

Enzymatic modifications of exopolysaccharides enhance bacterial persistence

Gregory B. Whitfield^{1,2†}, Lindsey S. Marmont^{1,2†} and P. Lynne Howell^{1,2*}

¹ Program in Molecular Structure and Function, Research Institute, The Hospital for Sick Children, Toronto, ON, Canada,

² Department of Biochemistry, Faculty of Medicine, University of Toronto, Toronto, ON, Canada

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*Correspondence:

P. Lynne Howell,
Program in Molecular Structure
and Function, Research Institute,
The Hospital for Sick Children,
686 Bay Street, Toronto,
ON M5G 0A4, Canada
howell@sickkids.ca

†These authors have contributed
equally to this work.

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Biofilms are surface-attached communities of bacterial cells embedded in a self-produced matrix that are found ubiquitously in nature. The biofilm matrix is composed of various extracellular polymeric substances, which confer advantages to the encapsulated bacteria by protecting them from eradication. The matrix composition varies between species and is dependent on the environmental niche that the bacteria inhabit. Exopolysaccharides (EPS) play a variety of important roles in biofilm formation in numerous bacterial species. The ability of bacteria to thrive in a broad range of environmental settings is reflected in part by the structural diversity of the EPS produced both within individual bacterial strains as well as by different species. This variability is achieved through polymerization of distinct sugar moieties into homo- or hetero-polymers, as well as post-polymerization modification of the polysaccharide. Specific enzymes that are unique to the production of each polymer can transfer or remove non-carbohydrate moieties, or in other cases, epimerize the sugar units. These modifications alter the physicochemical properties of the polymer, which in turn can affect bacterial pathogenicity, virulence, and environmental adaptability. Herein, we review the diversity of modifications that the EPS alginate, the Pel polysaccharide, *Vibrio* polysaccharide, cepacian, glycosaminoglycans, and poly-*N*-acetyl-glucosamine undergo during biosynthesis. These are EPS produced by human pathogenic bacteria for which studies have begun to unravel the effect modifications have on their physicochemical and biological properties. The biological advantages these polymer modifications confer to the bacteria that produce them will be discussed. The expanding list of identified modifications will allow future efforts to focus on linking these modifications to specific biosynthetic genes and biofilm phenotypes.

Keywords: Biofilm, exopolysaccharide, PNAG, PIA, alginate, PEL, VPS, cepacian

Abbreviations: Bcc, *Burkholderia cepacia* complex; CF, cystic fibrosis; CPS, capsular polysaccharide; EPS, exopolysaccharide; G-blocks, guluronate blocks; GAGs, glycosaminoglycans; GBS, Group B *Streptococcus*; GlcA, glucuronic acid; GulA, guluronate; GulNAcA, *N*-acetyl guluronate; HS, heparan sulfate; IdoA, iduronic acid; LPS, lipopolysaccharide; M-blocks, mannuronate blocks; ManA, mannuronate; MBOAT, membrane-bound O-acetyltransferase; MG-blocks, mixed mannuronate and guluronate blocks; PEL, pel polysaccharide; PIA, polysaccharide intercellular adhesin; PNAG, poly-*N*-acetylglucosamine; polyM, polymannuronate; ROS, reactive oxygen species; VPS, *Vibrio* polysaccharide.

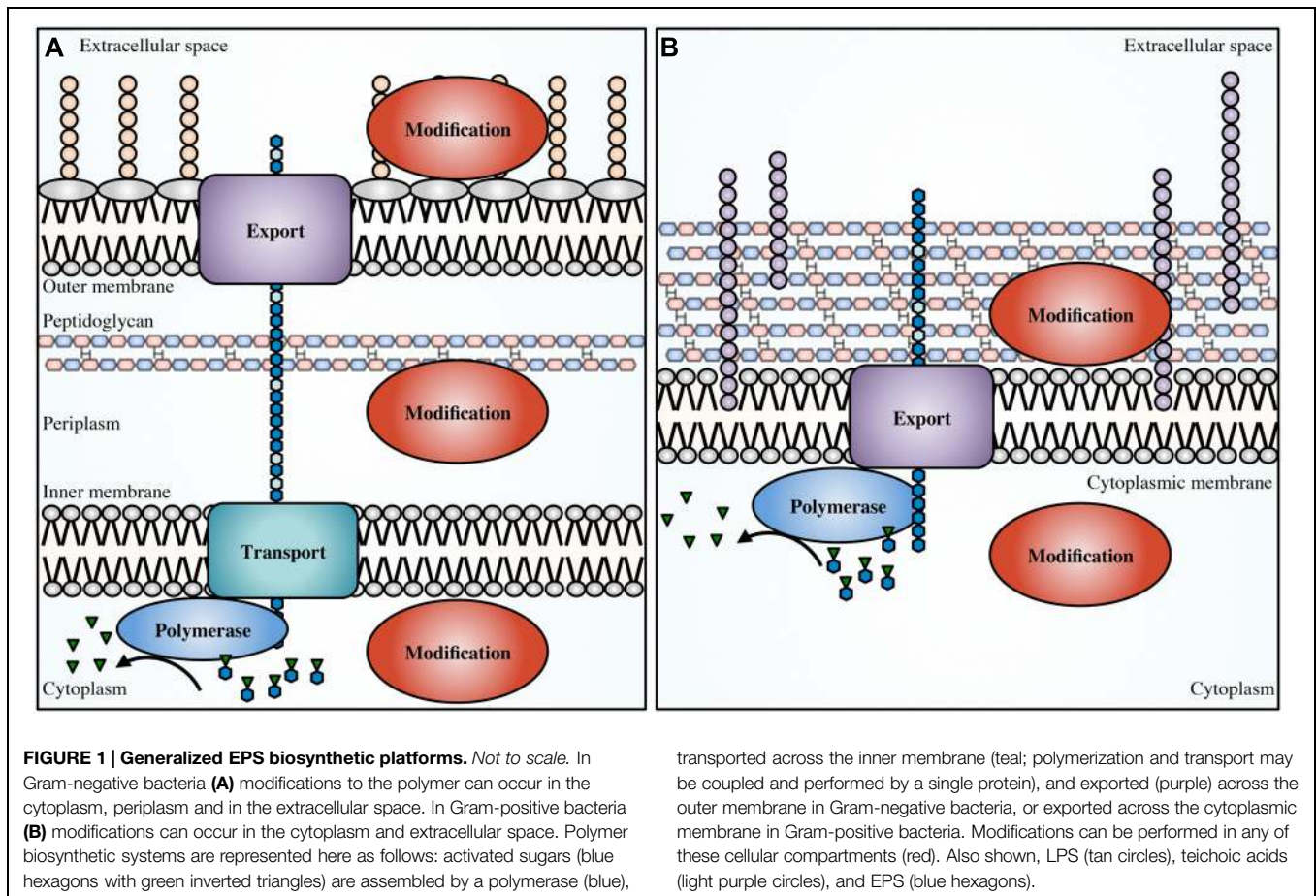
Introduction

Bacteria faced with fluctuating or stressful environmental conditions can undergo a number of physiological changes. One common tactic that bacteria use to adapt to their surroundings is to grow as a multicellular community or biofilm. Biofilm formation begins with attachment of the bacteria to a surface or, in the case of some infectious biofilms, embedding of the bacteria in host-derived tissue or mucous. This is followed by bacterial aggregation, colony development, and the secretion of self-produced polymeric substances, which form a matrix that encapsulates and protects the bacteria (Ohman, 1986; Allesen-Holm et al., 2006). This matrix is composed of nucleic acids, proteins, lipids, and extracellular polysaccharides (EPS; Flemming and Wingender, 2010), with the types and ratio of each component varying between bacterial species and environmental conditions. Once the biofilm has matured into a robust structure it becomes exceedingly difficult to eradicate, and is typically capable of enduring mechanical, biological, and chemical means of elimination. Bacteria form biofilms in nearly all environments studied to date (Bjarnsholt et al., 2013b), and are implicated in the contamination of surfaces as diverse as the International Space Station (Kim et al., 2013), ship hulls (Schultz et al., 2011), and oil storage and transfer infrastructure (Lenhart et al., 2014). Biofilms are also of major concern in medical settings, where

they are responsible for the chronic infection of burn wounds, eye and skin lacerations, and pneumonia in CF patients (Lyczak et al., 2000). The contamination of medical devices such as catheters, prosthetic joints, and ventilators (Veerachamy et al., 2014) has also been well documented. In these environments EPS often contribute to the formation, growth, and preservation of biofilm architecture and also serve to protect the bacteria against antibiotics, desiccation, and the host's immune defenses.

Biosynthesis of EPS begins in the cytoplasm with the generation of activated precursor sugars. These precursors are often taken from common cellular sugar pools and are modified for specific use in EPS biosynthesis pathways, prior to polymerization (**Figure 1**). In Gram-negative bacteria, the polymer is transported across the inner membrane to the periplasm during synthesis; whereas in Gram-positive bacteria the polymer is transported directly to the extracellular space. Modifications to the polymer can occur in the cytoplasm (Atkin et al., 2014) or the periplasm (Colvin et al., 2013; Baker et al., 2014; Little et al., 2014b; Wolfram et al., 2014) prior to export across the outer membrane, and in the extracellular space in both Gram-positive and Gram-negative bacteria (Rozeboom et al., 2008; Little et al., 2014a).

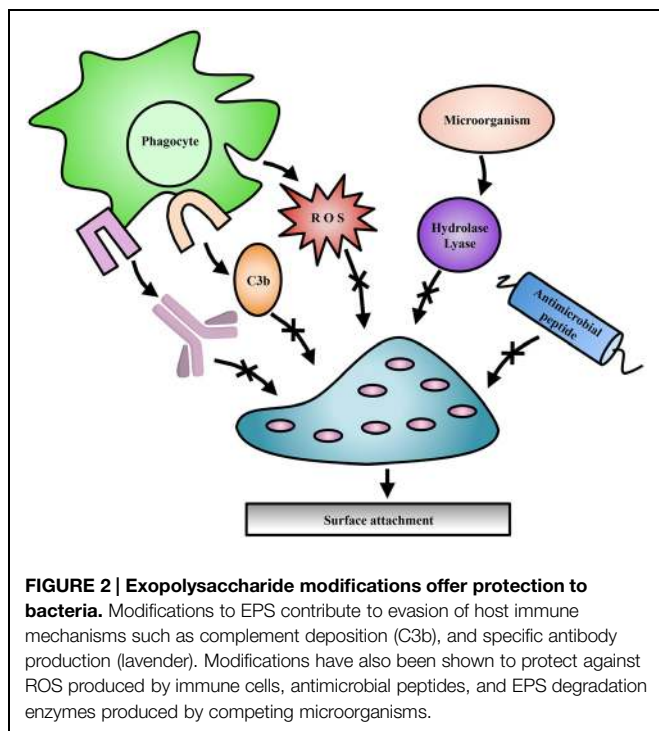
The chemical structure of EPS from different bacterial species, or even within the same organism, can vary greatly. Bacterial EPS are usually composed of hexose sugars, but pentose sugars



have also been identified. *Rhizobium huakuii* EPS contains ribose (Hisamatsu et al., 1997), while some marine bacteria produce EPS with xylose and ribose moieties (Kwon et al., 2002). EPS can be homo- or hetero-polymers, and have branching side chains (Byrd et al., 2009; Cescutti et al., 2010) or be simple linear sugar polymers (Linker and Jones, 1966; Maira-Litrán et al., 2002). They can be as short as dimers and trimers, or thousands of saccharide repeat units long (González et al., 1998), depending on the mechanisms of chain length regulation, and can even be woven together to form fibers (Benziman et al., 1980).

Exopolysaccharides can be modified by the action of transferases and hydrolases which add or remove functional groups such as acetyls, pyruvyls (Marzocca et al., 1991), glyceryls (Kuo et al., 1986), succinyls (Reuber and Walker, 1993), lactyls (Maalej et al., 2014), or a combination of these, leading to variations in polymer surface electrostatics and solubility. Additionally, epimerization can drastically alter the structural conformation of polysaccharides, affecting polymer interactions within the biofilm (Steigedal et al., 2008). Some of these modifications have been studied with respect to their importance in bacterial virulence, pathogenesis, biofilm formation, or symbiosis (Figure 2; Ridout et al., 1997), as well as their commercial utility in the food and cosmetic industries.

Despite this wealth of knowledge, there remain a number of unresolved questions regarding the biological implications of EPS modifications. In this review, we explore the modifications that biofilm-forming EPS produced by human pathogenic bacteria undergo and discuss the proteins involved in modification, as well as the role modifications play in bacterial persistence in the environment and host.



Alginate

Alginate synthesis has been characterized in several species of brown algae, as well as in the genera *Azotobacter* and *Pseudomonas* (Gorin and Spencer, 1966; Evans and Linker, 1973; Govan et al., 1981; Gacesa, 1988). Bacterial alginate is a high molecular weight, linear polysaccharide composed of β -1,4-linked D-ManA and variable amounts of its C5 epimer L-GuA (Smidsrød and Draget, 1996). In *Pseudomonas aeruginosa* and *Azotobacter vinelandii*, alginate is initially synthesized as polyM in the cytoplasm and is shuttled across the inner membrane to the periplasm, where it is randomly acetylated at the O2 and/or O3 hydroxyl positions (Table 1; Figure 3; Franklin and Ohman, 1993, 1996, 2002). ManA residues that are not acetylated serve as substrates for epimerization at the C5 position by the enzyme AlgG in the periplasm, leading to the formation of mixed ManA and GuA segments (MG-blocks) as well as non-epimerized sections (M-blocks; Gacesa, 1988; Jain et al., 2003).

Pseudomonas aeruginosa chronic lung infections in CF patients are the leading cause of morbidity and mortality. In these infections the production of alginate is often linked to poorer patient prognosis (Lyczak et al., 2000). In the lung, the clinical isolate *P. aeruginosa* FRD1 displays an alginate-overproducing, or mucoid phenotype due to mutations in negative regulatory elements, providing *P. aeruginosa* with the capability of adhering to respiratory tract epithelial cells and mucin (Marcus and Baker, 1985; Doig et al., 1987; Ramphal et al., 1987). In addition, alginate production has been linked to the hindrance of host cell-mediated phagocytosis and neutralization of ROS (Learn et al., 1987; Mai et al., 1993; Pier et al., 2001). Mucoid conversion is still not well understood, but *in vitro* experimentation has shown that nutrient and aeration levels (Buckmire, 1984; Krieg et al., 1986; Speert et al., 1990) and external stressors (Terry et al., 1991; Damron et al., 2011; Limoli et al., 2014) can induce mucoidy.

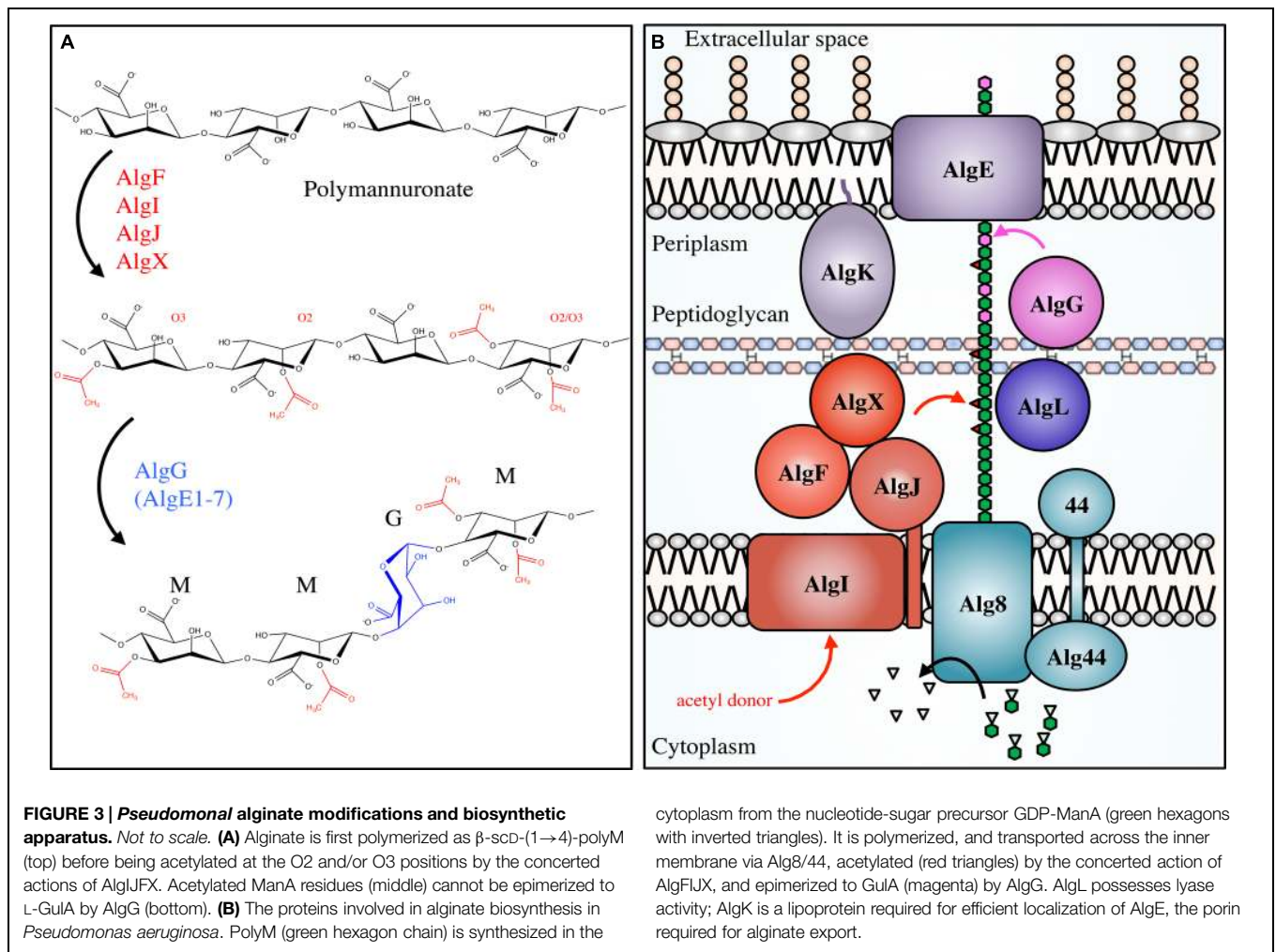
Azotobacter vinelandii is a soil-borne bacterium that is often used as a model organism for nitrogen fixation studies (Bulen et al., 1964). Although *A. vinelandii* is not a human pathogen, understanding the process of alginate biosynthesis in this organism has provided valuable insight into the biological significance of alginate production by *P. aeruginosa*. In *A. vinelandii*, alginate plays a unique and essential role where, under conditions of nutrient and environmental stress such as nitrogen starvation, *A. vinelandii* converts from a vegetative cell to a dormant cyst (Socolofsky and Wyss, 1961). Cyst development proceeds through deposition of a protective extracellular material composed primarily of alginate. The cyst layers are rich in proteins, lipids and carbohydrates, with the exine (outer layer) and intine (inner layer) containing carbohydrate material consisting of approximately 40 and 72% polyuronic acids, respectively (Lin and Sadoff, 1969). Cyst formation, much like the biofilm matrix, protects *A. vinelandii* from desiccation, and only when environmental conditions become more favorable will *A. vinelandii* convert back to the vegetative state by degrading the alginate barrier. In contrast to *P. aeruginosa*, *A. vinelandii* contains seven additional extracellular epimerases (AlgE1-7),

TABLE 1 | Exopolysaccharide modifying enzymes of pathogenic bacteria.

Protein name	GenBank Accession number	Organism(s) studied ^a	Function	Cellular localization	PDB code ^b	Additional comments	Reference
AlgG	NP_252235.1 AAP46694.1 YP_002798298.1	<i>P. aeruginosa</i> <i>P. fluorescens</i> <i>A. vinelandii</i>	Mannuronan C5-epimerase; introduces MG-blocks into polyM alginate	Periplasm	4NK6	Required for alginate production and epimerization in <i>P. aeruginosa</i> and <i>P. fluorescens</i> ; cannot epimerize acetylated alginate	Chitnis and Ohman (1990), Franklin et al. (1994), Rehm et al. (1996), Morea et al. (2001), Gimmestad et al. (2003), Jain et al. (2003), Wolfram et al. (2014)
AlgE1	YP_002802178	<i>A. vinelandii</i>	Mannuronan C5-epimerases; alginate lyase (AlgE2, AlgE7); varying MG- and G-block forming activities	Extracellular	AlgE4: 2PYG (active domain), 2AGM (regulatory domain) AlgE6: 2ML2 and 2ML3 (regulatory domains)	Ca ²⁺ -dependent; modular architecture composed of one or more epimerase active domains and activity enhancing regulatory domains	Ertesvåg et al. (1994, 1995, 1998), Ertesvåg and Valla (1999), Svanem et al. (1999, 2001), Aachmann et al. (2006), Rozeboom et al. (2008), Buchinger et al. (2014)
AlgJ	NP_252239.1	<i>P. aeruginosa</i>	Exhibits <i>in vitro</i> acetyltransferase activity	Periplasm - IM tethered	4O8V	Required for alginate acetylation	Franklin and Ohman (1996, 2002), Baker et al. (2014)
AlgF	NP_252240.1 YP_002798293.1	<i>P. aeruginosa</i> <i>A. vinelandii</i>	Acetylation; specific role unknown	Periplasm	ND	Required for alginate acetylation	Franklin and Ohman (1993), Shinabarger et al. (1993), Vazquez et al. (1999), Franklin and Ohman (2002)
AlgI	NP_252238.1	<i>P. aeruginosa</i>	Acetylation; predicted MBOAT	Inner membrane	ND	Required for alginate acetylation	Franklin and Ohman (1996, 2002), Franklin et al. (2004)
AlgX	NP_252236.1	<i>P. aeruginosa</i> <i>A. vinelandii</i>	Acetylation; exhibits <i>in vitro</i> acetyltransferase activity; terminal alginate acetylase	Periplasm	4KNC	Required for alginate production and acetylation	Monday and Schiller (1996), Robles-Price et al. (2004), Riley et al. (2013), Baker et al. (2014)
PelA	NP_251754.1	<i>P. aeruginosa</i>	Deacetylation; <i>in vitro</i> deacetylase activity	Periplasm	2VYO (30%; 511-794)	Modeled region of deacetylase, also has N-terminal hydrolase domain	Colvin et al. (2013)
BceO	YP_001116903	<i>B. cepacia</i> complex	Acetyltransferase	Inner membrane (predicted)	ND	Deletion of <i>bceS</i> – reduced acetylation	Ferreira et al. (2010)
BceS	YP_001116907						
BceU	YP_001116910						
VpsC	NP_230566.1	<i>V. cholerae</i>	Acetyltransferase (proposed)	ND	1T3D (92%; 2-172) 1T3D (97%; 1-140)	Modeled protein is an acetyltransferase	Fong et al. (2010)
VpsG	NP_230570.1						
PgaB	NP_415542.1	<i>E. coli</i>	Deacetylase; C-terminal carbohydrate binding module facilitates PNAG export	Outer membrane lipoprotein	4F9J/4F9D	Low catalytic efficiency allows for partial PNAG deacetylation	Wang et al. (2004), Itoh et al. (2008), Little et al. (2012, 2014b)
HmsF	NP_415542.1	<i>Y. pestis</i>	Deacetylase	Outer membrane	4F9D (88%; 43-646)	Modeled on PgaB	Forman et al. (2006)
IcaB	AAC06118.1 AAD52057.1	<i>S. epidermidis</i> <i>S. aureus</i>	Deacetylase	Extracellular	4WCJ	Low catalytic efficiency allows for partial PIA deacetylation	Pokrovskaya et al. (2013), Little et al. (2014a)
IcaC	AAC06119.1 AAD52058.1	<i>S. epidermidis</i> <i>S. aureus</i>	Succinyltransferase (proposed)	Membrane embedded	ND	Not experimentally determined	Atkin et al. (2014)

^aGenus abbreviations as follows: *P. aeruginosa*, *Pseudomonas fluorescens*; *A. vinelandii*, *Azotobacter vinelandii*; *B. cepacia* complex, *Bcc*; *V. cholerae*, *Vibrio cholerae*; *E. coli*, *Escherichia coli*; *Y. pestis*, *Yersinia pestis*; *S. epidermidis*, *Staphylococcus epidermidis*; *S. aureus*, *Staphylococcus aureus*.

^bAccession code for structure or structurally related protein, bold letters indicate that the structure has been experimentally determined. Where applicable, protein coverage (%) and residues modeled are listed in brackets. ND: not determined, Phyre² (Kelley and Sternberg, 2009) was unable to model the protein.



capable of generating polyguluronate segments (G-blocks) in addition to MG-blocks (Table 1; Fischer and Dorfel, 1955; Haug et al., 1966, 1967). Despite the importance of alginate in cyst formation (Campos et al., 1996), alginate production by *A. vinelandii* has been studied primarily for its potential use as an alternative source of commercial alginate in place of traditional seaweed harvesting approaches.

Given the importance of alginate for the virulence of *P. aeruginosa* in the CF lung, the protective characteristics of *A. vinelandii* cysts, and the use of bacterial alginate as a convenient substitute for commercial eukaryotic alginate sources, there has been a drive to understand the consequences of alginate acetylation and epimerization on these processes.

Alginate Acetylation

Alginate acetylation in *P. aeruginosa* requires the collective actions of the proteins AlgF, AlgI, AlgJ, and AlgX for the addition of acetyl groups to the O2 and/or O3 hydroxyl positions of polyM in the periplasm prior to epimerization and export (Table 1; Figure 3). Specifically, it is thought that AlgI transfers

an acetyl group from an as yet unidentified cytoplasmic donor to AlgJ or AlgX, where it may be passed between them before transfer to the polymer by AlgX (Riley et al., 2013; Baker et al., 2014). AlgF is an important part of the acetylation process but has not yet been assigned a role. Based on the peptidoglycan O-acetylation machinery (Moynihan and Clarke, 2011), it seems that the presence of a MBOAT for acetyl-donor transport (AlgI), and an acetyltransferase (AlgX) should be sufficient for polysaccharide O-acetylation. As acetylation occurs at both the O2 and O3 positions, one hypothesis is that the proteins AlgJ and AlgF govern specificity. Although they have not yet been shown to directly bind alginate, they may still be able to regulate the position and frequency of acetyl addition under different environmental conditions. Genetic and biochemical experiments targeting the degree of alginate acetylation in combination with structural data may provide insight into these questions.

One of the purposes of EPS production is to serve as a structural component of the biofilm matrix. Alginate lacking O-acetyl groups has been analyzed for its ability to form structured biofilms using an acetylation defective *P. aeruginosa* FRD1 mutant. These studies revealed that the mutant was only

TABLE 2 | Biological implications of EPS modifications.

Modification	Proteins involved	Organism studied	Implication of modification	Reference
Alginate				
Acetylation	AlgF, AlgI, AlgJ, AlgX	<i>Pseudomonas aeruginosa</i>	Required for surface attachment and formation of structured microcolonies Increased polymer viscosity Decreased neutrophil locomotion and lymphocyte transformation Reduced activation of complement and opsonic killing by phagocytes Scavenging of ROS Reduced susceptibility to enzymatic degradation Increased gel thickness	Nivens et al. (2001), Tielen et al. (2005) Tielen et al. (2005) Mai et al. (1993) Pier et al. (2001) Learn et al. (1987) Farrell and Tipton (2012) Skjåk-Braek et al. (1989)
Epimerization	AlgG, AlgE1-7 (<i>Azotobacter vinelandii</i>)	<i>P. aeruginosa</i> <i>A. vinelandii</i>	Improved gel forming ability (cohesion) Upregulation of virulence factors through Ca ²⁺ sequestration Maintain biofilm structure during changing environmental conditions Preserve N ₂ -fixing capability Required for formation of functional cyst coat	Grant et al. (1973), Donati et al. (2005) Horsman et al. (2012) Ertesvåg et al. (1998) Sabra et al. (2000) Steigedal et al. (2008)
PEL				
Deacetylation	PelA	<i>P. aeruginosa</i>	Required for biofilm formation (in PSL deficient strains)	Colvin et al. (2013)
Cepacian				
Acetylation	BceOSU	<i>Bcc</i>	Reduced susceptibility to enzymatic degradation Scavenging of ROS	Cescutti et al. (2006) Cuzzi et al. (2012)
Vibrio polysaccharide (VPS)				
Acetylation	VpsG	<i>Vibrio cholerae</i>	Required for robust biofilm formation and wild-type phenotypes	Fong et al. (2010)
Poly-N-acetyl-glucosamine (PNAG)				
Deacetylation	PgaB (<i>Escherichia coli</i>), HmsF (<i>Yersinia pestis</i>) IcaB (<i>Staphylococcus epidermidis</i> and <i>S. aureus</i>)	<i>S. epidermidis</i> <i>S. aureus</i> <i>E. coli</i> <i>Y. pestis</i>	Required for biofilm formation and surface attachment Resistance to human cationic antimicrobial peptides Resistance to neutrophil phagocytosis Persistence in mouse model of infection Required for biofilm formation and surface attachment Resistance to phagocytosis Persistence in mouse model of infection Required for export of polymer and biofilm formation Required for biofilm formation	Vuong et al. (2004a) Cerca et al. (2007) Itoh et al. (2008) Forman et al. (2006)
Succinylation	IcaC	<i>S. aureus</i>	Modulation improves <i>in vitro</i> fitness	Brooks and Jefferson (2014)

able to produce small, unstructured microcolonies that sparsely populated the examined surface, suggesting an attachment defect (Table 2; Nivens et al., 2001). In contrast, FRD1 formed extensive biofilm structures that exhibited significant structural heterogeneity. In a separate study, an aggregation defect was revealed when the capacity for an acetylation-deficient FRD1 mutant to adhere to a steel surface was tested (Tielen et al., 2005). Additionally, the viscosity of extracellular material from the acetylation-defective mutant was significantly reduced in comparison to FRD1, suggesting that the loss of *O*-acetyl groups led to weakening of inter- and intra-polymer interactions within the biofilm matrix. This is supported by rheological studies of FRD1 biofilms, which suggested that inter-chain alginate interactions occur primarily through physical entanglements

(Wloka et al., 2005). These entanglements supported an elastic biofilm architecture, which differed from *O*-acetylation-defective FRD1 mutants which produced weaker biofilms with reduced resistance to tensile forces. Based on these results, it was suggested that *O*-acetyl groups in alginate act as molecular hooks that improve the resistance of the entangled alginate structural network against applied forces (Wloka et al., 2005). While the importance of alginate acetyl groups for cell aggregation and microcolony formation *in vitro* is well established, the influence of *O*-acetyl groups on biofilm formation phenotypes in clinically relevant *P. aeruginosa* infections or related *in vivo* model systems of infection remain uncharacterized.

P. aeruginosa biofilm formation in the CF lung has been shown to provide significant protection from a variety of host immune

factors. For example, decreased locomotion of neutrophils, as well as reduced lymphocyte transformation, have been observed when these cell types are incubated with alginate (Simpson et al., 1988). However, chemical removal of acetyl groups from alginate led to a complete loss of these inhibitory effects on neutrophil and lymphocyte function, suggesting that alginate *O*-acetylation is essential for their suppression (Mai et al., 1993). The activation of complement is also affected by the presence of acetyl groups (Pier et al., 2001). This is not surprising given that interactions between alginate and the complement component C3b likely occurs through unsubstituted hydroxyl groups (Hostetter et al., 1982), suggesting that the addition of acetyl groups to alginate in *P. aeruginosa* may have evolved as a mechanism for complement evasion. Activation of the alternative pathway of complement can lead to phagocytic killing, which is also impaired by the presence of *O*-acetyl groups. Opsonic killing of the FRD1 *O*-acetylation deficient mutant by phagocytes was readily observed, while wild-type FRD1 was resistant to these attacks (Pier et al., 2001). Alginate is also known to scavenge ROS produced by phagocytic cells during infection. Hypochlorite is a common ROS produced by phagocytes, and the presence of alginate in mucoid *P. aeruginosa* provides a significant protective advantage against hypochlorite over non-mucoid cells *in vitro* (Learn et al., 1987). This protective effect was, in part, attributed to the *O*-acetyl groups, as chemically deacetylated alginate exhibited impaired hypochlorite scavenging. Furthermore, addition of hypochlorite to native alginate led to a decrease in viscosity, similar to that seen for the chemically deacetylated alginate, suggesting that hypochlorite may be specifically reacting with *O*-acetyl groups from native alginate (Learn et al., 1987).

When bacteria are contending for control of the same environment, they can release extracellular enzymes to degrade critical structural components of cohabiting organisms to give them a competitive advantage (Korotkov et al., 2012). This is observed in the CF lung, where instances of multi-species biofilms are common (Elias and Banin, 2012). During colonization of the CF lung, alginate acetyl groups may serve as a protective mechanism to prevent unwanted degradation of alginate within the biofilm by bacteria that could secrete an AlgL-like lyase as an offensive tactic. The *P. aeruginosa* alginate lyase AlgL preferentially degrades deacetylated alginate or polyM over mature, acetylated alginate (Farrell and Tipton, 2012). Furthermore, *O*-acetyl groups prevent the epimerization of ManA to GulA by the epimerases AlgE1-7 in *A. vinelandii*, which may allow for control over the degree of epimerization and, in turn, regulation of the cyst coat composition.

Alginate acetylation content ranges from 4 to 57%, depending on the percentage of ManA present (Skjåk-Braek et al., 1986). The degree of *O*-acetylation is often observed to vary not only between different alginate-producing organisms, but also between different strains of the same organism and even within the same strain under differing growth conditions (Marty et al., 1992; Peña et al., 2006). For example, modulation of carbon source during growth for a single alginate-producing *P. syringae* strain led to significant differences in acetyl content, ranging from 9 to 34% of total uronic acids bearing an acetyl group (Day, 1988). In another study, the alginate produced by several

different strains of *P. aeruginosa* grown on nutritionally distinct media was examined. This study revealed that between different strains *O*-acetyl content of alginate varied between 2 to 56% (Marty et al., 1992). Furthermore, in both studies alginate acetyl content changed over the course of a single growth experiment by as much as 40%, possibly owing to the availability of acetyl-CoA, the proposed acetyl donor (Lee and Day, 1998). In addition to acetyl-CoA availability, differences in acetyl content could conceivably be a means to optimize attachment, nutrient uptake, or nutrient diffusion within the biofilm in the face of different media compositions and nutrient sources. This notion is supported by findings which suggest that alginate *O*-acetylation can enhance the swelling ability of calcium alginate gels (Skjåk-Braek et al., 1989). Deacetylated alginate exhibited poor swelling ability in comparison to chemically acetylated variants, with increasing degrees of acetylation leading to greater swelling volume. Conversely, increased *O*-acetylation led to a decrease in the affinity of alginate gels for calcium ions (Skjåk-Braek et al., 1989). Thus, alginate acetyl content has specific consequences with respect to calcium ion binding and the thickness of alginate gels, which may influence nutrient diffusion in the biofilm. These findings could potentially be extrapolated to other components of the growth media, and suggests a mechanism by which alginate-producing bacteria could regulate the uptake of essential nutrients.

Alginate Epimerization

Pseudomonas aeruginosa has a single alginate C5-epimerase in the periplasm, AlgG. In *A. vinelandii*, there is an AlgG ortholog that performs the same function, and seven additional extracellular epimerases, AlgE1 through AlgE7 (Table 1). Alginate can form strong gels through interactions with GulA residues, mediated by Ca²⁺ ions. This feature was thought to be limited to alginates containing G-blocks, and would therefore exclude the MG-block alginates produced by *P. aeruginosa* (Grant et al., 1973). However, it is now thought that alginates containing exclusively MG-blocks can also form gels in the presence of Ca²⁺ (Donati et al., 2005), suggesting that epimerization by AlgG in *P. aeruginosa* may serve as a mechanism to improve the cohesion of alginate during biofilm formation. It was found that addition of CaCl₂ to growth media led to the production of biofilms that were 10- to 20-fold thicker than that produced in the absence of Ca²⁺ (Sarkisova et al., 2005). Ca²⁺-alginate interactions also regulate virulence factor expression, as chelation of Ca²⁺ by alginate induces expression of the Type 3 secretion system (Horsman et al., 2012). Therefore, it appears that there are mechanisms in place in *P. aeruginosa* for virulence factors to be upregulated by the expression of another, thus allowing for concerted actions that improve fitness (Table 2). Despite advances in understanding the interplay between Ca²⁺ and alginate in *P. aeruginosa*, and the extensive studies performed on acetyl-deficient alginate, there are no reports on the effects of epimerization on biofilm formation, pathogenicity, or virulence.

In contrast, the role of epimerization in *A. vinelandii* cyst formation has been well characterized. One hypothesis regarding

the ability of *A. vinelandii* to express multiple epimerases with unique activities is that these enzymes allow the alginate structures to be tailored to different layers of the cyst under diverse environmental conditions. For example, the epimerase AlgE1 has two catalytic domains that introduce primarily MG-blocks and G-blocks, respectively (Ertesvåg et al., 1998). Decreasing the availability of Ca^{2+} in the presence of AlgE1 *in vitro* led to greater incorporation of G-blocks into polyM alginate. This may provide a means *in vivo* to maintain the strength of Ca^{2+} -mediated inter-alginate bonds in the face of decreased environmental Ca^{2+} availability (Ertesvåg et al., 1998). Regulation of alginate structure is also observed during vegetative growth of *A. vinelandii*, where nitrogen fixation is mediated by the expression of highly oxygen-sensitive nitrogenases. In this state, alginate is utilized as a barrier to prevent the diffusion of oxygen into the cell. In the presence of increasing environmental O_2 concentrations, *A. vinelandii* was able to produce alginate with greater G-content, which led to the formation of a thicker, denser alginate layer around the cell and thus limited oxygen penetration (Sabra et al., 2000). The expression of different mannuronan C5-epimerases is also regulated over the course of the *A. vinelandii* life cycle, including during vegetative growth, cyst development, and cyst germination (Høidal et al., 2000). Although the exact biological function for the expression of specific epimerases at unique points in the life cycle of *A. vinelandii* has not been determined, preferential expression of AlgE7 during cyst germination could be linked to the apparent lyase activity of this enzyme, which may be utilized to degrade the cyst coat (Høidal et al., 2000).

Unlike AlgG in *P. aeruginosa*, the importance of the AlgE1-7 epimerases in the formation of the cyst coat and tolerance to desiccation has been explored. Inactivation of the AlgE1-7 epimerases, either through chromosomal deletion in *A. vinelandii* (Steigedal et al., 2008) or by inactivating the Type 1 secretion system responsible for their export (Gimmestad et al., 2006) led to the production of low G-content alginate, suggesting that the periplasmic *A. vinelandii* AlgG is active but not very efficient. In both cases, these mutants were unable to form a cyst coat and could not survive desiccation. In contrast, deletion of individual *algE* genes, with the exception of *algE3*, did not have an appreciable effect on the G-content of alginate. Deletion of *algE3* showed a significant reduction in G-content (Steigedal et al., 2008). However, since each of the individual *algE* deletion mutants was able to form a functional cyst and survive desiccation, it appears that no single epimerase is absolutely essential for cyst formation or germination. This suggests that the presence of multiple extracellular epimerases may increase redundancy of epimerase activity to ensure formation of a functional cyst coat (Steigedal et al., 2008). It remains to be determined whether cyst formation under unique stressful conditions may require specific epimerases, and little work has been done to date to examine the role of mannuronan C5-epimerases during the vegetative stage of *A. vinelandii* growth.

While a great deal is understood about the regulation of alginate biosynthesis and its modification at the genetic and protein level (Hay et al., 2014), the implications of alginate acetylation and epimerization in terms of biofilm formation,

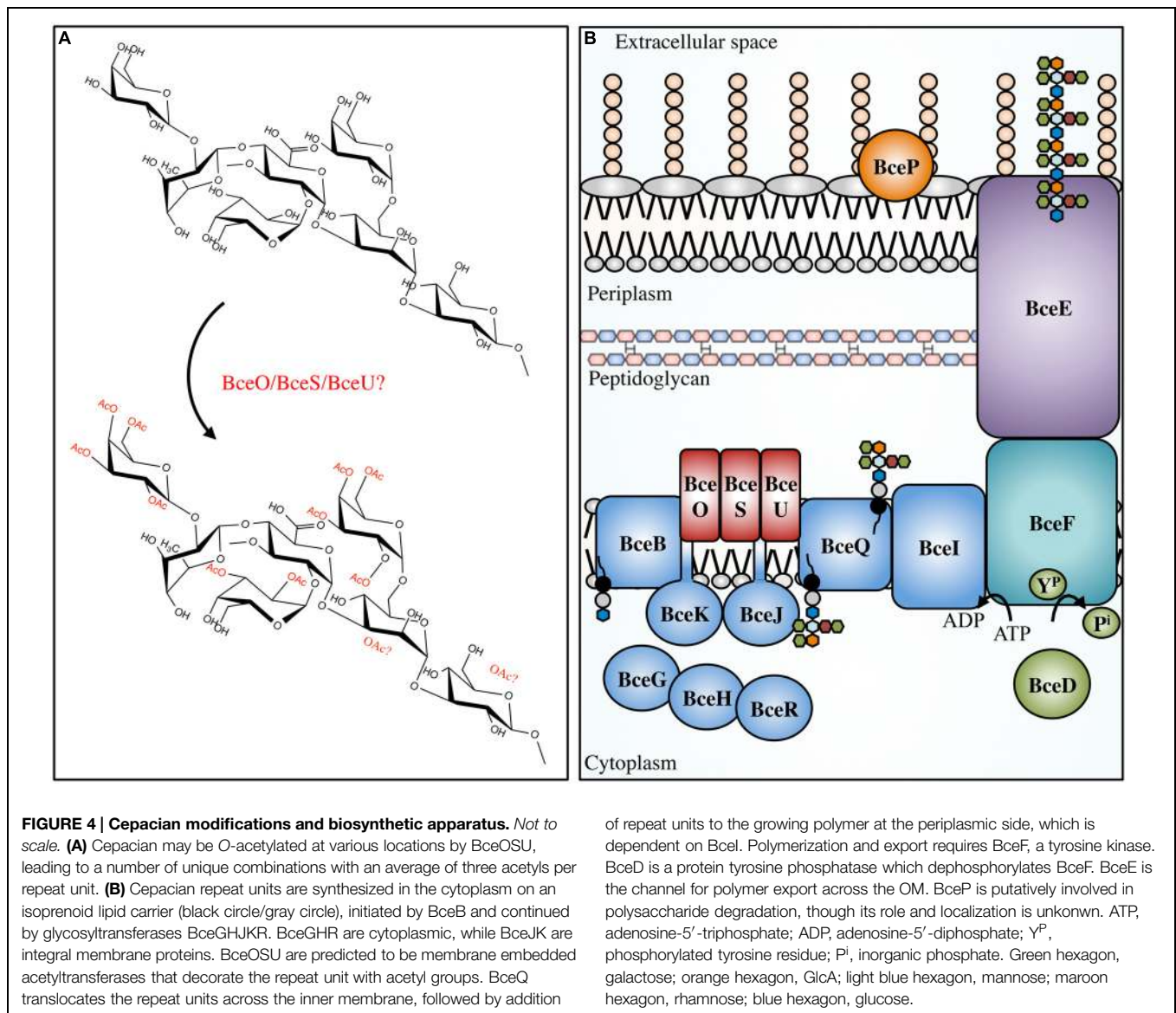
pathogenicity and virulence, environmental adaptability and survivability remain largely uncharacterized.

The Pel Polysaccharide

In addition to alginate, *P. aeruginosa* is capable of synthesizing two other polymers that have been implicated in biofilm formation, the Pel and Psl polysaccharides (PEL and PSL; Franklin et al., 2011). Unlike alginate, PEL and PSL are primarily associated with the establishment of non-mucoid biofilms. PSL is a neutral, branched polysaccharide with a five-sugar repeat unit composed of D-mannose, D-glucose, and L-rhamnose and is not thought to undergo any modifications after polymerization (Byrd et al., 2009). The exact structure of PEL is currently unknown, but it is predicted to be glucose rich (Friedman and Kolter, 2004; Ma et al., 2012). Colvin and colleagues have demonstrated that the PEL biosynthesis protein PelA has deacetylase activity *in vitro* and when residues predicted to be required for deacetylation were mutated, this activity was lost. Introduction of these PelA deacetylation mutations in *P. aeruginosa* PA14, which uses PEL as the primary EPS, led to a biofilm deficient phenotype and a lack of material recognizable by PEL-reactive antisera (Table 1; Colvin et al., 2013). Given the localization of PelA to the periplasm (Colvin et al., 2013), deacetylation of PEL following polymerization may be necessary for biofilm formation, and suggests that an acetylated sugar is likely a feature of the PEL. Our current understanding of PEL biosynthesis is limited, and it remains to be determined whether PelA acts directly on PEL, the degree of PEL deacetylation by PelA, and what effect this modification has on virulence.

Cepacian

The Bcc is a group of at least 17 different bacterial species, including beneficial environmental isolates, as well as rhizosphere parasites, and plant and animal pathogens (Mahenthalingam et al., 2002; Vanlaere et al., 2009). The Bcc have become increasingly important as opportunistic pathogens of immunocompromised individuals and those with CF (Mahenthalingam et al., 2005). In CF patients, Bcc infections occasionally develop into a form of necrotising pneumonia, known as cepacia syndrome, which often leads to patient death (Govan and Deretic, 1996). The majority of both clinical and environmental Bcc isolates produce the EPS cepacian (Ferreira et al., 2010); a known virulence factor that contributes significantly to bacterial pathogenicity. Cepacian is composed of glucose, GlcA, mannose, rhamnose, and galactose in a 1:1:1:1:3 ratio and is decorated with acetyl groups (Figure 4; Cérantola et al., 1999; Cescutti et al., 2000; Linker et al., 2001). The acetyl groups can be found at 12 different locations on the polymer repeating unit with an average of three acetyl groups present per repeat unit (Cescutti et al., 2011). The genes responsible for cepacian acetylation were discovered by Ferreira and colleagues, and include the putative acetyltransferases *bceO*, *bceS*, and *bceU* (Ferreira et al., 2010). Mutations in *bceS* produced cepacian with



approximately 20% fewer acetyl groups, implicating this protein in partial cepacian acetylation (Ferreira et al., 2010). The roles of *bceO* and *bceU* in cepacian acetylation have not been evaluated, nor has a triple mutant been generated to discern the fitness of an acetyl-deficient cepacian producer.

An enzyme with lyase activity that specifically degrades cepacian has been isolated from culture supernatants of *Bacillus* sp. This enzyme has significantly higher activity against the chemically deacetylated polymer than native cepacian, implicating acetylation in protective mechanisms (Table 2; Cescutti et al., 2006). As with alginate acetylation, cepacian acetylation may have evolved as a defensive mechanism to prevent polymer cleavage in the presence of competitive organisms. Furthermore, like alginate, cepacian acetyl groups have been shown to provide protection against ROS, specifically hypochlorite (Cuzzi et al., 2012). Acetyl groups were the first structural features to undergo damage following hypochlorite

treatment, which led to a loss of polymer-polymer interactions and cepacian unfolding, increasing the susceptibility of the cepacian backbone to hypochlorite degradation (Cuzzi et al., 2012). Consequently, cepacian acetylation improves polymer robustness to hypochlorite-mediated damage and increases the amount of polymer reactive groups that could neutralize hypochlorite prior to reaching the cellular surface. Further study of cepacian producing Bcc pathogens is necessary to determine, whether like alginate, there is a role for cepacian acetyl modification in immune evasion.

Vibrio Polysaccharide (VPS)

Vibrio cholerae is a human pathogen that causes the diarrhoeal disease cholera (Kaper et al., 1995; Faruque et al., 1998). This bacterium is a natural inhabitant of aquatic ecosystems, where

it forms biofilms on a variety of surfaces, including plankton, plants, crustaceans, insects, and sediment (Huq et al., 1983, 1995; Halpern et al., 2004; Broza et al., 2005). In areas where cholera is endemic, *V. cholerae* has been shown to form suspended biofilm-like aggregates in surface waters, however, when particles >20 μm in diameter are removed from water sources, the incidence of cholera can be reduced (Huq et al., 1996; Colwell et al., 2003). Furthermore, it has been shown that the average infectivity of the aggregate form of *V. cholerae* is significantly higher than that of planktonic cells (Faruque et al., 2006), and biofilm formation within aquatic ecosystems significantly improves *V. cholerae* fitness and persistence (Matz et al., 2005). A major component of the biofilm produced by *V. cholerae* is an EPS called VPS. This polymer is thought to be produced during infection and contributes to bacterial colonization and survival (Yildiz and Schoolnik, 1999; Fong et al., 2010). The chemical structure of VPS revealed a backbone containing the unusual constituent GulNACAGly: the amide formed from 2-acetamido-2-deoxy-L-guluronic acid and glycine (Figure 6; Yildiz et al., 2014). Of the genes involved in VPS biosynthesis, originally identified using a transposon mutagenesis screen (Yildiz and Schoolnik, 1999), two putative acetyltransferases, *vpsG* and *vpsC*, were identified (Fong et al., 2010). Deletion of *vpsG* results in reduced biofilm formation and altered biofilm-related phenotypes, as well as weak reactivity with VPS antisera, suggesting that it may modify the polymer, perhaps through acetylation (Table 1). In contrast, deletions of *vpsC* do not affect biofilm formation or VPS production, suggesting that *vpsC* is inactive, not expressed, or is performing some other function in VPS biosynthesis besides polymer modification (Fong et al., 2010). The chemical composition of VPS produced by *vpsG* and *vpsC* mutants was not studied for alterations in acetyl content. The presence of GulNAC in VPS may be the result of epimerization by the predicted GDP-mannose dehydrogenase *VpsB*, a conversion similar to the ManA to Gula epimerization catalyzed by AlgG in the biosynthesis of alginate by *P. aeruginosa* (Wolfram et al., 2014; Yildiz et al., 2014). The unusual glycine modification in VPS requires further exploration, as the enzyme responsible for its addition is presently unknown. Given the important role of VPS in *V. cholerae* pathogenesis and environmental persistence, and the recent determination of its precise chemical structure, we anticipate that the proteins involved in VPS modification will soon be identified and characterized.

Glycosaminoglycans (GAGs)

GAGs are a group of polymers that are typically composed of a disaccharide repeat unit containing an amino sugar and a hexuronic acid (Laurent and Fraser, 1992; Esko and Lindahl, 2001; DeAngelis, 2002; Silbert and Sugumaran, 2002). GAGs were initially thought to exist only in the animal kingdom, where they serve essential biological functions, however, there has been an emergence of GAG-like polymers amongst prokaryotes (Vann et al., 1981; Rodriguez et al., 1988; DeAngelis et al., 2002). Prokaryotic GAGs are typically less complex than their

eukaryotic counterparts due to an absence of modifications such as sulfation (Raedts et al., 2011). HS, for example, is an essential GAG in animals and is composed of repeating disaccharides of GlcA and GlcNAc (Kjellén and Lindahl, 1991). HS can be modified post-polymerization by a glucuronyl C5-epimerase, which converts GlcA to IdoA, as well as by the addition of sulfate groups to GlcNAc or IdoA moieties. Mouse embryos lacking the GlcA C5-epimerase display a lethal phenotype characterized by skeletal malformations and lung defects (Li et al., 2003), highlighting the importance of HS epimerization in murine development. Interestingly, the K5 antigen of *E. coli* O10:K5:H4 has an identical structure to heparosan, the unsulfated, non-epimerized backbone structure of HS (Vann et al., 1981). K5 heparosan is a form of molecular camouflage, as it imparts low immunogenicity to the bacterium in humans and hence increased pathogenicity (Vann et al., 1981).

Although sulfation has not yet been observed amongst prokaryotic GAGs, IdoA residues have been found to be constituents of bacterial GAGs (Figure 6). The identification of the bacteria glucuronyl C5-epimerase has proven elusive (Raedts et al., 2011), however, an enzyme (RED65_08024) from the marine bacterium *Bermanella marisrubri* that shares 37% sequence similarity with the human glucuronyl C5-epimerase has been characterized and shown *in vitro* to convert GlcA to IdoA in de-sulfated mouse HS (Raedts et al., 2013). This glucuronyl C5-epimerase represents the first prokaryotic protein capable of generating IdoA residues, and is the only identified epimerase that can function on bacterial polysaccharides post-polymerization, besides AlgG and AlgE1-7. Unfortunately, the EPS produced by *B. marisrubri* has not been characterized, so its target remains unknown. Nevertheless, the ability of bacteria to more closely replicate the structures of essential human polysaccharides by expression of homologous modification enzymes likely serves as a mechanism to mask their presence from the host immune system (Cress et al., 2014).

Poly- β -1,6-*N*-Acetyl-glucosamine (PNAG)

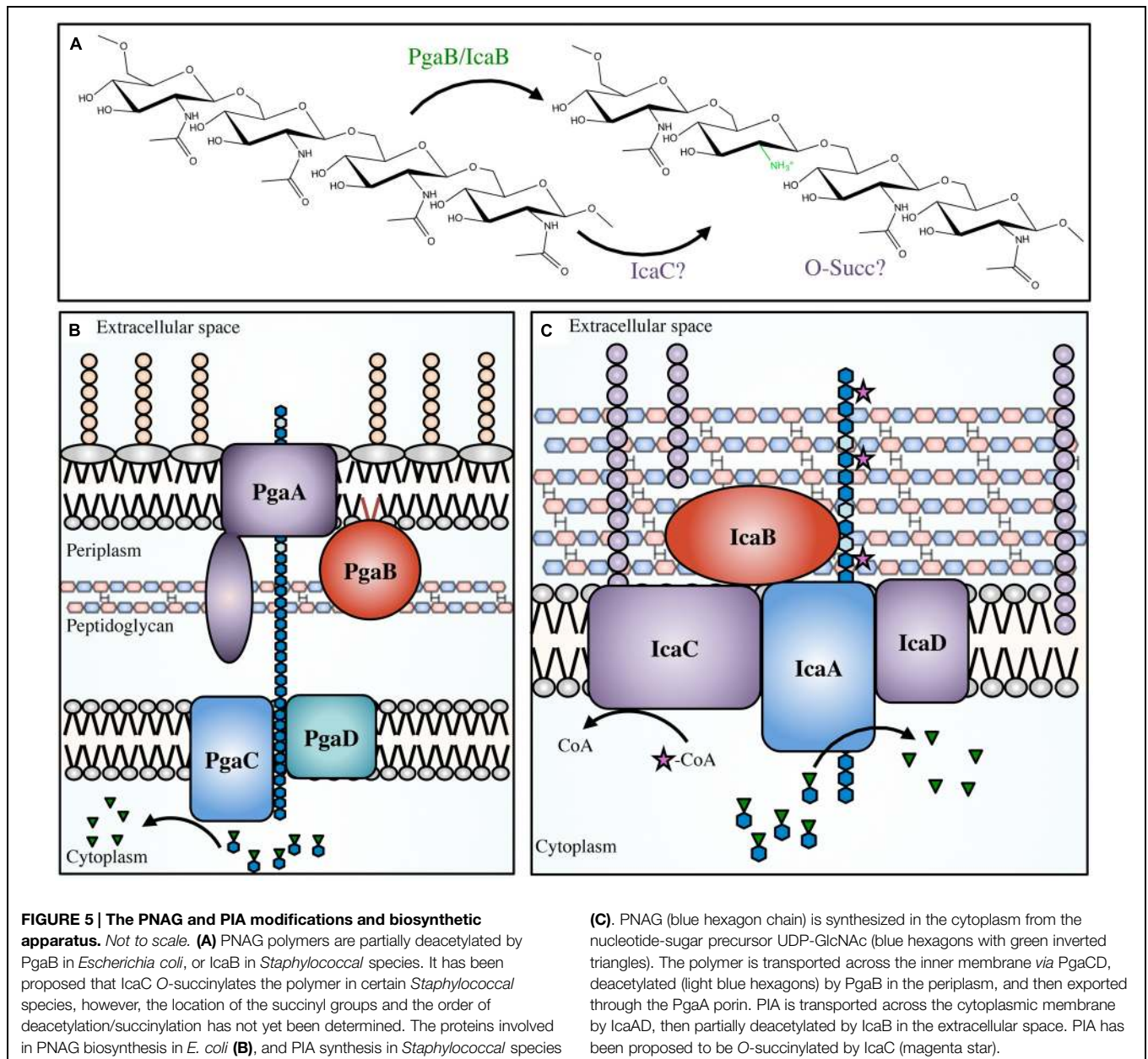
Poly- β -1,6-*N*-acetyl-glucosamine is a poly-GlcNAc polymer that is produced by a wide range of Gram-positive and Gram-negative bacterial pathogens, including *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Yersinia pestis*, *Bordetella* sp., *Acinetobacter baumannii*, *Actinobacillus pleuropneumoniae*, *Burkholderia cepacia* complex (Bcc), and *Aggregatibacter actinomycetemcomitans* (Cramton et al., 1999; Vuong et al., 2004b; Wang et al., 2004; Izano et al., 2007, 2008; Parise et al., 2007; Bobrov et al., 2008; Choi et al., 2009). These organisms are responsible for a wide spectrum of diseases, including but not limited to, hospital acquired infections, toxic shock syndrome, plague, and whooping cough. Depending on the source or organism in question, PNAG may also be referred to as PGA (polyglucosamine, in Gram-negative bacteria), PIA (in Gram-positive bacteria), poly-NAG, hms+ (in *Y. pestis*), or BPS (*Bordetella* polysaccharide, in *Bordetella* sp.). Given the differences in PNAG modifications between Gram-positive and

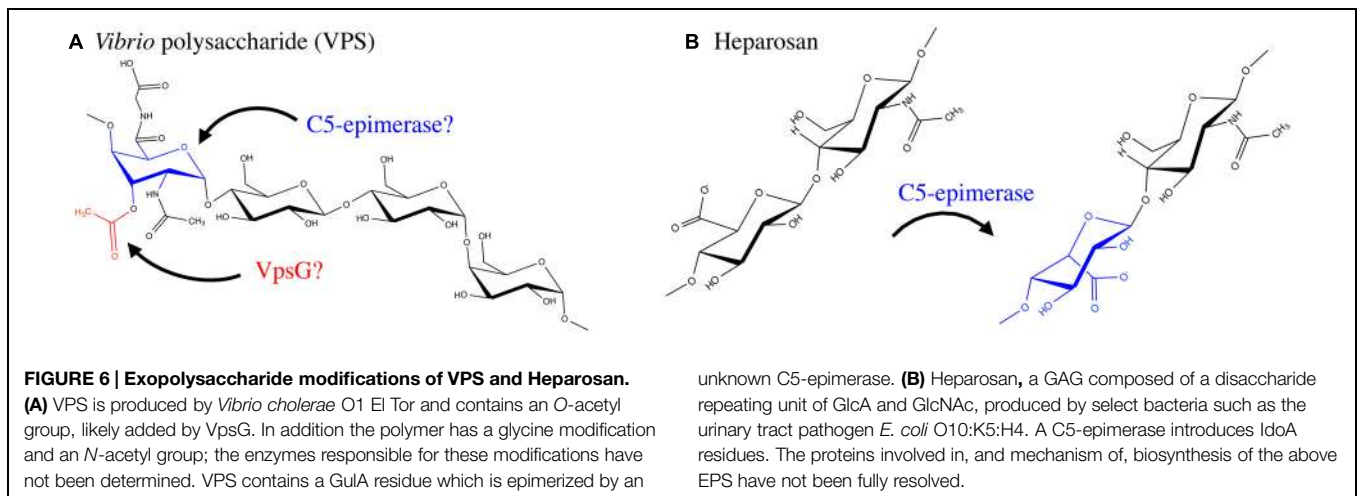
Gram-negative bacteria, as described below, we will use PGA and PIA to refer to PNAG polymer produced by Gram-negative and Gram-positive organisms, respectively.

Initially *S. epidermidis* was thought to produce several different polymers, but the discovery of the *icaADBC* operon (Heilmann et al., 1996a,b; Gerke et al., 1998) revealed that only a single polymer, PIA, was produced (Tojo et al., 1988; Christensen et al., 1990; Heilmann et al., 1996a; Mack et al., 1996; McKenney et al., 1998). PIA is a partially deacetylated β -1,6-GlcNAc polymer. In *S. epidermidis* and *S. aureus* 15–20% of the *N*-acetyls are removed by the extracellular, cell-surface associated polysaccharide deacetylase IcaB (Table 1; Figure 5; Vuong et al., 2004a; Cerca et al., 2007). In addition to deacetylation, approximately 6 and 10% of GlcNAc residues

in *S. epidermidis* and *S. aureus*, respectively, are *O*-succinylated (Joyce et al., 2003; Sadovskaya et al., 2005). This modification is thought to be performed by the membrane localized protein, IcaC (Atkin et al., 2014). Interestingly, a mechanism of phase variation, where bacteria modulate virulence phenotypes at the genome level in a rapid on/off fashion, was noted in *S. aureus* wherein slipped-strand mispairing led to inactivation of *icaC* (Brooks and Jefferson, 2014). This phenotype confers a fitness advantage that was not seen when the *ica* operon was deleted, which may be a response to modulate PIA *O*-succinylation and thus decrease the overall anionic charge of the polymer.

The production of PGA has been extensively characterized in *E. coli*, where the *pgaABCD* operon encodes the proteins necessary for its biosynthesis (Wang et al., 2004). In *E. coli*,





approximately 3–5% of *N*-acetyls are removed by the lipoprotein PgaB (Wang et al., 2004; Itoh et al., 2008; Little et al., 2012). The *N*-terminal domain of PgaB is homologous to IcaB in Gram-positive bacteria but the protein is located on the inner leaflet of the outer membrane. There is no IcaC ortholog in the *pgaABCD* operon, which is consistent with an observed lack of *O*-succinyl groups in PGA.

Partial deacetylation of PNAG by both IcaB and PgaB is important for a variety of biofilm-associated phenotypes in *S. epidermidis*, *S. aureus*, and *E. coli*. Deletion of *icaB* in *S. epidermidis* led to the production of fully acetylated PIA, suggesting that IcaB is not necessary for polymer production (Vuong et al., 2004a). However, the fully acetylated polymer was not retained at the cellular surface and was shed into the culture media, which led to deficiencies in biofilm formation and surface attachment (Table 2). The lack of deacetylation led to a loss of cationic charge in the polymer, which may be essential for interactions with the anionic cell surface of *S. epidermidis* (Vuong et al., 2004a). Furthermore, *icaB*-deficient mutants of *S. epidermidis* were more susceptible to human cationic antimicrobial peptides and phagocytosis by neutrophils, and were unable to persist in a mouse model of device-related infections (Vuong et al., 2004a). Deletion of *icaB* in *S. aureus* produced similar phenotypes (Cerca et al., 2007). Intriguingly, the production of wall teichoic acids, the predominant anionic component of the Gram-positive bacterial envelope, was dispensable for adherence of PIA to the cell surface of *S. aureus* (Vergara-Irigaray et al., 2008), suggesting that other less prevalent anionic species mediate this interaction.

In contrast to IcaB, inactivation of PgaB in *E. coli* prevented polymer export, suggesting that partial deacetylation is necessary for export through the predicted outer membrane porin PgaA (Itoh et al., 2008). This is in line with findings that suggest conformational changes in the C-terminal domain of PgaB, upon binding of deacetylated PNAG, assist in targeting PNAG for export (Little et al., 2014b). Deacetylation has also been studied in *Y. pestis*, where a PNAG-like polymer is thought to mediate biofilm formation. Biofilm formation in *Y. pestis* is crucial for

its zoonotic transmission (Jarrett et al., 2004). In the flea, the proventriculus, a feeding tube covered in spines that connects the midgut to the esophagus, provides a platform for the adhesion of *Y. pestis* aggregates. Subsequent colonization impedes blood passage and leads to transposition of *Y. pestis* from flea to mammal when a flea attempts excessive feeding due to a partial or completely blocked proventriculus (Jarrett et al., 2004). The *hmsHFRS* operon in *Y. pestis* is orthologous to the *pgaABCD* operon, where HmsF is the outer membrane localized deacetylase with structural similarity to PgaB (Forman et al., 2006). Mutation or deletion of *hmsF* led to a deficiency in biofilm formation. This suggests HmsF in *Y. pestis* may be analogous to PgaB in *E. coli* in terms of de-*N*-acetylation activity and importance for polysaccharide export and biofilm formation.

While PNAG production has been studied primarily in *S. aureus*, *S. epidermidis*, and *E. coli*, there are a multitude of additional pathogenic bacteria, fungi, and protozoans that may produce this polymer (Cywes-Bentley et al., 2013). PNAG could represent the first example of an EPS that is broadly utilized by pathogenic organisms as a mechanism to improve fitness in the environment or during infection.

Insights from Modification of Other Microbial Polysaccharides

The implications of EPS modifications in pathogenic bacteria have been studied to some extent, particularly in alginate and PNAG producing bacteria (Figure 2; Table 2). However, the breadth of our knowledge in this field remains limited. Despite this, comparable modifications found on LPS and CPSs have been studied extensively in an effort to identify vaccine targets, and can be used for comparison purposes to generate new hypotheses regarding EPS modifications (Cody et al., 2003; Pinto and Berti, 2014). In particular, the study of polysaccharide acetyl modifications has clarified their role in mediating a variety of survival mechanisms.

Many of the protective benefits of EPS acetyl modifications described above have been noted for other bacterial pathogens.

For instance, in *Haemophilus influenzae*, an opportunistic pathogen of the upper respiratory tract, acetylation of LPS by the acetyltransferase OafA leads to increased resistance to complement-mediated killing by human serum (Fox et al., 2005). Similarly, in *S. aureus*, the acetyltransferase Cap5H, which is responsible for the O-acetylation of type 5 CPS, confers protection against opsonophagocytic killing and improves propagation in a murine model of infection (Bhasin et al., 1998). Type 5 CPS producers also exhibit increased survival rates in murine models of bacteremia and renal abscess formation and resistance to killing in whole mouse blood and opsonophagocytic assays, in comparison to producers of the structurally similar type 8 CPS which have reduced levels of N-acetylation (Watts et al., 2005). Beyond the prokaryotic domain, acetyl modifications are also incorporated into the CPS of the pathogenic fungus *Cryptococcus neoformans* to evade complement activation (Fujihara et al., 1997), decrease the efficiency of capsule clearance by the host (Kozel et al., 2003), and inhibit neutrophil migration (Ellerbroek et al., 2004) during cryptococcosis. An excellent example of the benefits of polysaccharide acetyl modifications comes from a survey of clinical isolates of *Streptococcus pneumoniae* and *E. coli* K1, which found that the bacteria expressing acetyl-decorated polymers were more virulent and invasive than those that expressed polymers lacking the modification (Frasa et al., 1993; Melin et al., 2010). Therefore, the immunomodulatory characteristics of acetyl modifications are utilized by a wide range of pathogenic organisms and likely represent a general mechanism for survival and proliferation within the host.

The above notion is firmly supported by studies of serotype variation within the context of CPS biosynthesis. In *Streptococcus pneumoniae*, a causative agent of meningitis, bacteremia, and pneumonia, there are more than 90 different capsule serotypes with unique carbohydrate structures and biosynthetic loci. This has evolved, in part, as a mechanism to overcome serotype-specific host mechanisms of adaptive immunity that can efficiently clear infections. In some serotypes, such as 9V/9A, 11A/11E, and 15B/15C, the CPS structures differ only in the degree of O-acetylation (Jansson et al., 1987; Rutherford et al., 1991; Zartler et al., 2009). Mechanisms within *S. pneumoniae* have been revealed that allow for serotype switching during infection as a means to actively evade the host immune response. In the case of serotypes 9V and 11A, inactivating mutations in the acetyltransferase-encoding gene *wceJ* led to expression of non-acetylated 9A and 11E capsule (Calix and Nahm, 2010; Calix et al., 2011). Moreover, certain *wceJ* mutations only partially inhibit acetyltransferase activity, which have led to intermediate 9V/9A and 11A/11E phenotypes (Calix et al., 2011, 2014). In the case of 15B/15C serotype switching, the process is reversible due to slipped-strand mispairing of the acetyltransferase-encoding gene *wciZ* (van Selm et al., 2003). Regardless of the mechanism, this mid-infection serotype variation provides significant protective advantages to *S. pneumoniae* in terms of antibody evasion. For example, antibodies generated against O-acetylated serotype 15B were unreactive with non-acetylated 15C polymer (Rajam et al., 2007), and serotype 9V specific antibodies exhibited reduced specificity for 9A polymer (Calix et al., 2012). Furthermore,

10–20% of individuals receiving a *S. pneumoniae* vaccine targeted against the 9V polysaccharide did not generate antibodies targeting serotype 9A (McNeely et al., 1998). In addition, the ability of acetyl groups to mask protective epitopes of bacterial polysaccharides has been noted for the Vi antigen of *Salmonella typhi* (Szu et al., 1991), *Salmonella typhimurium* O-antigen (Kim and Slauch, 1999) and *Neisseria meningitidis* serogroup A, C, and Y CPS (Michon et al., 2000; Berry et al., 2002; Fusco et al., 2007). Therefore, through modulation of acetyl groups on the polymer, a wide variety of pathogenic bacteria are able to evade host-mediated mechanisms of adaptive immunity.

The above examples illustrate scenarios in which acetyl modification is an all-or-nothing response to adaptive immunity, however, in the case of GBS, acetyl levels on its sialic acid CPS can be fine-tuned by the actions of the acetyltransferase NeuD and the acylesterase NeuA (Lewis et al., 2006, 2007). Different degrees of O-acetylation in GBS CPS have been linked to different stages of invasion and infection. For instance, it is thought that during the asymptomatic stages of initial colonization and persistence in the human gastrointestinal and vaginal tracts, GBS produces an extensively acetylated form of CPS to protect against degradation by sialidases introduced by competing microbes in these environments (Weiman et al., 2009). However, highly acetylated CPS renders GBS more susceptible to killing by neutrophils and reduces virulence during stages of opportunistic infections (Weiman et al., 2010). Therefore, during infection it is thought that GBS produces a sparsely acetylated form of CPS that improves resistance to neutrophil-mediated killing through reduced neutrophil activation and production of pro-inflammatory cytokines, and enhances survival in the murine bladder (Kline et al., 2011). Interestingly, this variant of CPS is also able to promote the persistence of uropathogenic *E. coli* in co-culture urinary tract infection models (Kline et al., 2012). Therefore, in certain pathogens, specific degrees of polysaccharide acetylation allow for adaptation during different stages of colonization and infection.

The above examples of acetyl modulation in LPS and CPS not only reinforce the importance of EPS acetylation for pathogenicity and persistence, but also provide additional perspectives in considering the variability of this modification observed in alginate and cepacian. For instance, cepacian has on average three acetyl groups per repeat unit, each located on one of 12 potential positions (Cescutti et al., 2011). Therefore, each cepacian repeat unit can have one or more acetyl groups at any of 12 positions, generating an overwhelming number of unique acetyl decoration patterns. Given the importance of acetyl groups in forming or masking antibody epitopes, this level of diversity would make the generation of protective antibodies or the development of an effective vaccine extraordinarily difficult. Furthermore, production of such a heterogeneous polymer likely requires an arsenal of regulatory factors and/or acetyltransferases, very few of which have been discovered in the context of cepacian biosynthesis (Ferreira et al., 2011). Similar to *S. pneumoniae* and *N. meningitidis* CPS production, members of the Bcc may modulate the presentation of cepacian acetyl groups through an as yet unknown mechanism as a means to evade host adaptive immune mechanisms.

The degree of acetylation and epimerization of alginate has long been known to differ depending on the organism and strain, as well as the composition of the growth medium (Day, 1988; Marty et al., 1992; Peña et al., 2006). This reflects, in part, a need to adapt to the specific conditions imposed by different nutritional media, and may mirror other features of the environment from which the organism was isolated. In line with this concept, additional promoters within the alginate operon have been identified upstream of *algG* and *algIJF* in *P. aeruginosa*, suggesting that there may be modes of regulating the levels of these modifying enzymes independently of the rest of the alginate biosynthesis machinery (Paletta and Ohman, 2012). The upregulation of *O*-acetylation machinery would not only increase alginate acetyl content, but would also decrease the availability of substrate for AlgG and thus decrease epimerization levels. Conversely, upregulation of *algG* would increase the number of G-residues that cannot act as substrates for *O*-acetylation. Therefore, there is the potential for a complicated regulatory interplay between these processes, much like the reciprocal *O*-acetylation/de-*O*-acetylation of GBS CPS that allows for fine-tuning of acetyl levels at different stages of infection.

The degree of PNAG de-*N*-acetylation is not known to vary considerably, and the exact processes involved in VPS acetylation and epimerization, PIA *O*-succinylation, PEL deacetylation and GAG epimerization are poorly understood. However, the ability to perform these types of modifications in a random fashion may increase the difficulty in generating antibodies that recognize specific epitopes on these EPS, either during host adaptive immune responses or in vaccine development (Gening et al., 2010). As such, EPS modifications are capable of imparting beneficial characteristics upon polymers that improve persistence, survival, or evasion of the immune response in their cognate bacteria regardless of their frequency, mechanism of addition to the polymer, or chemical properties.

Reflection and Future Perspectives

Identifying and characterizing biofilm EPS is difficult and there are a number of hurdles that need to be overcome. One of the initial challenges involves culturing biofilm-forming bacteria. Identification of an appropriate medium and growth conditions is required to study EPS production of certain microorganisms in the laboratory (Stewart, 2012). Of those that can be cultured, it is imperative to use similar growth conditions when making experimental comparisons in the literature, as variations can affect the presence or degree of different polysaccharide modifications. With alginate, varying levels of acetylation and epimerization are observed depending on the culture conditions, as well as varying biofilm phenotypes of identical *P. aeruginosa* strains (Pier et al., 2001; Tielen et al., 2005). Additionally, conflicting studies on the levels of pyruvyl and *O*-acetyl modifications to xanthan gum were attributed to different media conditions (Bradshaw et al., 1983). Different media or culturing equipment may also affect experiments such as surface attachment assays. For example, different types of plastics were found to affect PIA-mediated surface attachment

in microtitre plates (Maira-Litràn et al., 2004). This suggests that during preliminary analyses, multiple types of media and different materials including plastics and glass should be tested to ensure the validity of observed biofilm phenotypes. Variations in the abundance or type of modifications on a given polymer under different experimental conditions can be difficult to quantify; however, this variation likely reflects the ability of different bacteria to adapt to unique situations. Many EPS-producing bacteria naturally exist in diverse environments and are also able to infect various hosts and survive in specific tissues. Additionally, during the course of infection, the environment within the host will change as immune mechanisms attempt to eradicate the bacteria and the surrounding tissue suffers damage. Variations in the degree of modifications under different growth conditions or stressors may therefore provide valuable insight into bacterial adaptation.

The majority of biofilm studies focusing on EPS modifications have been performed using *in vitro* model systems with monospecies cultures. However, the majority of biofilms from chronic infections differ significantly from those studied in the laboratory (Bjarnsholt et al., 2013a). One significant issue lies in the use of abiotic surfaces, such as cover slides or the plastic surfaces of 96-well plates for the growth and study of biofilms. While some systems can closely approximate the conditions encountered *in vivo*, such as flow cell models for catheter associated infections, biofilms from infections like CF pneumonia or those encountered in epidermal wounds are thought to involve attachment to host cells or host-derived molecules (Bjarnsholt et al., 2009). In these instances it is challenging to extrapolate results obtained *in vitro* to chronic biofilm infections, as modifications that appear important for attachment to abiotic surfaces may be disposable for attachment to host tissues, or vice versa (Leuck et al., 2014). Furthermore, many biofilms encountered in the clinic are not comprised of a single species of bacteria, but rather contain mixtures that can include pathogenic or non-pathogenic bacteria of host or environmental origin, as well as fungi (Wargo and Hogan, 2006; Elias and Banin, 2012). These types of microbial interactions have been studied extensively in the CF lung, where *P. aeruginosa* has been shown to interact with the Bcc opportunistic pathogens *B. cepacia* and *B. cenocepacia* (Chattoraj et al., 2010; Schwab et al., 2014), as well as the pathogenic fungus *Aspergillus fumigatus* (Chotirmall and McElvaney, 2014). In the case of mixed *P. aeruginosa* and *B. cenocepacia* biofilms, the production of alginate by *P. aeruginosa* has been shown to promote *B. cenocepacia* persistence in a mouse model of CF (Chattoraj et al., 2010). This highlights how the production of EPS in mixed species biofilms can have implications that extend beyond the source organism, and suggests that data obtained regarding the presence or absence of EPS modifications in monospecies biofilms *in vitro* may have additional significance in multispecies biofilms.

Following bacterial culturing, difficulties in polysaccharide isolation due to the compositional complexity of the biofilm matrix or polymer insolubility may necessitate extensive optimization of purification protocols. Typically, separation of the polymer from the cell surface may require the use of procedures that lyse the associated bacteria, introducing

additional contaminants (Bales et al., 2013). Therefore, it is preferable if possible to isolate EPS from culture supernatants; however, such polymers may exhibit different properties from their cell-associated counterparts leading to discrepant analysis (Maira-Litràn et al., 2002). In either case, once the polymer has been obtained from the cell surface or supernatant, contaminants such as DNA, RNA and protein must be removed. This can be achieved through enzymatic digestion or chemical precipitation of contaminants, or precipitation of EPS. Contaminating carbohydrates can be removed by chromatographic techniques such as size exclusion or ion exchange (Bales et al., 2013). In some cases, purification of EPS leads to insufficient yields for subsequent compositional or structural analysis, often as a result of polymer insolubility. As a result, harsher conditions may need to be employed to solubilize the polymer, including the use of strong acids or bases. During initial structural studies of PIA, strong alkaline purification conditions led to the incorrect identification of *N*-succinyl groups on the polymer (McKenney et al., 1999; Maira-Litràn et al., 2002), which was actually a degradation product of glucosamine monosaccharides (Sadovskaya et al., 2005). Additionally, phosphates have been reported in the monosaccharide composition of polymers, which are occasionally remnants from purification buffers, or teichoic acids in the case of Gram-positive bacteria (Sadovskaya et al., 2005). Harsher conditions can also partially or completely remove functional groups, such as acetyls, or even degrade the polymer, leading to incorrect calculations of molecular weight. The EPS described in this review are the select few whose structures have been determined, or for which we have

biochemical and genetic data supporting the importance of the polysaccharide modifications. With improved culturing, purification and structural determination procedures, additional EPS will be discovered and their modifications characterized. We will then be able to formulate trends between different types of modifications and their effect on biofilm formation, pathogenicity, and virulence. Furthermore, the details regarding the genes and proteins involved in the addition of polysaccharide modifications remain largely unavailable. Characterization of these genes and proteins will likely provide details on how the levels and types of modifications are regulated under different conditions. Such findings will have a significant impact on our understanding of bacterial pathogenicity, and may reveal novel drug targets aimed at inhibiting the biosynthesis of these important virulence factors.

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References

- Aachmann, F. L., Svanem, B. I. G., Güntert, P., Petersen, S. B., Valla, S., and Wimmer, R. (2006). NMR structure of the R-module: a parallel beta-roll subunit from an *Azotobacter vinelandii* mannuronan C-5 epimerase. *J. Biol. Chem.* 281, 7350–7356. doi: 10.1074/jbc.M510069200
- Allesen-Holm, M., Barken, K. B., Yang, L., Klausen, M., Webb, J. S., Kjelleberg, S., et al. (2006). A characterization of DNA release in *Pseudomonas aeruginosa* cultures and biofilms. *Mol. Microbiol.* 59, 1114–1128. doi: 10.1111/j.1365-2958.2005.05008.x
- Atkin, K. E., Macdonald, S. J., Brentnall, A. S., Potts, J. R., and Thomas, G. H. (2014). A different path: revealing the function of staphylococcal proteins in biofilm formation. *FEBS Lett.* 588, 1869–1872. doi: 10.1016/j.febslet.2014.04.002
- Baker, P., Ricer, T., Moynihan, P. J., Kitova, E. N., Walvoort, M. T. C., Little, D. J., et al. (2014). *P. aeruginosa* SGNH hydrolase-like proteins AlgJ and AlgX have similar topology but separate and distinct roles in alginate acetylation. *PLoS Pathog.* 10:e1004334. doi: 10.1371/journal.ppat.1004334
- Bales, P. M., Renke, E. M., May, S. L., Shen, Y., and Nelson, D. C. (2013). Purification and characterization of biofilm-associated EPS exopolysaccharides from ESKAPE organisms and other pathogens. *PLoS ONE* 8:e67950. doi: 10.1371/journal.pone.0067950
- Benziman, M., Haigler, C. H., Brown, R. M., White, A. R., and Cooper, K. M. (1980). Cellulose biogenesis: polymerization and crystallization are coupled processes in *Acetobacter xylinum*. *Proc. Natl. Acad. Sci. U.S.A.* 77, 6678–6682. doi: 10.1073/pnas.77.11.6678
- Berry, D. S., Lynn, F., Lee, C.-H., Frasca, C. E., and Bash, M. C. (2002). Effect of O acetylation of *Neisseria meningitidis* serogroup A capsular polysaccharide on development of functional immune responses. *Infect. Immun.* 70, 3707–3713. doi: 10.1128/IAI.70.7.3707-3713.2002
- Bhasin, N., Albus, A., Michon, F., Livolsi, P. J., Park, J. S., and Lee, J. C. (1998). Identification of a gene essential for O-acetylation of the *Staphylococcus aureus* type 5 capsular polysaccharide. *Mol. Microbiol.* 27, 9–21. doi: 10.1046/j.1365-2958.1998.00646.x
- Bjarnsholt, T., Alhede, M., Alhede, M., Eickhardt-Sørensen, S. R., Moser, C., Kühl, M., et al. (2013a). The in vivo biofilm. *Trends Microbiol.* 21, 466–474. doi: 10.1016/j.tim.2013.06.002
- Bjarnsholt, T., Ciofu, O., Molin, S., Givskov, M., and Høiby, N. (2013b). Applying insights from biofilm biology to drug development – can a new approach be developed? *Nat. Rev. Drug Discov.* 12, 791–808. doi: 10.1038/nrd4000
- Bjarnsholt, T., Jensen, P. Ø., Fiandaca, M. J., Pedersen, J., Hansen, C. R., Andersen, C. B., et al. (2009). *Pseudomonas aeruginosa* biofilms in the respiratory tract of cystic fibrosis patients. *Pediatr. Pulmonol.* 44, 547–558. doi: 10.1002/ppul.21011
- Bobrov, A. G., Kirillina, O., Forman, S., Mack, D., and Perry, R. D. (2008). Insights into *Yersinia pestis* biofilm development: topology and co-interaction of Hms inner membrane proteins involved in exopolysaccharide production. *Environ. Microbiol.* 10, 1419–1432. doi: 10.1111/j.1462-2920.2007.01554.x
- Bradshaw, I. J., Nisbet, B. A., Kerr, M. H., and Sutherland, I. W. (1983). Modified xanthan—its preparation and viscosity. *Carbohydr. Polym.* 3, 23–38. doi: 10.1016/0144-8617(83)90010-3
- Brooks, J. L., and Jefferson, K. K. (2014). Phase variation of poly-N-acetylglucosamine expression in *Staphylococcus aureus*. *PLoS Pathog.* 10:e1004292. doi: 10.1371/journal.ppat.1004292
- Broza, M., Gancz, H., Halpern, M., and Kashi, Y. (2005). Adult non-biting midges: possible windborne carriers of *Vibrio cholerae* non-O1 non-O139. *Environ. Microbiol.* 7, 576–585. doi: 10.1111/j.1462-2920.2005.00745.x
- Buchinger, E., Knudsen, D. H., Behrens, M. A., Pedersen, J. S., Aarstad, O. A., Tøndervik, A., et al. (2014). Structural and functional characterization of the R-modules in alginate C-5 epimerases AlgE4 and AlgE6 from *Azotobacter vinelandii*. *J. Biol. Chem.* 289, 31382–31396. doi: 10.1074/jbc.M114.567008
- Buckmire, F. L. (1984). Influence of nutrient media on the characteristics of the exopolysaccharide produced by three mucoid *Pseudomonas aeruginosa* strains. *Microbios* 41, 49–63.

- Bulen, W. A., Burns, R. C., and LeComte, J. R. (1964). Nitrogen fixation: cell-free system with extracts of *Azotobacter*. *Biochem. Biophys. Res. Commun.* 17, 265–271. doi: 10.1016/0006-291X(64)90395-X
- Byrd, M. S., Sadovskaya, I., Vinogradov, E., Lu, H., Sprinkle, A. B., Richardson, S. H., et al. (2009). Genetic and biochemical analyses of the *Pseudomonas aeruginosa* Psl exopolysaccharide reveal overlapping roles for polysaccharide synthesis enzymes in Psl and LPS production. *Mol. Microbiol.* 73, 622–638. doi: 10.1111/j.1365-2958.2009.06795.x
- Calix, J. J., Brady, A. M., Du, V. Y., Saad, J. S., and Nahm, M. H. (2014). Spectrum of pneumococcal serotype 11A variants results from incomplete loss of capsule O-acetylation. *J. Clin. Microbiol.* 52, 758–765. doi: 10.1128/JCM.02695-13
- Calix, J. J., and Nahm, M. H. (2010). A new pneumococcal serotype, 11E, has a variably inactivated wcjE gene. *J. Infect. Dis.* 202, 29–38. doi: 10.1086/653123
- Calix, J. J., Oliver, M. B., Sherwood, L. K., Beall, B. W., Hollingshead, S. K., and Nahm, M. H. (2011). *Streptococcus pneumoniae* serotype 9A isolates contain diverse mutations to wcjE that result in variable expression of serotype 9V-specific epitope. *J. Infect. Dis.* 204, 1585–1595. doi: 10.1093/infdis/jjr593
- Calix, J. J., Saad, J. S., Brady, A. M., and Nahm, M. H. (2012). Structural characterization of *Streptococcus pneumoniae* serotype 9A capsule polysaccharide reveals role of glycosyl 6-O-acetyltransferase wcjE in serotype 9V capsule biosynthesis and immunogenicity. *J. Biol. Chem.* 287, 13996–14003. doi: 10.1074/jbc.M112.346924
- Campos, M., Martínez-Salazar, J. M., Lloret, L., Moreno, S., Núñez, C., Espín, G., et al. (1996). Characterization of the gene coding for GDP-mannose dehydrogenase (algD) from *Azotobacter vinelandii*. *J. Bacteriol.* 178, 1793–1799.
- Cérantola, S., Lemassu-Jacquier, A., and Montrozier, H. (1999). Structural elucidation of a novel exopolysaccharide produced by a mucoid clinical isolate of *Burkholderia cepacia*. Characterization of a trisubstituted glucuronic acid residue in a heptasaccharide repeating unit. *Eur. J. Biochem.* 260, 373–383. doi: 10.1046/j.1432-1327.1999.00171.x
- Cerca, N., Jefferson, K. K., Maira-Litrán, T., Pier, D. B., Kelly-Quintos, C., Goldmann, D. A., et al. (2007). Molecular basis for preferential protective efficacy of antibodies directed to the poorly acetylated form of staphylococcal poly-N-acetyl-beta-(1-6)-glucosamine. *Infect. Immun.* 75, 3406–3413. doi: 10.1128/IAI.00078-07
- Cescutti, P., Bosco, M., Picotti, F., Impallomeni, G., Leitão, J. H., Richau, J. A., et al. (2000). Structural study of the exopolysaccharide produced by a clinical isolate of *Burkholderia cepacia*. *Biochem. Biophys. Res. Commun.* 273, 1088–1094. doi: 10.1006/bbrc.2000.3059
- Cescutti, P., Foschiatti, M., Furlanis, L., Lagatolla, C., and Rizzo, R. (2010). Isolation and characterization of the biological repeating unit of cepacian, the exopolysaccharide produced by bacteria of the *Burkholderia cepacia* complex. *Carbohydr. Res.* 345, 1455–1460. doi: 10.1016/j.carres.2010.03.029
- Cescutti, P., Impallomeni, G., Garozzo, D., and Rizzo, R. (2011). O-Acetyl complex on cepacian, the principal exopolysaccharide of *Burkholderia cepacia* complex bacteria. *Carbohydr. Res.* 346, 2905–2912. doi: 10.1016/j.carres.2011.10.011
- Cescutti, P., Scussolin, S., Herasimenka, Y., Impallomeni, G., Bicego, M., and Rizzo, R. (2006). First report of a lyase for cepacian, the polysaccharide produced by *Burkholderia cepacia* complex bacteria. *Biochem. Biophys. Res. Commun.* 339, 821–826. doi: 10.1016/j.bbrc.2005.11.104
- Chattoraj, S. S., Murthy, R., Ganesan, S., Goldberg, J. B., Zhao, Y., Hershenson, M. B., et al. (2010). *Pseudomonas aeruginosa* alginate promotes *Burkholderia cenocepacia* persistence in cystic fibrosis transmembrane conductance regulator knockout mice. *Infect. Immun.* 78, 984–993. doi: 10.1128/IAI.01192-09
- Chitnis, C. E., and Ohman, D. E. (1990). Cloning of *Pseudomonas aeruginosa* algG, which controls alginate structure. *J. Bacteriol.* 172, 2894–2900.
- Choi, A. H. K., Slamti, L., Avcı, F. Y., Pier, G. B., and Maira-Litrán, T. (2009). The pgaABCD locus of *Acinetobacter baumannii* encodes the production of poly-beta-1-6-N-acetylglucosamine, which is critical for biofilm formation. *J. Bacteriol.* 191, 5953–5963. doi: 10.1128/JB.00647-09
- Chotirmall, S. H., and McElvaney, N. G. (2014). Fungi in the cystic fibrosis lung: bystanders or pathogens? *Int. J. Biochem. Cell Biol.* 52, 161–173. doi: 10.1016/j.biocel.2014.03.001
- Christensen, G. D., Barker, L. P., Mawhinney, T. P., Baddour, L. M., and Simpson, W. A. (1990). Identification of an antigenic marker of slime production for *Staphylococcus epidermidis*. *Infect. Immun.* 58, 2906–2911.
- Cody, A. J., Field, D., Feil, E. J., Stringer, S., Deadman, M. E., Tsolaki, A. G., et al. (2003). High rates of recombination in otitis media isolates of non-typeable *Haemophilus influenzae*. *Infect. Genet. Evol.* 3, 57–66. doi: 10.1016/S1567-1348(02)00152-1
- Colvin, K. M., Alnabseya, N., Baker, P., Whitney, J. C., Howell, P. L., and Parsek, M. R. (2013). PelA deacetylase activity is required for Pel polysaccharide synthesis in *Pseudomonas aeruginosa*. *J. Bacteriol.* 195, 2329–2339. doi: 10.1128/JB.02150-12
- Colwell, R. R., Huq, A., Islam, M. S., Aziz, K. M. A., Yunus, M., Khan, N. H., et al. (2003). Reduction of cholera in Bangladeshi villages by simple filtration. *Proc. Natl. Acad. Sci. U.S.A.* 100, 1051–1055. doi: 10.1073/pnas.0237386100
- Cramton, S. E., Gerke, C., Schnell, N. F., Nichols, W. W., and Götz, F. (1999). The intercellular adhesion (ica) locus is present in *Staphylococcus aureus* and is required for biofilm formation. *Infect. Immun.* 67, 5427–5433.
- Cress, B. F., Englaender, J. A., He, W., Kasper, D., Linhardt, R. J., and Koffas, M. A. G. (2014). Masquerading microbial pathogens: capsular polysaccharides mimic host-tissue molecules. *FEMS Microbiol. Rev.* 38, 660–697. doi: 10.1111/1574-6976.12056
- Cuzzi, B., Cescutti, P., Furlanis, L., Lagatolla, C., Sturiale, L., Garozzo, D., et al. (2012). Investigation of bacterial resistance to the immune system response: cepacian depolymerisation by reactive oxygen species. *Innate Immun.* 18, 661–671. doi: 10.1177/1753425911435954
- Cywes-Bentley, C., Skurnik, D., Zaidi, T., Roux, D., Deoliveira, R. B., Garrett, W. S., et al. (2013). Antibody to a conserved antigenic target is protective against diverse prokaryotic and eukaryotic pathogens. *Proc. Natl. Acad. Sci. U.S.A.* 110, E2209–E2218. doi: 10.1073/pnas.1303573110
- Damron, F. H., Davis, M. R., Withers, T. R., Ernst, R. K., Goldberg, J. B., Yu, G., et al. (2011). Vanadate and trichosan synergistically induce alginate production by *Pseudomonas aeruginosa* strain PAO1. *Mol. Microbiol.* 81, 554–570. doi: 10.1111/j.1365-2958.2011.07715.x
- Day, D. F. (1988). Bacterial alginate control of enzyme modification. *Ann. N. Y. Acad. Sci.* 542, 107–110. doi: 10.1111/j.1749-6632.1988.tb25813.x
- DeAngelis, P. L. (2002). Evolution of glycosaminoglycans and their glycosyltransferases: implications for the extracellular matrices of animals and the capsules of pathogenic bacteria. *Anat. Rec.* 268, 317–326. doi: 10.1002/ar.10163
- DeAngelis, P. L., Gunay, N. S., Toida, T., Mao, W.-J., and Linhardt, R. J. (2002). Identification of the capsular polysaccharides of Type D and F *Pasteurella multocida* as unmodified heparin and chondroitin, respectively. *Carbohydr. Res.* 337, 1547–1552. doi: 10.1016/S0008-6215(02)00219-7
- Doig, P., Smith, N. R., Todd, T., and Irvin, R. T. (1987). Characterization of the binding of *Pseudomonas aeruginosa* alginate to human epithelial cells. *Infect. Immun.* 55, 1517–1522.
- Donati, I., Holtan, S., Mørch, Y. A., Borgogna, M., Dentini, M., and Skjak-Braek, G. (2005). New hypothesis on the role of alternating sequences in calcium-alginate gels. *Biomacromolecules* 6, 1031–1040. doi: 10.1021/bm049306e
- Elias, S., and Banin, E. (2012). Multi-species biofilms: living with friendly neighbors. *FEMS Microbiol. Rev.* 36, 990–1004. doi: 10.1111/j.1574-6976.2012.00325.x
- Ellerbroek, P. M., Lefeber, D. J., van Veghel, R., Scharringa, J., Brouwer, E., Gerwig, G. J., et al. (2004). O-acetylation of cryptococcal capsular glucuronoxylomannan is essential for interference with neutrophil migration. *J. Immunol.* 173, 7513–7520. doi: 10.4049/jimmunol.173.12.7513
- Ertesvåg, H., Doseth, B., Larsen, B., Skjak-Braek, G., and Valla, S. (1994). Cloning and expression of an *Azotobacter vinelandii* mannuronan C-5-epimerase gene. *J. Bacteriol.* 176, 2846–2853.
- Ertesvåg, H., Høidal, H. K., Hals, I. K., Rian, A., Doseth, B., and Valla, S. (1995). A family of modular type mannuronan C-5-epimerase genes controls alginate structure in *Azotobacter vinelandii*. *Mol. Microbiol.* 16, 719–731. doi: 10.1111/j.1365-2958.1995.tb02433.x
- Ertesvåg, H., Høidal, H. K., Skjak-Braek, G., and Valla, S. (1998). The *Azotobacter vinelandii* mannuronan C-5-epimerase AlgE1 consists of two separate catalytic domains. *J. Biol. Chem.* 273, 30927–30932. doi: 10.1074/jbc.273.47.30927
- Ertesvåg, H., and Valla, S. (1999). The A modules of the *Azotobacter vinelandii* mannuronan-C-5-epimerase AlgE1 are sufficient for both epimerization and binding of Ca²⁺. *J. Bacteriol.* 181, 3033–3038.

- Esko, J. D., and Lindahl, U. (2001). Molecular diversity of heparan sulfate. *J. Clin. Invest.* 108, 169–173. doi: 10.1172/JCI13530
- Evans, L. R., and Linker, A. (1973). Production and characterization of the slime polysaccharide of *Pseudomonas aeruginosa*. *J. Bacteriol.* 116, 915–924.
- Farrell, E. K., and Tipton, P. A. (2012). Functional characterization of AlgI, an alginate lyase from *Pseudomonas aeruginosa*. *Biochemistry* 51, 10259–10266. doi: 10.1021/bi301425r
- Faruque, S. M., Albert, M. J., and Mekalanos, J. J. (1998). Epidemiology, genetics, and ecology of toxigenic *Vibrio cholerae*. *Microbiol. Mol. Biol. Rev.* 62, 1301–1314.
- Faruque, S. M., Biswas, K., Udden, S. M. N., Ahmad, Q. S., Sack, D. A., Nair, G. B., et al. (2006). Transmissibility of cholera: in vivo-formed biofilms and their relationship to infectivity and persistence in the environment. *Proc. Natl. Acad. Sci. U.S.A.* 103, 6350–6355. doi: 10.1073/pnas.0601277103
- Ferreira, A. S., Leitão, J. H., Silva, I. N., Pinheiro, P. F., Sousa, S. A., Ramos, C. G., et al. (2010). Distribution of cepacian biosynthesis genes among environmental and clinical *Burkholderia* strains and role of cepacian exopolysaccharide in resistance to stress conditions. *Appl. Environ. Microbiol.* 76, 441–450. doi: 10.1128/AEM.01828-09
- Ferreira, A. S., Silva, I. N., Oliveira, V. H., Cunha, R., and Moreira, L. M. (2011). Insights into the role of extracellular polysaccharides in *Burkholderia* adaptation to different environments. *Front. Cell Infect. Microbiol.* 1:16. doi: 10.3389/fcimb.2011.00016
- Fischer, F. G., and Dorfel, H. (1955). Die polyuronsäuren der braunalgen (Kohlenhydrate der Algen I). *Biol. Chem.* 302, 186–203. doi: 10.1515/bchm2.1955.302.1-2.186
- Flemming, H.-C., and Wingender, J. (2010). The biofilm matrix. *Nat. Rev. Microbiol.* 8, 623–633. doi: 10.1038/nrmicro2415
- Fong, J. C. N., Syed, K. A., Klose, K. E., and Yildiz, F. H. (2010). Role of *Vibrio polysaccharide* (vps) genes in VPS production, biofilm formation and *Vibrio cholerae* pathogenesis. *Microbiology* 156, 2757–2769. doi: 10.1099/mic.0.040196-0
- Forman, S., Bobrov, A. G., Kirillina, O., Craig, S. K., Abney, J., Fetherston, J. D., et al. (2006). Identification of critical amino acid residues in the plague biofilm Hms proteins. *Microbiology* 152, 3399–3410. doi: 10.1099/mic.0.29224-0
- Fox, K. L., Yildirim, H. H., Deadman, M. E., Schweda, E. K. H., Moxon, E. R., and Hood, D. W. (2005). Novel lipopolysaccharide biosynthetic genes containing tetranucleotide repeats in *Haemophilus influenzae*, identification of a gene for adding O-acetyl groups. *Mol. Microbiol.* 58, 207–216. doi: 10.1111/j.1365-2958.2005.04814.x
- Franklin, M. J., Chitnis, C. E., Gacesa, P., Sonesson, A., White, D. C., and Ohman, D. E. (1994). *Pseudomonas aeruginosa* AlgG is a polymer level alginate C5-mannuronan epimerase. *J. Bacteriol.* 176, 1821–1830.
- Franklin, M. J., Douthit, S. A., and McClure, M. A. (2004). Evidence that the algI/algJ gene cassette, required for O acetylation of *Pseudomonas aeruginosa* alginate, evolved by lateral gene transfer. *J. Bacteriol.* 186, 4759–4773. doi: 10.1128/JB.186.14.4759-4773.2004
- Franklin, M. J., Nivens, D. E., Weadge, J. T., and Howell, P. L. (2011). Biosynthesis of the *Pseudomonas aeruginosa* Extracellular Polysaccharides, Alginate, Pel, and Psl. *Front. Microbiol.* 2:167. doi: 10.3389/fmicb.2011.00167
- Franklin, M. J., and Ohman, D. E. (1993). Identification of algF in the alginate biosynthetic gene cluster of *Pseudomonas aeruginosa* which is required for alginate acetylation. *J. Bacteriol.* 175, 5057–5065.
- Franklin, M. J., and Ohman, D. E. (1996). Identification of algI and algJ in the *Pseudomonas aeruginosa* alginate biosynthetic gene cluster which are required for alginate O acetylation. *J. Bacteriol.* 178, 2186–2195.
- Franklin, M. J., and Ohman, D. E. (2002). Mutant analysis and cellular localization of the AlgI, AlgJ, and AlgF proteins required for O acetylation of alginate in *Pseudomonas aeruginosa*. *J. Bacteriol.* 184, 3000–3007. doi: 10.1128/JB.184.11.3000-3007.2002
- Frasa, H., Procee, J., Torensma, R., Verbruggen, A., Algra, A., Rozenberg-Arska, M., et al. (1993). *Escherichia coli* in bacteremia: O-acetylated K1 strains appear to be more virulent than non-O-acetylated K1 strains. *J. Clin. Microbiol.* 31, 3174–3178.
- Friedman, L., and Kolter, R. (2004). Genes involved in matrix formation in *Pseudomonas aeruginosa* PA14 biofilms. *Mol. Microbiol.* 51, 675–690. doi: 10.1046/j.1365-2958.2003.03877.x
- Fujihara, H., Kagaya, K., and Fukazawa, Y. (1997). Anti-chemotactic activity of capsular polysaccharide of *Cryptococcus neoformans* in vitro. *Microbiol. Immunol.* 41, 657–664. doi: 10.1111/j.1348-0421.1997.tb01908.x
- Fusco, P. C., Farley, E. K., Huang, C.-H., Moore, S., and Michon, F. (2007). Protective meningococcal capsular polysaccharide epitopes and the role of O acetylation. *Clin. Vaccine Immunol.* 14, 577–584. doi: 10.1128/CVI.00009-07
- Gacesa, P. (1988). Alginates. *Carbohydr. Polym.* 8, 161–182. doi: 10.1016/0144-8617(88)90001-X
- Gening, M. L., Maira-Litràn, T., Kropec, A., Skurnik, D., Grout, M., Tsvetkov, Y. E., et al. (2010). Synthetic [beta]-(1->6)-linked N-acetylated and nonacetylated oligoglucosamines used to produce conjugate vaccines for bacterial pathogens. *Infect. Immun.* 78, 764–772. doi: 10.1128/IAI.01093-09
- Gerke, C., Kraft, A., Süßmuth, R., Schweitzer, O., and Götz, F. (1998). Characterization of the N-acetylglucosaminyltransferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. *J. Biol. Chem.* 273, 18586–18593. doi: 10.1074/jbc.273.29.18586
- Gimmestad, M., Sletta, H., Ertesvåg, H., Bakkevig, K., Jain, S., Suh, S. J., et al. (2003). The *Pseudomonas fluorescens* AlgG protein, but not its mannuronan C-5-epimerase activity, is needed for alginate polymer formation. *J. Bacteriol.* 185, 3515–3523. doi: 10.1128/JB.185.12.3515-3523.2003
- Gimmestad, M., Steigedal, M., Ertesvåg, H., Moreno, S., Christensen, B. E., Espin, G., et al. (2006). Identification and characterization of an *Azotobacter vinelandii* type I secretion system responsible for export of the AlgE-type mannuronan C-5-epimerases. *J. Bacteriol.* 188, 5551–5560. doi: 10.1128/JB.00236-06
- González, J. E., Semino, C. E., Wang, L. X., Castellano-Torres, L. E., and Walker, G. C. (1998). Biosynthetic control of molecular weight in the polymerization of the octasaccharide subunits of succinoglycan, a symbiotically important exopolysaccharide of *Rhizobium meliloti*. *Proc. Natl. Acad. Sci. U.S.A.* 95, 13477–13482. doi: 10.1073/pnas.95.23.13477
- Gorin, P., and Spencer, J. (1966). Exocellular alginic acid from *Azotobacter vinelandii*. *Can. J. Chem.* 44, 993–998. doi: 10.1139/v66-147
- Govan, J. R., and Deretic, V. (1996). Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol. Rev.* 60, 539–574.
- Govan, J. R., Fyfe, J. A., and Jarman, T. R. (1981). Isolation of alginate-producing mutants of *Pseudomonas fluorescens*, *Pseudomonas putida* and *Pseudomonas mendocina*. *J. Gen. Microbiol.* 125, 217–220. doi: 10.1099/00221287-125-1-217
- Grant, G. T., Morris, E. R., Rees, D. A., Smith, P., and Thom, D. (1973). Biological interactions between polysaccharides and divalent cations: the egg-box model. *FEBS Lett.* 32, 195–198. doi: 10.1016/0014-5793(73)80770-7
- Halpern, M., Broza, Y. B., Mittler, S., Arakawa, E., and Broza, M. (2004). Chironomid egg masses as a natural reservoir of *Vibrio cholerae* non-O1 and non-O139 in freshwater habitats. *Microb. Ecol.* 47, 341–349. doi: 10.1007/s00248-003-2007-6
- Haug, A., Larsen, B., and Smidsrød, O. (1966). A study of the constitution of alginic acid by partial acid hydrolysis. *Acta Chem. Scand.* 20, 183–190. doi: 10.3891/acta.chem.scand.20-0183
- Haug, A., Larsen, B., and Smidsrød, O. (1967). Alkaline degradation of alginate. *Acta Chem. Scand.* 21, 2859–2870. doi: 10.3891/acta.chem.scand.21-2859
- Hay, I. D., Wang, Y., Moradali, M., Rehman, Z. U., and Rehm, B. H. A. (2014). Genetics and regulation of bacterial alginate production. *Environ. Microbiol.* 16, 2997–3011. doi: 10.1111/1462-2920.12389
- Heilmann, C., Gerke, C., Perdreau-Remington, F., and Götz, F. (1996a). Characterization of Tn917 insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. *Infect. Immun.* 64, 277–282.
- Heilmann, C., Schweitzer, O., Gerke, C., Vanittanakom, N., Mack, D., and Götz, F. (1996b). Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. *Mol. Microbiol.* 20, 1083–1091. doi: 10.1111/j.1365-2958.1996.tb02548.x
- Hisamatsu, M., Nomura, S., Shutsrirung, A., Obata, H., Teranishi, K., Yamada, T., et al. (1997). Structural characterization of a new acidic exopolysaccharide and cyclic (1->2) β -glucan produced by *Rhizobium huakuii* forming nodules on *Astragalus sinicus*. *J. Ferment. Bioeng.* 83, 315–320. doi: 10.1016/S0922-338X(97)80135-3
- Hoidal, H. K., Glaerum Svanem, B. I., Gimmestad, M., and Valla, S. (2000). Mannuronan C-5 epimerases and cellular differentiation of *Azotobacter vinelandii*. *Environ. Microbiol.* 2, 27–38. doi: 10.1046/j.1462-2920.2000.00074.x

- Horsman, S. R., Moore, R. A., and Lewenza, S. (2012). Calcium chelation by alginate activates the type III secretion system in mucoid *Pseudomonas aeruginosa* biofilms. *PLoS ONE* 7:e46826. doi: 10.1371/journal.pone.0046826
- Hostetter, M. K., Thomas, M. L., Rosen, F. S., and Tack, B. F. (1982). Binding of C3b proceeds by a transesterification reaction at the thioester site. *Nature* 298, 72–75. doi: 10.1038/298072b0
- Huq, A., Colwell, R. R., Chowdhury, M. A., Xu, B., Moniruzzaman, S. M., Islam, M. S., et al. (1995). Coexistence of *Vibrio cholerae* O1 and O139 Bengal in plankton in Bangladesh. *Lancet* 345, 1249. doi: 10.1016/S0140-6736(95)92038-2
- Huq, A., Small, E. B., West, P. A., Huq, M. I., Rahman, R., and Colwell, R. R. (1983). Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. *Appl. Environ. Microbiol.* 45, 275–283.
- Huq, A., Xu, B., Chowdhury, M. A., Islam, M. S., Montilla, R., and Colwell, R. R. (1996). A simple filtration method to remove plankton-associated *Vibrio cholerae* in raw water supplies in developing countries. *Appl. Environ. Microbiol.* 62, 2508–2512.
- Toh, Y., Rice, J. D., Goller, C., Pannuri, A., Taylor, J., Meisner, J., et al. (2008). Roles of pgaABCD genes in synthesis, modification, and export of the *Escherichia coli* biofilm adhesin poly-beta-1,6-N-acetyl-D-glucosamine. *J. Bacteriol.* 190, 3670–3680. doi: 10.1128/JB.01920-07
- Izano, E. A., Sadvokaya, I., Vinogradov, E., Mulks, M. H., Velliagounder, K., Ragunath, C., et al. (2007). Poly-N-acetylglucosamine mediates biofilm formation and antibiotic resistance in *Actinobacillus pleuropneumoniae*. *Microb. Pathog.* 43, 1–9. doi: 10.1016/j.micpath.2007.02.004
- Izano, E. A., Sadvokaya, I., Wang, H., Vinogradov, E., Ragunath, C., Ramasubbu, N., et al. (2008). Poly-N-acetylglucosamine mediates biofilm formation and detergent resistance in *Aggregatibacter actinomycetemcomitans*. *Microb. Pathog.* 44, 52–60. doi: 10.1016/j.micpath.2007.08.004
- Jain, S., Franklin, M. J., Ertesvåg, H., Valla, S., and Ohman, D. E. (2003). The dual roles of AlgG in C-5-epimerization and secretion of alginate polymers in *Pseudomonas aeruginosa*. *Mol. Microbiol.* 47, 1123–1133. doi: 10.1046/j.1365-2958.2003.03361.x
- Jansson, P. E., Lindberg, B., Lindquist, U., and Ljungberg, J. (1987). Structural studies of the capsular polysaccharide from *Streptococcus pneumoniae* types 15B and 15C. *Carbohydr. Res.* 162, 111–116. doi: 10.1016/0008-6215(87)80205-7
- Jarrett, C. O., Deak, E., Isherwood, K. E., Oyston, P. C., Fischer, E. R., Whitney, A. R., et al. (2004). Transmission of *Yersinia pestis* from an infectious biofilm in the flea vector. *J. Infect. Dis.* 190, 783–792. doi: 10.1086/422695
- Joyce, J. G., Abeygunawardana, C., Xu, Q., Cook, J. C., Hepler, R., Przysiecki, C. T., et al. (2003). Isolation, structural characterization, and immunological evaluation of a high-molecular-weight exopolysaccharide from *Staphylococcus aureus*. *Carbohydr. Res.* 338, 903–922. doi: 10.1016/S0008-6215(03)00045-4
- Kaper, J. B., Morris, J. G., and Levine, M. M. (1995). Cholera. *Clin. Microbiol. Rev.* 8, 48–86.
- Kelley, L. A., and Sternberg, M. J. E. (2009). Protein structure prediction on the Web: a case study using the Phyre server. *Nat. Protoc.* 4, 363–371. doi: 10.1038/nprot.2009.2
- Kim, M. L., and Schlauch, J. M. (1999). Effect of acetylation (O-factor 5) on the polyclonal antibody response to *Salmonella typhimurium* O-antigen. *FEMS Immunol. Med. Microbiol.* 26, 83–92. doi: 10.1111/j.1574-695X.1999.tb01375.x
- Kim, W., Tengra, F. K., Young, Z., Shong, J., Marchand, N., Chan, H. K., et al. (2013). Spaceflight promotes biofilm formation by *Pseudomonas aeruginosa*. *PLoS ONE* 8:e62437. doi: 10.1371/journal.pone.0062437
- Kjellén, L., and Lindahl, U. (1991). Proteoglycans: structures and interactions. *Annu. Rev. Biochem.* 60, 443–475. doi: 10.1146/annurev.bi.60.070191.002303
- Kline, K. A., Schwartz, D. J., Gilbert, N. M., Hultgren, S. J., and Lewis, A. L. (2012). Immune modulation by group B *Streptococcus* influences host susceptibility to urinary tract infection by uropathogenic *Escherichia coli*. *Infect. Immun.* 80, 4186–4194. doi: 10.1128/IAI.00684-12
- Kline, K. A., Schwartz, D. J., Lewis, W. G., Hultgren, S. J., and Lewis, A. L. (2011). Immune activation and suppression by group B streptococcus in a murine model of urinary tract infection. *Infect. Immun.* 79, 3588–3595. doi: 10.1128/IAI.00122-11
- Korotkov, K. V., Sandkvist, M., and Hol, W. G. J. (2012). The type II secretion system: biogenesis, molecular architecture and mechanism. *Nat. Rev. Microbiol.* 10, 336–351. doi: 10.1038/nrmicro2762
- Kozel, T. R., Levitz, S. M., Dromer, F., Gates, M. A., Thorkildson, P., and Janbon, G. (2003). Antigenic and biological characteristics of mutant strains of *Cryptococcus neoformans* lacking capsular O acetylation or xylosyl side chains. *Infect. Immun.* 71, 2868–2875. doi: 10.1128/IAI.71.5.2868-2875.2003
- Krieg, D. P., Bass, J. A., and Mattingly, S. J. (1986). Aeration selects for mucoid phenotype of *Pseudomonas aeruginosa*. *J. Clin. Microbiol.* 24, 986–990.
- Kuo, M.-S., Mort, A. J., and Dell, A. (1986). Identification and location of l-glycerate, an unusual acyl substituent in gellan gum. *Carbohydr. Res.* 156, 173–187. doi: 10.1016/S0008-6215(00)90109-5
- Kwon, K. K., Lee, H. S., Jung, S., Yim, J., Lee, J., and Lee, H. K. (2002). Isolation and identification of biofilm-forming marine bacteria on glass surfaces in dae-ho dike, Korea. *J. Microbiol.* 40, 260–266.
- Laurent, T. C., and Fraser, J. R. (1992). Hyaluronan. *FASEB J.* 6, 2397–2404.
- Learn, D. B., Brestel, E. P., and Seetharama, S. (1987). Hypochlorite scavenging by *Pseudomonas aeruginosa* alginate. *Infect. Immun.* 55, 1813–1818.
- Lee, J. W., and Day, F. (1998). The separation of alginate biosynthesis and acetylation in *Pseudomonas syringae*. *Can. J. Microbiol.* 44, 394–398. doi: 10.1139/w98-008
- Lenhart, T. R., Duncan, K. E., Beech, I. B., Sunner, J. A., Smith, W., Bonifay, V., et al. (2014). Identification and characterization of microbial biofilm communities associated with corroded oil pipeline surfaces. *Biofouling* 30, 823–835. doi: 10.1080/08927014.2014.931379
- Leuck, A.-M., Johnson, J. R., and Dunny, G. M. (2014). A widely used in vitro biofilm assay has questionable clinical significance for enterococcal endocarditis. *PLoS ONE* 9:e107282. doi: 10.1371/journal.pone.0107282
- Lewis, A. L., Cao, H., Patel, S. K., Diaz, S., Ryan, W., Carlin, A. F., et al. (2007). NeuA sialic acid O-acetyltransferase activity modulates O-acetylation of capsular polysaccharide in group B *Streptococcus*. *J. Biol. Chem.* 282, 27562–27571. doi: 10.1074/jbc.M700340200
- Lewis, A. L., Hensler, M. E., Varki, A., and Nizet, V. (2006). The group B streptococcal sialic acid O-acetyltransferase is encoded by neuD, a conserved component of bacterial sialic acid biosynthetic gene clusters. *J. Biol. Chem.* 281, 11186–11192. doi: 10.1074/jbc.M513772200
- Li, J.-P., Gong, F., Hagner-McWhirter, A., Forsberg, E., Abrink, M., Kisilevsky, R., et al. (2003). Targeted disruption of a murine glucuronyl C5-epimerase gene results in heparan sulfate lacking L-iduronic acid and in neonatal lethality. *J. Biol. Chem.* 278, 28363–28366. doi: 10.1074/jbc.C300219200
- Limoli, D. H., Rockel, A. B., Host, K. M., Jha, A., Kopp, B. T., Hollis, T., et al. (2014). Cationic antimicrobial peptides promote microbial mutagenesis and pathoadaptation in chronic infections. *PLoS Pathog.* 10:e1004083. doi: 10.1371/journal.ppat.1004083
- Lin, L. P., and Sadoff, H. L. (1969). Chemical composition of *Azotobacter vinelandii* cysts. *J. Bacteriol.* 100, 480–486.
- Linker, A., Evans, L. R., and Impallomeni, G. (2001). The structure of a polysaccharide from infectious strains of *Burkholderia cepacia*. *Carbohydr. Res.* 335, 45–54. doi: 10.1016/S0008-6215(01)00205-1
- Linker, A., and Jones, R. S. (1966). A new polysaccharide resembling alginic acid isolated from pseudomonads. *J. Biol. Chem.* 241, 3845–3851.
- Little, D. J., Bamford, N. C., Podrovska, V., Robinson, H., Nitz, M., and Howell, P. L. (2014a). Structural basis for the de-N-acetylation of poly-beta-1,6-N-acetyl-D-glucosamine in Gram-positive bacteria. *J. Biol. Chem.* 289, 35907–35917. doi: 10.1074/jbc.M114.611400
- Little, D. J., Li, G., Ing, C., DiFrancesco, B. R., Bamford, N. C., Robinson, H., et al. (2014b). Modification and periplasmic translocation of the biofilm exopolysaccharide poly-beta-1,6-N-acetyl-d-glucosamine. *Proc. Natl. Acad. Sci. U.S.A.* 111, 11013–11018. doi: 10.1073/pnas.1406388111
- Little, D. J., Poloczek, J., Whitney, J. C., Robinson, H., Nitz, M., and Howell, P. L. (2012). The structure- and metal-dependent activity of *Escherichia coli* PgaB provides insight into the partial de-N-acetylation of poly-beta-1,6-N-acetyl-D-glucosamine. *J. Biol. Chem.* 287, 31126–31137. doi: 10.1074/jbc.M112.390005
- Lyczak, J. B., Cannon, C. L., and Pier, G. B. (2000). Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Microbes Infect.* 2, 1051–1060. doi: 10.1016/S1286-4579(00)01259-4
- Ma, L., Wang, J., Wang, S., Anderson, E. M., Lam, J. S., Parsek, M. R., et al. (2012). Synthesis of multiple *Pseudomonas aeruginosa* biofilm matrix exopolysaccharides is post-transcriptionally regulated. *Environ. Microbiol.* 14, 1995–2005. doi: 10.1111/j.1462-2920.2012.02753.x
- Maalej, H., Boisset, C., Hmidet, N., Buon, L., Heyraud, A., and Nasri, M. (2014). Purification and structural data of a highly substituted exopolysaccharide

- from *Pseudomonas stutzeri* AS22. *Carbohydr. Polym.* 112, 404–411. doi: 10.1016/j.carbpol.2014.06.003
- Mack, D., Fischer, W., Krokotsch, A., Leopold, K., Hartmann, R., Egge, H., et al. (1996). The intercellular adhesion involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear beta-1,6-linked glucosaminoglycan: purification and structural analysis. *J. Bacteriol.* 178, 175–183.
- Mahenthalingam, E., Baldwin, A., and Vandamme, P. (2002). Burkholderia cepacia complex infection in patients with cystic fibrosis. *J. Med. Microbiol.* 51, 533–538.
- Mahenthalingam, E., Urban, T. A., and Goldberg, J. B. (2005). The multifarious, multireplicon *Burkholderia cepacia* complex. *Nat. Rev. Microbiol.* 3, 144–156. doi: 10.1038/nrmicro1085
- Mai, G. T., Seow, W. K., Pier, G. B., McCormack, J. G., and Thong, Y. H. (1993). Suppression of lymphocyte and neutrophil functions by *Pseudomonas aeruginosa* mucoid exopolysaccharide (alginate): reversal by physicochemical, alginate, and specific monoclonal antibody treatments. *Infect. Immun.* 61, 559–564.
- Maira-Litrán, T., Kropec, A., Abeygunawardana, C., Joyce, J., Mark, G., Goldmann, D. A., et al. (2002). Immunochemical properties of the staphylococcal poly-N-acetylglucosamine surface polysaccharide. *Infect. Immun.* 70, 4433–4440.
- Maira-Litrán, T., Kropec, A., Goldmann, D., and Pier, G. B. (2004). Biologic properties and vaccine potential of the staphylococcal poly-N-acetyl glucosamine surface polysaccharide. *Vaccine* 22, 872–879. doi: 10.1016/j.vaccine.2003.11.033
- Marcus, H., and Baker, N. R. (1985). Quantitation of adherence of mucoid and nonmucoid *Pseudomonas aeruginosa* to hamster tracheal epithelium. *Infect. Immun.* 47, 723–729.
- Marty, N., Dournes, J. L., Chabanon, G., and Montrozier, H. (1992). Influence of nutrient media on the chemical composition of the exopolysaccharide from mucoid and non-mucoid *Pseudomonas aeruginosa*. *FEMS Microbiol. Lett.* 77, 35–44. doi: 10.1111/j.1574-6968.1992.tb05486.x
- Marzocca, M. P., Harding, N. E., Petroni, E. A., Cleary, J. M., and Ielpi, L. (1991). Location and cloning of the ketol pyruvate transferase gene of *Xanthomonas campestris*. *J. Bacteriol.* 173, 7519–7524.
- Matz, C., McDougald, D., Moreno, A. M., Yung, P. Y., Yildiz, F. H., and Kjelleberg, S. (2005). Biofilm formation and phenotypic variation enhance predation-driven persistence of *Vibrio cholerae*. *Proc. Natl. Acad. Sci. U.S.A.* 102, 16819–16824. doi: 10.1073/pnas.0505350102
- McKenney, D., Hübner, J., Muller, E., Wang, Y., Goldmann, D. A., and Pier, G. B. (1998). The ica locus of *Staphylococcus epidermidis* encodes production of the capsular polysaccharide/adhesin. *Infect. Immun.* 66, 4711–4720.
- McKenney, D., Pouliot, K. L., Wang, Y., Murthy, V., Ulrich, M., Döring, G., et al. (1999). Broadly protective vaccine for *Staphylococcus aureus* based on an in vivo-expressed antigen. *Science* 284, 1523–1527. doi: 10.1126/science.284.5419.1523
- McNeely, T. B., Staub, J. M., Rusk, C. M., Blum, M. J., and Donnelly, J. J. (1998). Antibody responses to capsular polysaccharide backbone and O-acetate side groups of *Streptococcus pneumoniae* type 9V in humans and rhesus macaques. *Infect. Immun.* 66, 3705–3710.
- Melin, M., Trzciński, K., Antonio, M., Meri, S., Adegbola, R., Kaijalainen, T., et al. (2010). Serotype-related variation in susceptibility to complement deposition and opsonophagocytosis among clinical isolates of *Streptococcus pneumoniae*. *Infect. Immun.* 78, 5252–5261. doi: 10.1128/IAI.00739-10
- Michon, F., Huang, C. H., Farley, E. K., Hronowski, L., Di, J., and Fusco, P. C. (2000). Structure activity studies on group C meningococcal polysaccharide-protein conjugate vaccines: effect of O-acetylation on the nature of the protective epitope. *Dev. Biol. (Basel)* 103, 151–160.
- Monday, S. R., and Schiller, N. L. (1996). Alginate synthesis in *Pseudomonas aeruginosa*: the role of AlgL (alginate lyase) and AlgX. *J. Bacteriol.* 178, 625–632.
- Morea, A., Mathee, K., Franklin, M. J., Giacomini, A., O'Regan, M., and Ohman, D. E. (2001). Characterization of algG encoding C5-epimerase in the alginate biosynthetic gene cluster of *Pseudomonas fluorescens*. *Gene* 278, 107–114. doi: 10.1016/S0378-1119(01)00685-0
- Moynihan, P. J., and Clarke, A. J. (2011). O-Acetylated peptidoglycan: controlling the activity of bacterial autolysins and lytic enzymes of innate immune systems. *Int. J. Biochem. Cell Biol.* 43, 1655–1659. doi: 10.1016/j.biocel.2011.08.007
- Nivens, D. E., Ohman, D. E., Williams, J., and Franklin, M. J. (2001). Role of alginate and its O acetylation in formation of *Pseudomonas aeruginosa* microcolonies and biofilms. *J. Bacteriol.* 183, 1047–1057. doi: 10.1128/JB.183.3.1047-1057.2001
- Ohman, D. E. (1986). Molecular genetics of exopolysaccharide production by mucoid *Pseudomonas aeruginosa*. *Eur. J. Clin. Microbiol.* 5, 6–10. doi: 10.1007/BF02013452
- Paletta, J. L., and Ohman, D. E. (2012). Evidence for two promoters internal to the alginate biosynthesis operon in *Pseudomonas aeruginosa*. *Curr. Microbiol.* 65, 770–775. doi: 10.1007/s00284-012-0228-y
- Parise, G., Mishra, M., Itoh, Y., Romeo, T., and Deora, R. (2007). Role of a putative polysaccharide locus in *Bordetella* biofilm development. *J. Bacteriol.* 189, 750–760. doi: 10.1128/JB.00953-06
- Peña, C., Hernández, L., and Galindo, E. (2006). Manipulation of the acetylation degree of *Azotobacter vinelandii* alginate by supplementing the culture medium with 3-(N-morpholino)-propane-sulfonic acid. *Let. Appl. Microbiol.* 43, 200–204. doi: 10.1111/j.1472-765X.2006.01925.x
- Pier, G. B., Coleman, F., Grout, M., Franklin, M., and Ohman, D. E. (2001). Role of alginate O acetylation in resistance of mucoid *Pseudomonas aeruginosa* to opsonic phagocytosis. *Infect. Immun.* 69, 1895–1901. doi: 10.1128/IAI.69.3.1895-1901.2001
- Pinto, V., and Berti, F. (2014). Exploring the Group B *Streptococcus* capsular polysaccharides: the structural diversity provides the basis for development of NMR-based identity assays. *J. Pharm. Biomed. Anal.* 98, 9–15. doi: 10.1016/j.jpba.2014.05.004
- Pokrovskaya, V., Poloczek, J., Little, D. J., Griffiths, H., Howell, P. L., and Nitz, M. (2013). Functional Characterization of *Staphylococcus epidermidis* IcaB, a De-N-acetylase important for biofilm formation. *Biochemistry* 52, 5463–5471. doi: 10.1021/bi400836g
- Raedts, J., Kengen, S. W. M., and van der Oost, J. (2011). Occurrence of L-iduronic acid and putative D-glucuronyl C5-epimerases in prokaryotes. *Glycoconj. J.* 28, 57–66. doi: 10.1007/s10719-011-9324-7
- Raedts, J., Lundgren, M., Kengen, S. W. M., Li, J.-P., and van der Oost, J. (2013). A novel bacterial enzyme with D-glucuronyl C5-epimerase activity. *J. Biol. Chem.* 288, 24332–24339. doi: 10.1074/jbc.M113.476440
- Rajam, G., Carlone, G. M., and Romero-Steiner, S. (2007). Functional antibodies to the O-acetylated pneumococcal serotype 15B capsular polysaccharide have low cross-reactivities with serotype 15C. *Clin. Vaccine Immunol.* 14, 1223–1227. doi: 10.1128/CVI.00184-07
- Ramphal, R., Guay, C., and Pier, G. B. (1987). *Pseudomonas aeruginosa* adhesins for tracheobronchial mucin. *Infect. Immun.* 55, 600–603.
- Rehm, B. H., Ertesvåg, H., and Valla, S. (1996). A new *Azotobacter vinelandii* mannuronan C-5-epimerase gene (algG) is part of an alg gene cluster physically organized in a manner similar to that in *Pseudomonas aeruginosa*. *J. Bacteriol.* 178, 5884–5889.
- Reuber, T. L., and Walker, G. C. (1993). Biosynthesis of succinoglycan, a symbiotically important exopolysaccharide of *Rhizobium meliloti*. *Cell* 74, 269–280. doi: 10.1016/0092-8674(93)90418-P
- Ridout, M. J., Brownsey, G. J., York, G. M., Walker, G. C., and Morris, V. J. (1997). Effect of o-acyl substituents on the functional behaviour of *Rhizobium meliloti* succinoglycan. *Int. J. Biol. Macromol.* 20, 1–7. doi: 10.1016/S0141-8130(96)01140-3
- Riley, L. M., Weadge, J. T., Baker, P., Robinson, H., Codée, J. D. C., Tipton, P. A., et al. (2013). Structural and functional characterization of *Pseudomonas aeruginosa* AlgX: role of AlgX in alginate acetylation. *J. Biol. Chem.* 288, 22299–22314. doi: 10.1074/jbc.M113.484931
- Robles-Price, A., Wong, T. Y., Sletta, H., Valla, S., and Schiller, N. L. (2004). AlgX is a periplasmic protein required for alginate biosynthesis in *Pseudomonas aeruginosa*. *J. Bacteriol.* 186, 7369–7377. doi: 10.1128/JB.186.21.7369-7377.2004
- Rodriguez, M. L., Jann, B., and Jann, K. (1988). Structure and serological characteristics of the capsular K4 antigen of *Escherichia coli* O5:K4:H4, a fructose-containing polysaccharide with a chondroitin backbone. *Eur. J. Biochem.* 177, 117–124. doi: 10.1111/j.1432-1033.1988.tb14351.x
- Rozeboom, H. J., Bjerkan, T. M., Kalk, K. H., Ertesvåg, H., Holtan, S., Aachmann, F. L., et al. (2008). Structural and mutational characterization of the catalytic A-module of the mannuronan C-5-epimerase AlgE4 from *Azotobacter vinelandii*. *J. Biol. Chem.* 283, 23819–23828. doi: 10.1074/jbc.M804119200
- Rutherford, T. J., Jones, C., Davies, D. B., and Elliott, A. C. (1991). Location and quantitation of the sites of O-acetylation on the capsular polysaccharide from *Streptococcus pneumoniae* type 9V by ¹H-N.m.r. spectroscopy: comparison

- with type 9A. *Carbohydr. Res.* 218, 175–184. doi: 10.1016/0008-6215(91)84096-W
- Sabra, W., Zeng, A. P., Lünsdorf, H., and Deckwer, W. D. (2000). Effect of oxygen on formation and structure of *Azotobacter vinelandii* alginate and its role in protecting nitrogenase. *Appl. Environ. Microbiol.* 66, 4037–4044. doi: 10.1128/AEM.66.9.4037-4044.2000
- Sadovskaya, I., Vinogradov, E., Flahaut, S., Kogan, G., and Jabbouri, S. (2005). Extracellular carbohydrate-containing polymers of a model biofilm-producing strain, *Staphylococcus epidermidis* RP62A. *Infect. Immun.* 73, 3007–3017. doi: 10.1128/IAI.73.5.3007-3017.2005
- Sarkisova, S., Patrauchan, M. A., Berglund, D., Nivens, D. E., and Franklin, M. J. (2005). Calcium-induced virulence factors associated with the extracellular matrix of mucoid *Pseudomonas aeruginosa* biofilms. *J. Bacteriol.* 187, 4327–4337. doi: 10.1128/JB.187.13.4327-4337.2005
- Schultz, M. P., Bendick, J. A., Holm, E. R., and Hertel, W. M. (2011). Economic impact of biofouling on a naval surface ship. *Biofouling* 27, 87–98. doi: 10.1080/08927014.2010.542809
- Schwab, U., Abdullah, L. H., Perlmutter, O. S., Albert, D., Davis, C. W., Arnold, R. R., et al. (2014). Localization of *Burkholderia cepacia* complex bacteria in cystic fibrosis lungs and interactions with *Pseudomonas aeruginosa* in hypoxic mucus. *Infect. Immun.* 82, 4729–4745. doi: 10.1128/IAI.01876-14
- Shinabarger, D., May, T. B., Boyd, A., Ghosh, M., and Chakrabarty, A. M. (1993). Nucleotide sequence and expression of the *Pseudomonas aeruginosa* algF gene controlling acetylation of alginate. *Mol. Microbiol.* 9, 1027–1035. doi: 10.1111/j.1365-2958.1993.tb01232.x
- Silbert, J. E., and Sugumaran, G. (2002). Biosynthesis of chondroitin/dermatan sulfate. *IUBMB Life* 54, 177–186. doi: 10.1080/15216540214923
- Simpson, J. A., Smith, S. E., and Dean, R. T. (1988). Alginate inhibition of the uptake of *Pseudomonas aeruginosa* by macrophages. *J. Gen. Microbiol.* 134, 29–36. doi: 10.1099/00221287-134-1-29
- Skjåk-Braek, G., Grasdalen, H., and Larsen, B. (1986). Monomer sequence and acetylation pattern in some bacterial alginates. *Carbohydr. Res.* 154, 239–250. doi: 10.1016/S0008-6215(00)90036-3
- Skjåk-Braek, G., Zanetti, F., and Paoletti, S. (1989). Effect of acetylation on some solution and gelling properties of alginates. *Carbohydr. Res.* 185, 131–138. doi: 10.1016/0008-6215(89)84028-5
- Smidsrød, O., and Draget, K. I. (1996). Chemistry and physical properties of alginates. *Carbohydr. Eur.* 14, 6–13.
- Socolofsky, M. D., and Wyss, O. (1961). Cysts of *Azotobacter*. *J. Bacteriol.* 81, 946–954.
- Speert, D. P., Farmer, S. W., Campbell, M. E., Musser, J. M., Selander, R. K., and Kuo, S. (1990). Conversion of *Pseudomonas aeruginosa* to the phenotype characteristic of strains from patients with cystic fibrosis. *J. Clin. Microbiol.* 28, 188–194.
- Steigedal, M., Sletta, H., Moreno, S., Maerk, M., Christensen, B. E., Bjerkan, T., et al. (2008). The *Azotobacter vinelandii* AlgE mannuronan C-5-epimerase family is essential for the in vivo control of alginate monomer composition and for functional cyst formation. *Environ. Microbiol.* 10, 1760–1770. doi: 10.1111/j.1462-2920.2008.01597.x
- Stewart, E. J. (2012). Growing unculturable bacteria. *J. Bacteriol.* 194, 4151–4160. doi: 10.1128/JB.00345-312
- Svanem, B. I., Skjåk-Braek, G., Ertesvåg, H., and Valla, S. (1999). Cloning and expression of three new *Azotobacter vinelandii* genes closely related to a previously described gene family encoding mannuronan C-5-epimerases. *J. Bacteriol.* 181, 68–77.
- Svanem, B. I., Strand, W. I., Ertesvåg, H., Skjåk-Braek, G., Hartmann, M., Barbeyron, T., et al. (2001). The catalytic activities of the bifunctional *Azotobacter vinelandii* mannuronan C-5-epimerase and alginate lyase AlgE7 probably originate from the same active site in the enzyme. *J. Biol. Chem.* 276, 31542–31550. doi: 10.1074/jbc.M102562200
- Szu, S. C., Li, X. R., Stone, A. L., and Robbins, J. B. (1991). Relation between structure and immunologic properties of the Vi capsular polysaccharide. *Infect. Immun.* 59, 4555–4561.
- Terry, J. M., Piña, S. E., and Mattingly, S. J. (1991). Environmental conditions which influence mucoid conversion *Pseudomonas aeruginosa* PAO1. *Infect. Immun.* 59, 471–477.
- Tielen, P., Strathmann, M., Jaeger, K. -E., Flemming, H. -C., and Wingender, J. (2005). Alginate acetylation influences initial surface colonization by mucoid *Pseudomonas aeruginosa*. *Microbiol. Res.* 160, 165–176. doi: 10.1016/j.micres.2004.11.003
- Tojo, M., Yamashita, N., Goldmann, D. A., and Pier, G. B. (1988). Isolation and characterization of a capsular polysaccharide adhesin from *Staphylococcus epidermidis*. *J. Infect. Dis.* 157, 713–722. doi: 10.1093/infdis/157.4.713
- Vanlaere, E., Baldwin, A., Gevers, D., Henry, D., De Brandt, E., Lipuma, J. J., et al. (2009). Taxon K, a complex within the *Burkholderia cepacia* complex, comprises at least two novel species, *Burkholderia contaminans* sp. nov. and *Burkholderia lata* sp. nov. *Int. J. Syst. Evol. Microbiol.* 59, 102–111. doi: 10.1099/ijs.0.001123-0
- Vann, W. F., Schmidt, M. A., Jann, B., and Jann, K. (1981). The structure of the capsular polysaccharide (K5 antigen) of urinary-tract-infective *Escherichia coli* 010:K5:H4. A polymer similar to desulfo-heparin. *Eur. J. Biochem.* 116, 359–364. doi: 10.1111/j.1432-1033.1981.tb05343.x
- van Selm, S., van Cann, L. M., Kolkman, M. A. B., van der Zeijst, B. A. M., and van Putten, J. P. M. (2003). Genetic basis for the structural difference between *Streptococcus pneumoniae* serotype 15B and 15C capsular polysaccharides. *Infect. Immun.* 71, 6192–6198. doi: 10.1128/IAI.71.11.6192-6198.2003
- Vazquez, A., Moreno, S., Guzmán, J., Alvarado, A., and Espín, G. (1999). Transcriptional organization of the *Azotobacter vinelandii* algXLVIFA genes: characterization of algF mutants. *Gene* 232, 217–222. doi: 10.1016/S0378-1119(99)00119-5
- Veerachamy, S., Yarlagadda, T., Manivasagam, G., and Yarlagadda, P. K. (2014). Bacterial adherence and biofilm formation on medical implants: a review. *Proc. Inst. Mech. Eng. H* 228, 1083–1099. doi: 10.1177/0954411914556137
- Vergara-Irigaray, M., Maira-Litrán, T., Merino, N., Pier, G. B., Penadés, J. R., and Lasa, I. (2008). Wall teichoic acids are dispensable for anchoring the PNAG exopolysaccharide to the *Staphylococcus aureus* cell surface. *Microbiology (Reading, Engl.)* 154, 865–877. doi: 10.1099/mic.0.2007/013292-0
- Vuong, C., Kocianova, S., Voyich, J. M., Yao, Y., Fischer, E. R., DeLeo, F. R., et al. (2004a). A crucial role for exopolysaccharide modification in bacterial biofilm formation, immune evasion, and virulence. *J. Biol. Chem.* 279, 54881–54886. doi: 10.1074/jbc.M411374200
- Vuong, C., Voyich, J. M., Fischer, E. R., Braughton, K. R., Whitney, A. R., DeLeo, F. R., et al. (2004b). Polysaccharide intercellular adhesin (PIA) protects *Staphylococcus epidermidis* against major components of the human innate immune system. *Cell. Microbiol.* 6, 269–275. doi: 10.1046/j.1462-5822.2004.00367.x
- Wang, X., Preston, J. F., and Romeo, T. (2004). The pgaABCD locus of *Escherichia coli* promotes the synthesis of a polysaccharide adhesin required for biofilm formation. *J. Bacteriol.* 186, 2724–2734. doi: 10.1128/JB.186.9.2724-2734.2004
- Wargo, M. J., and Hogan, D. A. (2006). Fungal–bacterial interactions: a mixed bag of mingling microbes. *Curr. Opin. Microbiol.* 9, 359–364. doi: 10.1016/j.mib.2006.06.001
- Watts, A., Ke, D., Wang, Q., Pillay, A., Nicholson-Weller, A., and Lee, J. C. (2005). *Staphylococcus aureus* strains that express serotype 5 or serotype 8 capsular polysaccharides differ in virulence. *Infect. Immun.* 73, 3502–3511. doi: 10.1128/IAI.73.6.3502-3511.2005
- Weiman, S., Dahesh, S., Carlin, A. F., Varki, A., Nizet, V., and Lewis, A. L. (2009). Genetic and biochemical modulation of sialic acid O-acetylation on group B *Streptococcus*: phenotypic and functional impact. *Glycobiology* 19, 1204–1213. doi: 10.1093/glycob/cwp111
- Weiman, S., Uchiyama, S., Lin, F.-Y. C., Chaffin, D., Varki, A., Nizet, V., et al. (2010). O-Acetylation of sialic acid on Group B *Streptococcus* inhibits neutrophil suppression and virulence. *Biochem. J.* 428, 163–168. doi: 10.1042/BJ20100232
- Wloka, M., Rehage, H., Flemming, H. -C., and Wingender, J. (2005). Structure and rheological behaviour of the extracellular polymeric substance network of mucoid *Pseudomonas aeruginosa* biofilms. *Biofilms* 2, 275–283. doi: 10.1017/S1479050506002031
- Wolfram, F., Kitova, E. N., Robinson, H., Walvoort, M., Codée, J. D. C., Klassen, J. S., et al. (2014). Catalytic mechanism and mode of action of the periplasmic alginate epimerase AlgG. *J. Biol. Chem.* 289, 6006–6019. doi: 10.1074/jbc.M113.533158
- Yildiz, F., Fong, J., Sadovskaya, I., Grard, T., and Vinogradov, E. (2014). Structural characterization of the extracellular polysaccharide from *Vibrio*

- cholerae* O1 El-Tor. *PLoS ONE* 9:e86751. doi: 10.1371/journal.pone.0086751
- Yildiz, F. H., and Schoolnik, G. K. (1999). *Vibrio cholerae* O1 El Tor: identification of a gene cluster required for the rugose colony type, exopolysaccharide production, chlorine resistance, and biofilm formation. *Proc. Natl. Acad. Sci. U.S.A.* 96, 4028–4033. doi: 10.1073/pnas.96.7.4028
- Zartler, E. R., Porambo, R. J., Anderson, C. L., Chen, L. H., Yu, J., and Nahm, M. H. (2009). Structure of the capsular polysaccharide of pneumococcal serotype 11A reveals a novel acetyl glycerol that is the structural basis for 11A subtypes. *J. Biol. Chem.* 284, 7318–7329. doi: 10.1074/jbc.M807952200

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