several antibiofilm polysaccharides have been identified in cell-free extracts isolated directly from mature *in vitro* cultured biofilms. Preparation of cell-free biofilm extracts from 122 natural *E. coli* isolates screened against a panel of seven biofilm-forming Gram-positive and Gram-negative bacteria showed that 20% of the tested biofilm extracts contained molecules inhibiting biofilm formation, including the aforementioned group II capsular polysaccharide (Rendueles *et al.*, 2011). Specifically, extracts derived from strains Ec111 and Ec300 exhibited non-biocidal antibiofilm activity that was not present in the supernatants of planktonic cultures. The biofilm-associated activity in both extracts was found to be due to mannose-rich high-molecular-weight polysaccharides termed Ec111p and Ec300p, which inhibited biofilm formation by Gram-positive bacteria (*S. aureus*, *S. epidermidis*, *E. faecalis*) but not by Gram-negative bacteria (*E. coli*, *Enterobacter cloacae*, *P. aeruginosa*, *K. pneumoniae*). It should be stressed that polysaccharides extracted and concentrated from *E. coli* Ec111 and Ec300 planktonic culture supernatants also displayed antibiofilm activity, therefore suggesting that these antibiofilm polysaccharides are not *sensu stricto* biofilm-specific molecules, but are produced at higher levels within biofilms than under planktonic conditions (Rendueles *et al.*, 2011).

In a similar study, Bendaoud and colleagues (2011) screened extracts prepared from lawns of bacteria grown on agar. Bacterial colonies on agar, also known as colony biofilms, exhibit many properties characteristic of biofilms cultured in broth, including high cell density, EPS production, spatially dependent microbial growth, chemical gradients and reduced susceptibility to antibiotics (Anderl *et al.*, 2000; Walters *et al.*, 2003; McBain, 2009). Colony biofilm extracts from the oral bacterium *Kingella kingae* were found to inhibit biofilm formation by *S. aureus* and *S. epidermidis*, and by *K. kingae* itself (Fig. 1B), as well as by *K. pneumoniae* and *Candida albicans*, without inhibit-

ing growth. Two exopolysaccharides were present in the extract, and one of these – a high-molecular-weight polysaccharide named PAM galactan – was purified and shown to exhibit antibiofilm activity. The antibiofilm activity was also detected in *K. kingae* planktonic culture supernatants, but at much lower levels than in colony biofilm extracts (Fig. 1C). These studies therefore showed that bacterial biofilms constitute untapped sources of natural bioactive molecules antagonizing adhesion or biofilm formation of other bacteria.

#### *Antibiofilm properties of lipopolysaccharide*

In Gram-negative bacteria, lipid-linked polysaccharides such as lipopolysaccharides (LPS) can play direct and indirect roles in biofilm formation (Beloin *et al.*, 2006; Hori and Matsumoto, 2010). In addition, LPS mediates cohesion and stabilization of bacterial biofilms, and a reduction in LPS results in biofilm structure alteration and reduced adhesion (Lau *et al.*, 2009). For instance, LPS has been reported to be essential for colonization of *Arabidopsis thaliana* hydathodes by plant pathogen *Xanthomonas campestris* (Hugouvieux *et al.*, 1998). In contrast, LPS from *Vibrio cholerae* is able to partially inhibit *in vitro* adhesion on colonic cell lines HT29-18N2 (Benitez *et al.*, 1997). As other antibiofilm polysaccharides previously discussed, LPS is also able to inhibit biofilm formation of competing strains. Bandara and colleagues (2010) measured the effect of LPS from different bacteria (*P. aeruginosa*, *K. pneumoniae*, *Serratia marcescens* and *S. typhimurium*) on biofilm formation by six different species of *Candida*. Some LPS inhibited *Candida* biofilm formation, while others stimulated initial adhesion, suggesting species-specific modulation of *Candida* biofilm maturation.

#### **Modes of action**

None of the bacterial antibiofilm polysaccharides identified to date have been shown to exhibit bacteriostatic or bactericidal activity. Their antibiofilm activity, therefore, is likely to be mediated by mechanisms other than growth inhibition. There are three hypothetical non-biocidal modes of action. The evidence so far suggests that most antibiofilm polysaccharides act as surfactant molecules that modify the physical characteristics of bacterial cells and abiotic surfaces. Some studies also indicate that polysaccharides might act as signalling molecules that modulate gene expression of recipient bacteria (Kim *et al.*, 2009). Another possible mode of action is competitive inhibition of multivalent carbohydrate–protein interactions (Wittschier *et al.*, 2007). Thus, antibiofilm polysaccharides might block lectins or sugar binding proteins present on the surface of bacteria, or block tip

adhesins of fimbriae and pili. For example, lectin-dependent adhesion of pathogenic *P. aeruginosa* to human cells is efficiently inhibited by galactomannans (Zinger-Yosovich and Gilboa-Garber, 2009).

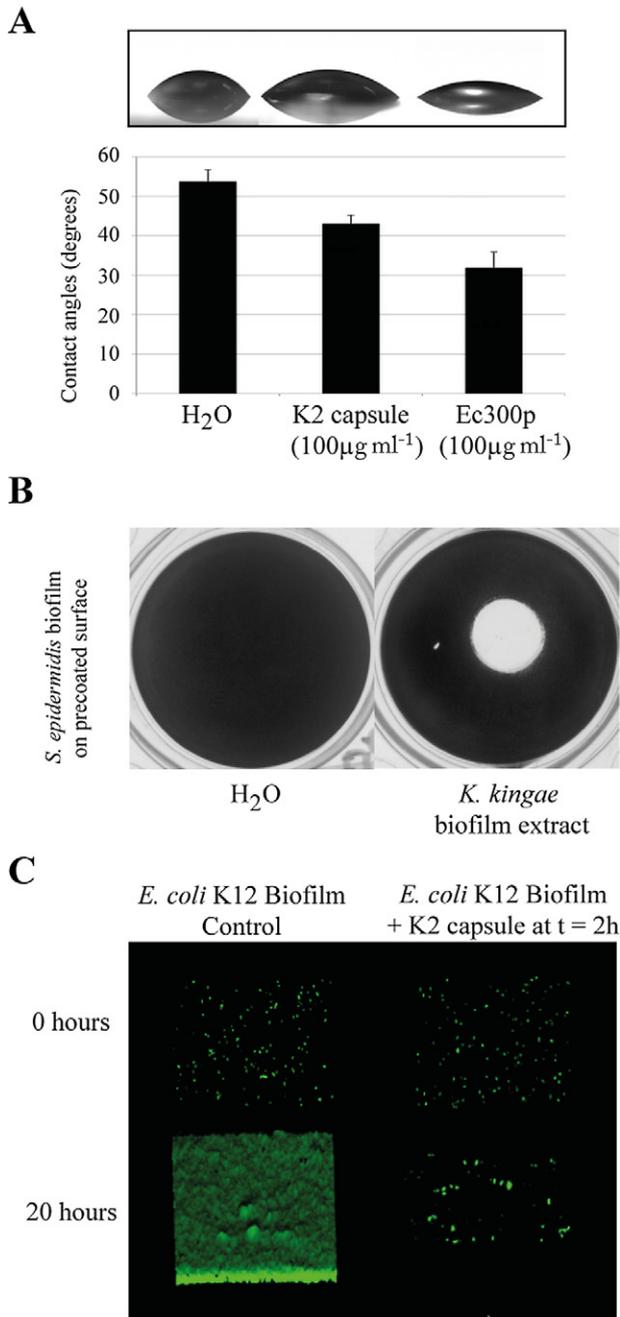
#### *Alteration of abiotic surface properties*

Biosurfactants and bioemulsifiers have been shown to alter the physico-chemical properties of surfaces by modifying the wettability and charge of the surface and hence affecting the interaction of bacteria with the surface (Neu, 1996; Banat *et al.*, 2010). This mechanism of biofilm inhibition is similar to the mode of action of rhamnolipid surfactants produced by *P. aeruginosa* (Davey *et al.*, 2003) as well as several biosurfactants and bioemulsifiers produced by marine bacteria that display antibiofilm activity against pathogenic bacteria (Kiran *et al.*, 2010). Physical measurements have directly demonstrated that bacterial antibiofilm polysaccharides can alter the properties of abiotic surfaces. For example, cationic colloids brought into contact with *E. coli* K2 culture supernatants led to a rapid charge inversion, indicative of their highly anionic nature (Valle *et al.*, 2006). In addition, both K2 and Ec300p polysaccharides lowered the interfacial energy of glass surfaces, increasing the hydrophilicity of the surface (Valle *et al.*, 2006; Rendueles *et al.*, 2011) (Fig. 2A). Similarly, purified *S. phocae* P180 EPS, which is highly viscous in solution, exhibited emulsifying activity against n-hexadecane and flocculating activity against an activated carbon suspension (Kanmani *et al.*, 2011), both of which are indicative of biosurfactant activity.

Studies utilizing culture supernatants or purified antibiofilm polysaccharides as surface coatings have provided further evidence that antibiofilm polysaccharides modify the physical properties of abiotic surfaces. Precoating microtiter plate wells with *B. licheniformis* SP1 culture supernatants, for example, inhibited biofilm formation by *E. coli* (Sayem *et al.*, 2011). Similarly, pretreatment of glass surfaces with *E. coli* K2 supernatants reduced biofilm formation by *E. coli*, *S. aureus*, *S. epidermidis*, *E. faecalis*, *K. pneumoniae* and *P. aeruginosa* in microfermentors (Valle *et al.*, 2006), and pretreatment of glass slides with purified *E. coli* Ec300p inhibited *S. aureus* biofilm formation in a flow reactor (Rendueles *et al.*, 2011). Evaporation coating of *K. kingae* colony biofilm extract onto polystyrene surfaces also produced an anti-adhesive film that resisted biofilm formation by *S. epidermidis* and *A. actinomycetemcomitans* (Fig. 2B).

#### *Alteration of biotic surface properties*

Evidence suggests that antibiofilm polysaccharides not only modify abiotic surfaces but also alter the physical properties of Gram-negative and Gram-positive bacterial



**Fig. 2.** Mode of action of antibiofilm polysaccharides. A. Alteration of abiotic surfaces. Determination of the surface contact angle of a drop of water on untreated [double-distilled water (dH<sub>2</sub>O)], K2 group II capsule or Ec300p-treated microscope slides showed an increased hydrophilicity of the surfaces. B. Surface coating. Biofilm formation by *S. epidermidis* on polystyrene surfaces coated with *K. kingae* colony biofilm extract. The extract forms an anti-adhesive layer where it contacted the surface. C. Alteration of biotic surfaces. GFP-tagged *E. coli* K12 was inoculated in a flow cell and monitored by confocal microscopy. Addition of K2 culture supernatant after 2 h of growth shows an alteration of development of K12 biofilm after 20 h of growth.

cell surfaces. *Escherichia coli* and *P. fluorescens* cells grown in the presence of *B. licheniformis* SP1 culture supernatant, for example, exhibited decreased cell surface hydrophobicity (Sayem *et al.*, 2011). Consistent with this hypothesis, cell-to-cell autoaggregation mediated by cell-surface adhesins has been shown to be inhibited by *E. coli* K2 polysaccharide (Fig. 2C). *Escherichia coli* K2 culture supernatants inhibited *E. coli* autoaggregation mediated by any of four different intercellular adhesion factors: conjugative pili, curli, Ag43 adhesin or cellulose (Valle *et al.*, 2006). *Lactobacillus acidophilus* r-EPS (Kim *et al.*, 2009), *S. phocae* PI80 EPS (Kanmani *et al.*, 2011) and *Vibrio* sp. A101 (Jiang *et al.*, 2011) were also found to inhibit intercellular adhesion but not growth of planktonic *E. coli* cells. Interestingly, purified A101 polysaccharide inhibited intercellular adhesion by both *P. aeruginosa* and *S. aureus* cells, and was able to disaggregate *P. aeruginosa* cells but not *S. aureus* cells (Jiang *et al.*, 2011). Similarly, purified Ec300p polysaccharide inhibited *S. aureus* biofilm formation, but not autoaggregation (Rendueles *et al.*, 2011). In addition to inhibiting intercellular adhesion, antibiofilm polysaccharides have also been shown to inhibit binding of bacterial cells to various biotic surfaces. For instance, crude *L. acidophilus* r-EPS inhibited attachment of *E. coli* O157:H7 to cultured HT-29 human colon adenocarcinoma cells (Kim *et al.*, 2009).

#### Downregulation of adhesion factors

Using transcriptome analysis, Kim and colleagues (2009) showed that *L. acidophilus* A4 polysaccharide caused downregulation of several *E. coli* O157:H7 genes related to biofilm formation. These included *crl*, *csgA* and *csgB*, which are required for the synthesis of curli adhesive surface fibres, and *cheY*, which encodes a response regulator. Both curli fibres and CheY have been shown to play a role in maintaining *E. coli* biofilm architecture. This suggests that *L. acidophilus* A4 polysaccharide may also act as an interspecies cell-to-cell signal that downregulates biofilm formation in other species. The *E. coli* receptor for *L. acidophilus* A4 polysaccharide is not known.

#### Disruption of preformed biofilms

Ultimately, most biofilms undergo detachment or dispersion, releasing planktonic bacteria that colonize other surfaces. Several different mechanisms have been implicated in this process, including cell death, matrix-degrading enzymes and induction of cellular motility (Karatan and Watnick, 2009; Kaplan, 2010). Several antibiofilm polysaccharides have also been shown to enhance or trigger biofilm dispersal. Purified A101 polysaccharide, for example, was able to disperse

*P. aeruginosa* biofilms (Jiang *et al.*, 2011). Similarly, biofilm extracts containing *K. kingae* PAM galactan dispersed *S. epidermidis* biofilms (Bendaoud *et al.*, 2011), whereas *B. licheniformis* SP1 culture supernatants, and purified *E. coli* Ec300p and K2 polysaccharides, did not disperse any preformed biofilms. In spite of recent research on the mechanisms of action of antibiofilm polysaccharides and other biosurfactants, the precise mechanisms by which they break down preformed biofilms are yet to be elucidated.

## Potential biological functions

### Bacterial competition

Beyond a structural role classically assigned to matrix polysaccharides, antibiofilm polysaccharides found associated with mono- or multispecies communities could contribute to colonization resistance, protecting biofilms from invading species (Fig. 3A). For instance, production of *E. coli* Ec300p anti-adhesion polysaccharide results in the very rapid exclusion of *S. aureus* in mixed *E. coli*/*S. aureus* biofilms, and Ec300 biofilms producing Ec300p are significantly protected from colonization by incoming *S. aureus* (Rendueles *et al.*, 2011). Similarly, the presence of *P. aeruginosa* cells expressing Pel or Psl reduced biofilm formation by *S. epidermidis* in dual-species biofilms *in vitro* (Pihl *et al.*, 2010). These findings suggest that antibiofilm polysaccharides may play a role in bacterial competition and niche exclusion in multispecies biofilms.

### Regulation

Another potential role of antibiofilm polysaccharides is in regulation of biofilm formation (Fig. 3B). Most antibiofilm polysaccharides not only affect a broad spectrum of competing bacteria but also affect the producer strains. For example, *E. coli* strains that produce K2 capsular polysaccharide, and *K. kingae* strains that produce PAM galactan, have been shown to inhibit their own biofilm formation (Valle *et al.*, 2006; Bendaoud *et al.*, 2011). This suggests that K2 and PAM antibiofilm polysaccharides may be involved in regulating biofilm architecture such as the formation of water channels or the dispersal of cells from the biofilm colony. An alternative hypothesis is that antibiofilm polysaccharides could regulate the producer's own adhesion, therefore enabling the bacteria to reduce fitness costs or non-productive interactions with surrounding surfaces. In the later case, *in vivo* regulation of anti-adhesion polysaccharide expression could contribute to fine-tuning of the producer's adhesion to surfaces.

### Signalling

In plants, rhizobial polysaccharides and oligosaccharides are well-known communication molecules able to induce

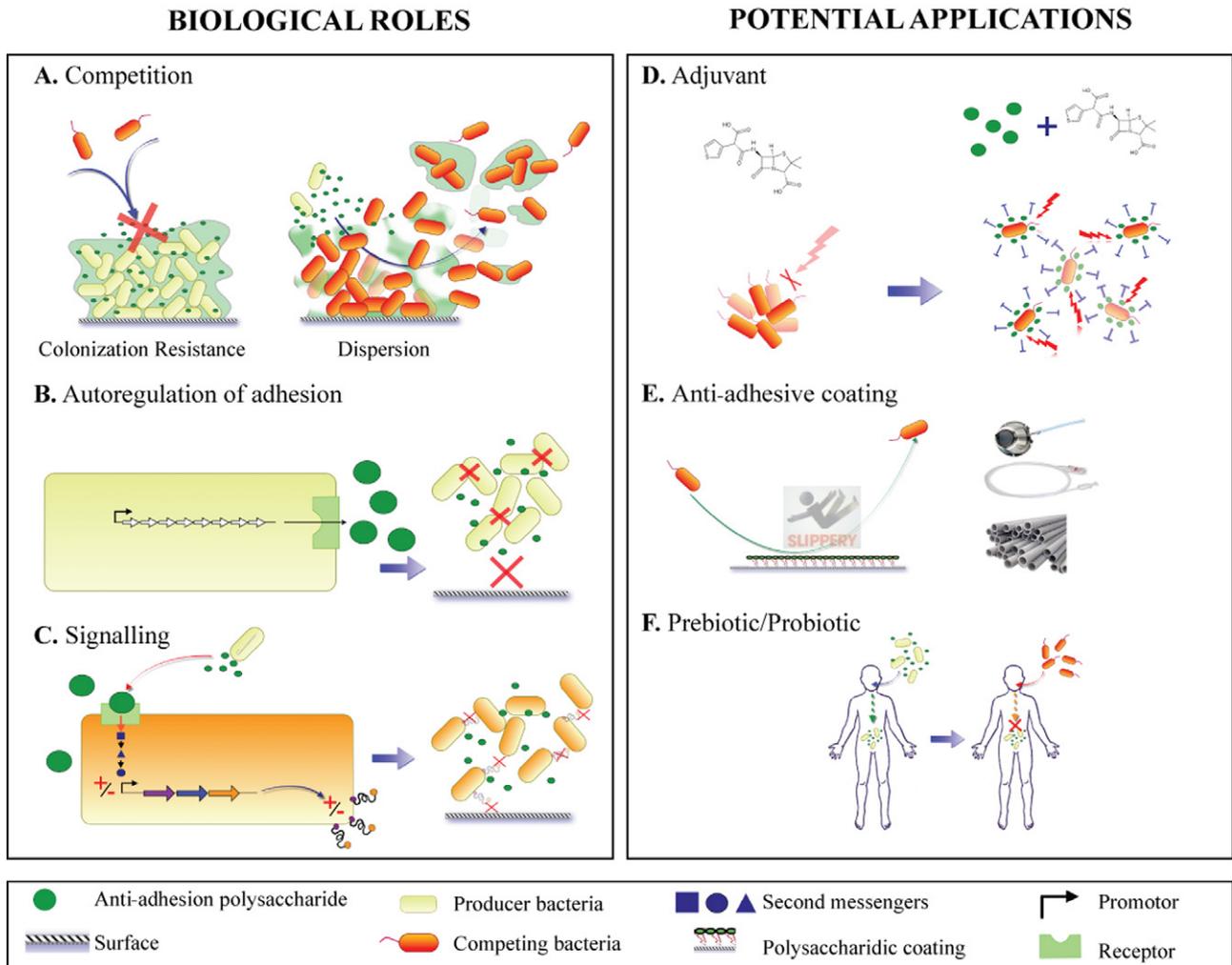
several natural processes such as plant nodulation, an important step in host-symbiont interactions (Frayse *et al.*, 2003). In bacteria, very few studies have directly addressed the role of polysaccharides as signalling molecules, but it has been shown that they can modulate gene expression of neighbouring species (see above; Fig. 3C). Some authors suggest that regulation of gene expression by polysaccharides may be an indirect effect, resulting from high osmolarity due to the presence of EPS, which may then itself act as a signal that triggers genetic responses (Berry *et al.*, 1989). Nevertheless, recent studies have shown that certain bacteria sense the presence of polysaccharides in the extracellular medium via specific receptors and modulate gene expression via alternative sigma factors (Nataf *et al.*, 2010).

## Non-bacterial antibiofilm polysaccharides

Antibiofilm polysaccharide production seems to be a well-conserved ability throughout nature. Evidence suggests that some algal, plant and animal polysaccharides may also exhibit antibiofilm activity (Table 2). Funoran, a sulfated polysaccharide extracted from the seaweed *Gloiopeltis furcata*, inhibited binding of *S. mutans*, *Streptococcus sobrinus*, *P. gingivalis*, *Fusobacterium nucleatum* and *Actinomyces* sp. to saliva-coated hydroxyapatite *in vitro*, prevented colonization of rats by *Streptococcus cricetus* and *S. sobrinus*, and reduced caries scores in rats (Saeki, 1994; Saeki *et al.*, 1996a,b). Funoran also inhibited plaque development in human subjects when administered as a chewing gum (Sato *et al.*, 1998). In addition, several polysaccharides derived from milk have been shown to block LecA-dependent binding of *P. aeruginosa* to human cells (Zinger-Yosovich and Gilboa-Garber, 2009; Zinger-Yosovich *et al.*, 2010; 2011). LecA is a galactoside-binding adhesin that has been shown to contribute to *P. aeruginosa* biofilm architecture under different environmental conditions (Diggle *et al.*, 2006). Various polysaccharides isolated from plants including okra fruit (Lengsfeld *et al.*, 2004; Wittschier *et al.*, 2007), *Aloe vera* (Xu *et al.*, 2010), licorice root (Wittschier *et al.*, 2007; 2009), ginseng (Lee *et al.*, 2004; 2006; 2009a), blackcurrant (Wittschier *et al.*, 2007), as well as polysaccharides isolated from the microalga *Chlorella* and *Spirulina* (Loke *et al.*, 2007), have been shown to inhibit binding of *Helicobacter pylori* to gastric cells and mucin *in vitro*. *Spirulina* polysaccharides were also shown to inhibit colonization of mice by *H. pylori* (Loke *et al.*, 2007).

### Oligosaccharides as anti-adhesion compounds

Plant and human oligosaccharides have long been known to block bacterial adhesion to surfaces and therefore limit biofilm formation (Lane *et al.*, 2010). Many reports reveal



**Fig. 3.** Biological roles and potential applications of anti-adhesion polysaccharides.

#### BIOLOGICAL ROLES.

**A. Competition.** Anti-adhesion polysaccharides can inhibit biofilm formation or enhance biofilm dispersal. They are also involved in colonization resistance against invading or competing bacteria, hence providing an ecological advantage to the producer bacteria.

**B. Anti-adhesion polysaccharides** are secreted into the extracellular medium. Bacteria producing such polysaccharides can be also susceptible to their own antibiofilm polysaccharide and therefore self-regulate their adhesion behaviour.

**C. Competing bacteria** can sense secreted anti-adhesion polysaccharides and respond to it by altering their own gene expression, for instance, by downregulating expression of their adhesion factors.

#### POTENTIAL APPLICATIONS.

**D. Adjuvant.** Several studies point out that anti-adhesion polysaccharides enhance antibiotic functions when administered together. For instance, they can rupture cell-to-cell interactions, rendering antibiotic effect more efficient.

**E. Anti-adhesive coating.** Surfaces coated or grafted with anti-adhesion polysaccharides could be used on indwelling medical devices (here, totally implanted veinous catheters and silicone tubing) or industrial settings (here industrial tubes).

**F. Prebiotic/Probiotic.** Bacteria producing anti-adhesion polysaccharides could be used as probiotics in order to outcompete pathogens, for instance in the gastrointestinal tract. Moreover, biodegradable oligosaccharides are currently used as prebiotics to confer health advantages.

the potential of using oligosaccharides to inhibit pathogen adhesion to host cells and subsequent colonization. For example, galactooligosaccharides have been shown to reduce adhesion of enteropathogenic *E. coli* and *Cronobacter sakazakii* to several intestinal cell lines (Shoaf *et al.*, 2006; Quintero *et al.*, 2011). In addition, oligosaccharides isolated from milk inhibit adhesion of several diarrhoeal pathogens such as *E. coli*, *V. cholerae* and *Sal-*

*monella tyris* to Caco-2 intestinal cells and were shown to inhibit type IV pili-mediated adhesion of *Neisseria meningitidis in vitro* (Hakkarainen *et al.*, 2005; Coppa *et al.*, 2006). Oligosaccharides were also shown to inhibit adhesion of several respiratory pathogens, including *Yersinia pestis*, *Legionella pneumophila*, *Bacillus anthracis* and *Burkholderia pseudomallei* (Thomas and Brooks, 2004a,b).

**Table 2.** Non-bacterial polysaccharides that exhibit anti-adhesive activity against bacteria.

| Source   | Activities   | References   |
|--|--|--|
| <i>Abelmoschus esculentus</i> (okra fruit)         | Inhibits binding of <i>H. pylori</i> and <i>Campylobacter jejuni</i> to human stomach tissue <i>in vitro</i> . Inhibits binding of <i>C. jejuni</i> to chicken stomach tissue <i>in vitro</i> .  | Lengsfeld <i>et al.</i> (2004); Wittschier <i>et al.</i> (2007)        |
| <i>Aloe vera</i> (plant)                           | Inhibits binding of <i>H. pylori</i> to human gastric cancer cells <i>in vitro</i> .   | Xu <i>et al.</i> (2010)  |
| <i>Aralia continentalis</i> (Manchurian spikenard) | Inhibits adhesion of <i>S. mutans</i> to saliva-coated hydroxyapatite beads.   | Lee <i>et al.</i> (2011)   |
| Bovine   | Milk oligosaccharides inhibit binding of <i>N. meningitidis</i> pili to bovine thyroglobulin.  | Hakkarainen <i>et al.</i> (2005)                                       |
| <i>Camellia sinensis</i> (green tea)               | Inhibition of <i>H. pylori</i> , <i>Propionibacterium acnes</i> and <i>S. aureus</i> to host cell lines.   | Lee <i>et al.</i> (2009b)  |
| <i>Chlorella</i> (microalga)                       | Inhibits binding of <i>H. pylori</i> to porcine gastric mucin <i>in vitro</i> .  | Loke <i>et al.</i> (2007)  |
| <i>Gllopelitis turcata</i> (seaweed)               | Inhibits binding of <i>S. sobrinus</i> , <i>S. sobrinus</i> , <i>P. gingivalis</i> , <i>F. nucleatum</i> and <i>Actinomyces</i> to saliva-coated hydroxyapatite <i>in vitro</i> . Prevents colonization of rats by <i>S. cricetus</i> and <i>S. sobrinus</i> , and reduces caries score. Inhibits plaque development in human subjects when administered as a chewing gum. | Saeki (1994); Saeki <i>et al.</i> (1996a,b); Sato <i>et al.</i> (1998) |
| <i>Glycyrrhiza glabra</i> (licorice root)          | Inhibits binding of <i>H. pylori</i> to human stomach tissue <i>in vitro</i> . Inhibits binding of <i>P. gingivalis</i> to rat oesophageal tissue <i>in vitro</i> .  | Wittschier <i>et al.</i> (2007; 2009)                                  |
| Human  | Polysulfated polysaccharides inhibit binding of several <i>H. pylori</i> strains to murine macrophage cell lines.  | Lutay <i>et al.</i> (2011)   |
| Human  | Heparin blocks adhesion of <i>H. pylori</i> and enterohaemorrhagic <i>E. coli</i> to macrophages and colonic epithelium respectively.  | Gu <i>et al.</i> (2008); Lutay <i>et al.</i> (2011)                    |
| <i>Panax ginseng</i> (plant)                       | Inhibits agglutination of human erythrocytes by <i>H. pylori</i> , <i>P. gingivalis</i> , <i>A. actinomycetemcomitans</i> , <i>P. acnes</i> and <i>S. aureus</i> . Inhibits binding of <i>H. pylori</i> to human gastric cancer cells <i>in vitro</i> .  | Lee <i>et al.</i> (2004; 2006; 2009a)                                  |
| <i>Fibes nigrum</i> (blackcurrant seed)            | Inhibits binding of <i>H. pylori</i> to human stomach tissue <i>in vitro</i> .   | Wittschier <i>et al.</i> (2007)  |
| Soybean tempe                                      | Inhibits adhesion of enterotoxigenic <i>E. coli</i> to intestinal cells.   | Roubos-van den Hil <i>et al.</i> (2010)                                |
| <i>Spirulina</i> (microalga)                       | Inhibits binding of <i>H. pylori</i> to porcine gastric mucin <i>in vitro</i> . Inhibits colonization of mice by <i>H. pylori</i> .  | Loke <i>et al.</i> (2007)  |
| <i>Vitis coignetiae</i> (crimson glory grape vine) | Inhibits adhesion of <i>S. mutans</i> to saliva-coated hydroxyapatite beads.   | Yano <i>et al.</i> (2012)  |

## Potential applications

Microbial polysaccharides have long been used for their biological, chemical and physical properties (reviewed by Sutherland, 1998). Antibiofilm polysaccharides could be applied in medical and industrial settings, in which antibiotic tolerance within biofilms is a growing concern. Moreover, toxicity issues and the rapid emergence of resistance associated with biocides have fostered interest in non-biocidal biofilm strategies. As non-biocidal agents destabilize the biofilm matrix without killing cells or inhibiting cell growth, they do not affect bacterial fitness and are less likely to develop resistance. Antibiofilm polysaccharides also have a number of characteristics such as biocompatibility and biodegradability that would be favourable for any medical or industrial application. Most bacterial antibiofilm polysaccharides also exhibit broad-spectrum biofilm-inhibiting activity, and some are able to disperse preformed biofilms. Potential applications of antibiofilm polysaccharides are illustrated in Fig. 3.

Purified *Vibrio* sp. A101 polysaccharide has been shown to decrease the minimum biofilm eradication concentration of amikacin, tobramycin and gentamicin against *P. aeruginosa* biofilms (Jiang *et al.*, 2011), which suggests that antibiofilm polysaccharides or oligosaccharides may be useful as adjuvants in traditional antibiotic treatments (Fig. 3D). This could be a promising strategy to reduce the emergence of antibiotic resistance in clinical settings. Several antibiofilm polysaccharides have been shown to function as efficient anti-adhesive coatings (Valle *et al.*, 2006; Bendaoud *et al.*, 2011; Rendueles *et al.*, 2011; Sayem *et al.*, 2011). This approach could potentially reduce the incidence of medical device-related infections (Fig. 3E). *Lactobacillus acidophilus* r-EPS has been shown to inhibit attachment of *E. coli* to cultured human colon adenocarcinoma cells (Kim *et al.*, 2009), raising the possibility that strains producing antibiofilm polysaccharides could also potentially be used as probiotics delivering saccharidic prebiotics (Fig. 3F). Taken together, these properties may make antibiofilm polysaccharides suitable for the treatment and prevention of a variety of biofilm-related infections, especially those caused by multispecies biofilms.

Among the antibiofilm polysaccharides identified to date, only the structure of *K. kingae* PAM galactan has been fully elucidated (Bendaoud *et al.*, 2011). Defining of the chemical and structural basis of antibiofilm polysaccharides will allow chemical synthesis, molecular mimicry and potential large-scale applications in medical and industrial settings. Adhesion-specific inhibition of lectins recognizing specific monosaccharides would directly lead to inability to adhere to a surface and inhibit subsequent biofilm formation. The use of *ad hoc* surface grafted glycan arrays to identify to sugar monomers that bind to

lectins, that is lectin specificity, is a promising strategy (Liang and Wu, 2009). Once identified, specific carbohydrate-based inhibitors could be designed and used to prevent bacterial fimbriae from binding to their receptors. For instance, a crystallographic analysis of the *E. coli* FimH adhesin showed that exogenous alpha D-mannosides display greater affinity for the adhesin than mannose (Bouckaert *et al.*, 2005). Other strategies may take into account the multiplicity of bacterial lectins on the cell surface, and are focused on the development of multivalent inhibitors through the creation of supramolecular structures such as glycopolymers, glycodendrimers or glyconanoparticles (Korea *et al.*, 2011).

### Future questions

Recent screens of culture supernatants and biofilm extracts for antibiofilm activities have yielded hit rates as high as 20% (Valle *et al.*, 2006; Bendaoud *et al.*, 2011; Sayem *et al.*, 2011), suggesting we have only begun to explore the diversity of the contribution of bacterial antibiofilm polysaccharides in interspecies interactions (Fig. 3). Whereas use of non-biocidal antibiofilm compounds in medicine and industry represents an appealing strategy, much research is still needed to validate the use of such molecules as alternative anti-infectious treatments in environmental or clinical settings. Focus should for instance be put on clarifying the relationship between polysaccharide structures and antibiofilm activities. While determination of antibiofilm polysaccharide composition and structure will enable their controlled industrial-scale production, it could also clarify their mechanism of action and provide leads for developing analogues with enhanced antibiofilm activity. Further studies should also address the biological roles of antibiofilm polysaccharides and their impact on population dynamics *in vivo*. While determinant for future applications, these studies could also provide new and valuable insights into processes driving co-evolution in multispecies biofilm consortia.

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