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Bacterial capsular polysaccharides with antibiofilm activity share common biophysical and electrokinetic properties

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AUTHOR CONTRIBUTIONS:

J. B.-B., N.M., P.T., J.F.L.D. and J.-M.G. designed the experiments. L.T., C.B. and B.R. contributed to the initial experiments. J. B.-B., J.T., M.B., A.B., C.C., Y.W., J.-M.G. and P.T. performed the experiments. J. B.-B., J.T., M.B., A.B., P.T., N.M., J.F.L.D. and J.-M.G. analyzed the data. J. B.-B., J.F.L.D. and J.-M.G. wrote the paper with significant contribution from C.B. J.T., A.B., P.T., B.R. and N.M.

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Classification: Biological Sciences, Microbiology; / Physical Sciences, Biophysics

Keywords: Biofilms; antibiofilm; biosurfactants; soft macromolecule electrokinetics

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Main Text
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Table 1

1 **Abstract**

2

3 Bacterial biofilms are surface-attached communities that are difficult to eradicate
4 due to a high tolerance to antimicrobial agents. The use of non-biocidal surface-
5 active compounds to prevent the initial adhesion and aggregation of bacterial
6 pathogens is a promising alternative to antibiotic treatments and several
7 antibiofilm compounds have been identified, including some capsular
8 polysaccharides released by various bacteria. However, the lack of chemical and
9 mechanistic understanding of the activity of these high-molecular-weight
10 polymers limits their use for control of biofilm formation. Here, we screened a
11 collection of 32 purified capsular polysaccharides and identified seven new
12 compounds with non-biocidal activity against biofilms formed by *Escherichia coli*
13 and/or *Staphylococcus aureus*. We analyzed the polysaccharide mobility under
14 applied electric field conditions and showed that active and inactive
15 polysaccharide polymers display distinct electrokinetic properties and that all
16 active macromolecules shared high intrinsic viscosity features. Based on these
17 characteristics, we identified two additional antibiofilm capsular polysaccharides
18 with high density of electrostatic charges and their permeability to fluid flow. Our
19 study therefore provides insights into key biophysical properties discriminating
20 active from inactive polysaccharides. This characterization of a specific
21 electrokinetic signature for polysaccharides displaying antibiofilm activity opens
22 new perspectives to identify or engineer non-biocidal surface-active
23 macromolecules to control biofilm formation in medical and industrial settings.

24

25

26

1 **Significance statement**

2 Some bacteria produce non-biocidal capsular polysaccharides that reduce the
3 adhesion of bacterial pathogens to surfaces. Due to a lack of molecular and
4 structural definition, the basis of their antiadhesion activity is unknown, thus
5 hindering their prophylactic use for biofilm control. Here, we identified nine new
6 active compounds and compared their composition, structure and biophysical
7 properties with other inactive capsular polysaccharides. Despite the absence of
8 specific molecular motif, we demonstrate that all active polysaccharides share
9 common electrokinetic properties that distinguish them from inactive polymers.
10 This characterization of the biophysical properties of antibiofilm bacterial
11 polysaccharide provides key insights to engineer non-biocidal and bio-inspired
12 surface-active compounds to control bacterial adhesion in medical and industrial
13 settings.

14

15

1 **MAIN TEXT**

2

3 **Introduction**

4

5 Bacterial biofilms are widespread surface-attached or aggregated bacteria that
6 can negatively impact human activities when developing on medical or industrial
7 surfaces (1, 2). Due to their high tolerance to antibiotics, biofilms are difficult to
8 eradicate and the prevention of biofilm-associated infections is a major health
9 and economic issue (3, 4). Strategies to prevent biofilm formation often targets
10 the initial steps of bacterial adhesion using surfaces coated by biocidal agents
11 such as broad-spectrum antibiotics or heavy metals (5). These approaches are
12 limited by the rapid masking of the coated surfaces by bacterial and organic
13 debris, and are associated with worrisome selection of resistance upon repeated
14 contact with treated surfaces (6).

15

16 Several studies have shown that non-antibiotic anti-adhesion strategies could
17 also efficiently interfere with bacterial biofilm formation (7-14). The design of bio-
18 inspired materials with anti-adhesion surface properties has been proposed to
19 constitute an effective solution to protect patient care equipment, control
20 pathogen colonization, and therefore impede key steps of infection, from initial
21 surface contact to subsequent bacteria-bacteria interactions (15-17). Non-
22 biocidal and bio-sourced strategies are also actively explored, including
23 approaches preventing and/or disrupting biofilms based on the use of quorum
24 sensing inhibitors that interfere with bacterial communications (18). Bacteria also
25 secrete biosurfactants altering material surface properties such as wettability and
26 charge (19, 20). These surface-active compounds reduce surface contacts and
27 contribute to bacterial motility or are involved in competitive interactions between
28 bacteria (12). Whereas many of these molecules correspond to small lipopeptides,
29 recent studies showed that high molecular weight capsular polysaccharides
30 released by various bacteria could prevent adhesion and subsequent cell
31 aggregation and formation of biofilm by a wide range of Gram+ and Gram-
32 bacteria. These include several nosocomial pathogens such as *Escherichia coli*,

1 *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*,
2 *Staphylococcus epidermidis* and *Enterococcus faecalis* (7, 9, 11, 21-24).

3

4 Unlike secreted bacterial antagonistic macromolecules such as colicins, toxins,
5 phages and a few toxic surface-active compounds (25, 26), these antibiofilm
6 polysaccharides are non-biocidal (11). They impair bacteria-biotic/abiotic surface
7 interactions mediated by adhesion factors such as pili, adhesins or extracellular
8 matrix polymers via the modification of the wettability, charge and overall
9 bacteria-surface contact properties (7, 12, 18). The use of these non-biocidal
10 antibiofilm macromolecules was proposed as a promising resistance-free
11 approach to reduce pathogenic biofilms and biofilm-associated bacterial
12 infections while avoiding side effects caused by broad spectrum biocides (11, 23,
13 27). However, the limited description of such antibiofilm macromolecules and
14 their associated chemical and structural activities severely hinders their
15 prophylactic use for bacterial biofilm control.

16

17 Here, we investigated the antibiofilm activity of a panel of 32 purified Gram+ or
18 Gram- bacterial capsular polysaccharides of known composition and structure.
19 Among those, we identified nine new non-biocidal polysaccharides inhibiting
20 biofilm formation by prototypical nosocomial pathogens, including *E. coli* and *S.*
21 *aureus*. This enabled us to perform a structure-function comparison of enough
22 active and inactive polysaccharides and to show that antibiofilm capsular
23 polysaccharides are characterized by a high intrinsic viscosity and a specific
24 electrokinetic signature. Our study therefore identified key chemical and
25 biophysical properties of bacterial antibiofilm polysaccharides, providing insights
26 paving the way for engineering of new and well-defined non-biocidal surface-
27 active macromolecules to control biofilm formation in medical and industrial
28 settings.

29

30

31

1 **Results**

2

3 **Size integrity is a key parameter of antibiofilm polysaccharide activity**

4 To investigate the potential relationships between composition, structure, size
5 and activity of non-biocidal bacterial antibiofilm polysaccharides, we first
6 determined the structure of Group 2 capsule (G2cps), a previously identified
7 hydrophilic and negatively charged antiadhesion polysaccharide active on both
8 Gram+ and Gram- bacteria, which is naturally produced and released, notably,
9 by uropathogenic *E. coli* strains (7). G2cps was purified by High-Performance
10 Anion-Exchange Chromatography – Pulsed Amperometric Detection (HPAEC-
11 PAD), High Performance Size-Exclusion Chromatography coupled to Static Light
12 Scattering (HPSEC-LS) and analyzed by ^1H and ^{13}C nuclear two dimensional ^1H -
13 ^{31}P experiment magnetic resonance (NMR) studies. These analyses showed that
14 G2cps polysaccharide consists of repeating units composed of O-acetylated and
15 glycerol phosphate residues with an average molecular weight of 800 kDa as
16 illustrated in Figure 1A. This structure is similar to that of *E. coli* polysaccharides
17 K2 and K62 (also named K2ab) (28). We then gradually reduced the size of
18 G2cps polysaccharide while preserving its structural integrity using radical
19 oxidation hydrolysis (Supporting Figure S1). The determination of antibiofilm
20 activity of full length and fragmented G2cps showed that even minor reduction of
21 polysaccharide size resulted in a loss of G2cps activity (Figure 1B), indicating
22 that the conservation of the size of the G2cps polymer is critical for its
23 antiadhesion properties.

24

25 **Screening a collection of bacterial capsular polysaccharides reveals new** 26 **non-biocidal antibiofilm compounds**

27

28 To further attempt to identify G2cps structural or composition features associated
29 with its activity, we screened a collection of 30 different high molecular-weight
30 bacterial polysaccharides to detect new macromolecules with G2cps-like
31 antibiofilm properties. Most of them are capsular polysaccharides of known origin,
32 composition and structure produced and purified from different strains of
33 *Streptococcus pneumoniae*, *Salmonella enterica* serovar Typhi, *Haemophilus*

1 *influenzae*, *Neisseria meningitidis*, and used as antigens in several
2 polysaccharide and glycoconjugate human vaccines (Supporting Table S1). We
3 showed that, at equal concentration (100µg/mL), Vi, MenA and MenC
4 polysaccharides were as active as G2cps in inhibiting *E. coli* biofilm formation but
5 were less active on *S. aureus* biofilms (Figure 2A, B). Moreover, whereas PRP
6 and PnPS3 were active on both bacteria, the activity of PnPS12F was restricted
7 to *E. coli* and that of PnPS18C to *S. aureus* (Figure 2A, B). The comparison of
8 the activity of Vi, PRP, PnPS3, MenA and MenC showed that Vi is the most active
9 polysaccharide and all 5 polysaccharides are non-biocidal (Supporting Figures
10 S2 and S3). Finally, we confirmed the activity of Vi, MenA, PRP and PnPS3 on a
11 panel of biofilm-forming Gram+ or Gram- bacteria, including a biofilm-forming *E.*
12 *coli* K-12 MG1655 carrying a F conjugative plasmid, *Enterobacter cloacae* 1092,
13 *K. pneumoniae* Kp21, *S. aureus* 15981 and *S. epidermidis* O47 (Supporting
14 [Figure S4](#)).

15

16 **Polysaccharide conformation and high intrinsic viscosity are predictive** 17 **indicators of antibiofilm activity.**

18 To identify the structural and biophysical properties that potentially correlate with
19 polysaccharide antibiofilm activity, we determined the molecular weight (*Mw*) and
20 the intrinsic viscosity ($[\eta]$) of active (Vi, MenA, MenC, PnPS3, PRP, G2cps) and
21 inactive (PnPS1, PnPS8, PnPS19A, PnPS9V, PnPS7F, PnPS22F and PnPS14)
22 polysaccharides by HPSEC (Table 1). Intrinsic viscosity $[\eta]$ of a given
23 polysaccharide reflects its contribution to the viscosity η of the whole solution, i.e.
24 $[\eta]=\frac{(\eta-\eta_o)}{(\eta_o \phi)}$, where η_o is the solution viscosity in the absence of
25 polysaccharide and ϕ stands for the volume fraction of polysaccharides in
26 solution. Accordingly, $[\eta]$ depends on the conformation adopted by the
27 polysaccharides in solution. This conformation being itself mediated by several
28 physicochemical parameters, including the electrostatic charges carried by the
29 polysaccharide and the charge distribution within the macromolecular body. The
30 combination of *Mw* and $[\eta]$ parameters are indicative of the volume (per mass
31 unit) occupied by the polysaccharides in solution. This analysis revealed a
32 remarkable correlation between intrinsic viscosity and broad-spectrum antibiofilm

1 activity. Indeed, all inactive macromolecules systematically display the lowest,
2 whereas active polysaccharides were characterized by a high (> 7 dl/g) intrinsic
3 viscosity (Supporting Figure S5). Moreover, molecular weight M_w and intrinsic
4 viscosity $[\eta]$ of polysaccharides with intermediate narrow-spectrum activity
5 (PnPS18C and PnPS12F) cover range of values measured for both broad-
6 spectrum active and non-active macromolecules. To determine whether high
7 intrinsic viscosity could be indicative of a potential antibiofilm activity, we
8 screened additional purified bacterial capsular polysaccharides of our collection
9 and we identified two such non-biocidal polysaccharides, MenY and MenW135,
10 presenting high intrinsic viscosity (Table 1 and Supporting Figures S3 and S5).
11 Although MenY and MenW135 differ in their primary composition from Vi, MenA,
12 MenC and G2cps, they both exhibited similar broad-spectrum antibiofilm activity
13 (Figure 3 and Supporting Figure S6). These results indicated that specific
14 polysaccharide conformation, reflected by a high intrinsic viscosity (29), is a
15 determinant of antibiofilm activity.

16

17 **Active antibiofilm polysaccharides are highly negatively charged** 18 **macromolecules**

19 To further identify the molecular bases of the biophysical properties displayed by
20 active polysaccharides, we first compared the composition of the broad-spectrum
21 polysaccharides (Vi, MenA, MenW, MenY, MenC, G2cps, PRP, PnPS3) but could
22 not identify a chemical feature common to all active molecules. Alternatively, we
23 tested whether an increased surface hydrophilicity, previously associated with
24 G2cps polysaccharide properties (7, 9), could correlate with the antibiofilm activity
25 of the newly identified active polysaccharides. However, the determination of the
26 surface contact angle of a drop of water on hydrophilic glass and hydrophobic
27 plastic surfaces treated with either distilled deionized water or active and inactive
28 polysaccharides did not reveal any correlation between surface
29 hydrophilicity/hydrophobicity balance and antibiofilm activity (Supporting Figure
30 S7). As an additional support of this finding, the characterization of
31 polysaccharide-coated surfaces by Atomic Force Microscopy (AFM) operated in
32 force spectroscopy mode using CH_3 -functionalized nanometric tips did not reveal

1 any correlation between antibiofilm activity, structural surface patterns of
2 adsorbed polysaccharides and surface hydrophobicity/hydrophilicity (Supporting
3 Figure S8). We also evaluated the macromolecular charge, defined by a charge
4 index i corresponding to the ratio between the number of acidic groups (carried
5 by uronic and neuraminic acid groups or by phosphate groups) and the number
6 of monosaccharide residues in a repeating unit (Table 1). The determination of
7 this charge index showed that all active polysaccharides (Vi, MenA, MenW135,
8 MenY, MenC, G2cps, PRP, PnPS3) correspond to highest i -values with $i = 0.5$ or
9 1. By contrast, macromolecules without anti-biofilm activity and with narrow-
10 spectrum activity could not be differentiated based on their respective i -values.
11 For instance, PnPS18C (narrow activity) for instance, shares similar value of i (=
12 0.2) with PnPS9V and PnPS22F (no activity). These results suggested that the
13 density of negative charges carried by the polysaccharides could be a key
14 determinant of the biophysical properties associated with broad-spectrum
15 antibiofilm activity.

16

17 **Active and inactive antibiofilm polysaccharides display distinct** 18 **electrokinetic patterns**

19 Previous reports documented the link between structure, surface charge
20 organization and electrokinetic properties of macromolecules (30-32) as derived
21 from electrophoresis. Bacterial polysaccharides are paradigms of ‘soft’
22 macromolecules, i.e. polyelectrolytic assemblies defined by a 3-dimensional
23 charge distribution, permeable to ions from the background electrolyte solution
24 and to the electroosmotic flow developed under electrophoresis measuring
25 conditions (30-32).

26 To determine whether the electrokinetic properties of polysaccharides (which
27 includes both charge density and flow permeability features) are linked or not to
28 their anti-biofilm activity, we performed blind measurements of the electrophoretic
29 mobility (μ) as a function of NaNO₃ electrolyte concentration in solution (1mM to
30 100mM range) for eight active, broad spectrum (Vi, MenA, MenW135, G2cps,
31 PRP, PnPS3) or narrow spectrum (PnPS18C and PnPS12F) polysaccharides.
32 The electrophoretic mobility of these active polysaccharides was then compared

1 to those of seven inactive polysaccharides (PnPS1, PnPS9V, PnPS8, PnPS19A,
2 PnPS7F, PnPS22F and PnPS14) (Figure 4). All tested polysaccharides displayed
3 the characteristic electrophoretic signature expected for soft macromolecules,
4 with an electrophoretic mobility tending to a constant, non-zero mobility plateau
5 value (noted below as μ^*) at sufficiently large salt concentrations (>30 mM). This
6 property is the direct consequence of the penetration of the electroosmotic flow
7 within the charged polysaccharide globular structure (33). Further qualitative
8 inspection of the sets of electrokinetic data collected for the polysaccharides of
9 interest revealed two main electrokinetic patterns. The first one corresponded to
10 the 7 tested inactive macromolecules whose electrophoretic mobility
11 systematically tends to value of μ^* satisfying $0.5 < |\mu^*| < 1.5 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. For
12 these macromolecules, the absolute value of the electrophoretic mobility
13 decreases with increasing electrolyte concentration as a result of screening of the
14 polysaccharide charges by the electrolyte ions. This feature is also shared by the
15 active macromolecules (PnPS3, PRP and G2cps) with the noticeable difference
16 that their asymptotic mobility value $|\mu^*|$ is significantly larger with μ^* now
17 satisfying the inequality $|\mu^*| > 2 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ (Figure 4A). The second observed
18 electrokinetic pattern applies to macromolecules with narrow (PnPS18C and
19 PnPS12F) or broad-spectrum (Vi, MenA, MenW135) activities and for which the
20 electrophoretic mobility μ poorly depends on background electrolyte
21 concentration. Strikingly, active macromolecules with narrow spectrum antibiofilm
22 activity are defined by electrophoretic mobilities ($|\mu^*| < 0.5 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$) that
23 are much lower in magnitude compared to those measured for broad-spectrum
24 antibiofilm polysaccharides (MenA, MenW135 and Vi, $|\mu^*| > 2 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$)
25 (Figure 4B). These results demonstrated clearly that active capsular
26 polysaccharides are characterized by a specific electrokinetic signature.

27

28 **The electrokinetic signature of antibiofilm polysaccharides is associated to** 29 **their high flow permeability and high density of electrostatic charges**

30 To further explore the electrokinetic properties of active capsular polysaccharides
31 we interpreted quantitatively the dependence of the electrophoretic mobility (μ) of
32 the tested macromolecules on background electrolyte concentration with soft

1 surface electrokinetic theory. This theory was developed by Ohshima for the
2 electrophoresis of soft colloids (33) in the Hermans-Fujita's limit that is applicable
3 to soft polyelectrolytes (34) (eq 1 in Materials and Method section). To that end,
4 the required hydrodynamic radii of the macromolecules of interest were
5 determined by Dynamic Light Scattering (DLS) after conversion of the measured
6 diffusion coefficients by means of Stokes-Einstein equation (See Methods and
7 Supporting Table S2). This quantitative analysis highlighted an excellent
8 reconstruction of the electrophoresis data measured for all tested
9 macromolecules tested (Figure 4) and, most importantly, a remarkable
10 classification of their activity (Figure 5) according to their electrostatic and flow
11 permeability properties.

12 These properties are expressed by two key parameters retrieved from theoretical
13 fitting of electrophoretic data to eq 1, namely: ρ_o , which is the net density of
14 negative charges carried by the macromolecule, and λ_o the so-called softness
15 parameter. $1/\lambda_o$ (also called the Brinkman length) corresponds to the extent of
16 penetration of the electroosmotic flow within the macromolecule. $1/\lambda_o$ is intimately
17 correlated to the polysaccharide structural compacity and by the degree of
18 entanglement of its constituting chains. All macromolecules with narrow spectrum
19 activity (PnPS18C and PnPS12F) are in the lower left zone within the diagram
20 reporting the determined value of ρ_o as a function of the corresponding $1/\lambda_o$
21 (Figure 5). By contrast, polysaccharides with a broad-spectrum activity (e.g. Vi,
22 MenA, MenW135, PRP, PnPS3, G2cps) combine high charge density and high
23 Brinkman length scale, and they are thus found at the upper right region in the
24 ρ_o - $1/\lambda_o$ representation (Figure 5). All tested inactive macromolecules (PnPS1,
25 PnPS9V, PnPS8, PnPS19A, PnPS7F, PnPS22F and PnPS14) are positioned in
26 a region intermediate between those of the broad-spectrum active and narrow-
27 spectrum active macromolecules. Taken together, our results indicate that
28 despite the lack of specific molecular motif associated with antibiofilm properties,
29 the combination of a loose structure (i.e. a large permeability to flow due to a
30 large amount of intraparticulate voids) and a high density of carried electrostatic
31 charges is critical for polysaccharides to exhibit antibiofilm activity.

1 DISCUSSION

2
3 The inhibition of bacterial adhesion using surface-active compounds is
4 considered a promising approach to prevent the key initial steps of bacterial
5 biofilm formation. In this study, we identified nine new non-biocidal antibiofilm
6 macromolecules among a collection of 30 different high molecular-weight
7 bacterial capsular polysaccharides of known structure and composition. This
8 allowed us to compare their chemical, structural and electrokinetic properties to
9 identify key molecular and biophysical determinants discriminating active
10 polysaccharides from inactive ones.

11 The identified active polysaccharides are composed of a high number of
12 repeating oligosaccharides with different composition and structure. This
13 suggests that, beyond the specific composition of the active polysaccharide, the
14 determinant of their antibiofilm activity could lie in higher order molecular features.
15 Consistently, we showed that, in the case of G2cps, the size integrity of the
16 polysaccharide is critical to maintain antibiofilm activity. We also demonstrated
17 that a high intrinsic viscosity is a property shared by broad spectrum active
18 macromolecules, which displayed the highest charge index i among all tested
19 polysaccharides tested. The hydrodynamic radii measured for all tested
20 macromolecules was shown to cover a relatively narrow range, between ca. 15
21 and 40 nm in hydrodynamic diameter (Supporting Table S2). Such small
22 variations in particle size cannot account for the well differentiated range of
23 intrinsic viscosity measured for non-active and active macromolecules (1 to 6 and
24 7 to 12 dl/g, respectively, see Supporting Figure S5), especially since both active
25 and non-active polysaccharides display identical molecular weight ranges
26 (Supporting Figure S5). In contrast, the high intrinsic viscosities measured for
27 active macromolecules correlate with their charge index i , indicative of a high
28 charge density. This correlation could be due to the fact that viscosimetric
29 properties of particle dispersions depend on their electrostatic characteristics that
30 govern the extent of so-called primary and secondary electroviscous effects (35,
31 36). These effects are a direct consequence of the presence of charged electric
32 double layers (EDL) at the macromolecule/solution interface and of ensuing
33 particle-particle and particle-fluid electrohydrodynamic interactions: the larger the

1 density of particle charges, the more significant electro-viscous effects become
2 (38,39). As a consequence, the viscosity of a solution containing macromolecules
3 of similar size can significantly differ according to the density of their carried
4 electrostatic charges (35, 36). A large flow penetration within the particles body
5 (as revealed by a high Brinkman length, $1/\lambda_o$, see Figure 5) is consistent with the
6 existence of a relatively loose structure adopted by the macromolecules and a
7 reduced frictional force they exert on electro-osmotic flow during electrophoresis,
8 with a resulting significant electrophoretic mobility. Altogether, the identified
9 properties of loose macromolecular structure combined with a high charge
10 density and a related high intrinsic viscosity correlate with antibiofilm activity.

11

12 Analysis of the electrokinetic data revealed very distinct electrokinetic patterns
13 associated with broad and narrow spectrum of antibiofilm activities, whereas
14 inactive macromolecules exhibited an intermediate electrokinetic behavior
15 (Figure 5). This suggests that the high and low magnitudes of the polysaccharide
16 charges could determine their broad and narrow activity, respectively, since a
17 charge density with magnitude lying in between these two extremes led to a loss
18 of activity. However, we previously showed that, despite its high negative charge,
19 G2cps displayed low affinity for cationic dyes (7), suggesting that its interaction
20 with surrounding biotic or abiotic environment is not only driven by
21 electrostatics ,but may also include remodeling of its surface properties. This
22 could possibly include changes in surface hydration or steric repulsion, and
23 subsequent limitation of bacterial adhesion (7). This indicates that consideration
24 of polysaccharide electrostatic properties alone is not enough to account for their
25 antibiofilm activity. Consistently, analysis of our electrokinetic data sets suggests
26 indeed that the very organization of the polymer chains and the resulting flow
27 permeability properties of the polysaccharidic macromolecules (as qualitatively
28 indicated by the magnitude of their Brinkman length) play an important role in
29 defining their antibiofilm activity. Considering that only charges located in the
30 peripheral region of the macromolecules are probed by electrophoresis (35), the
31 location of these charges and their degree of exposition to the outer solution are
32 likely important factors underlying antibiofilm activity.

1 Then, how does polysaccharide molecular composition correlate with antibiofilm
2 activity? Most active macromolecules display a high density of negative charges
3 that could contribute to the electrostatic repulsion of negatively charged bacteria.
4 However, bacterial surface structures such as pili, fimbriae, lipopolysaccharides
5 or even poly-cationic exopolysaccharides are known to overcome these repulsive
6 forces and promote bacterial adhesion and aggregation (37-39). We therefore
7 hypothesize that the directionality of the interactions on surfaces (40) could be a
8 critical determinant of broad spectrum antibiofilm polysaccharide activity. The
9 proper exposition of electrostatic charges could optimize the steric repulsion
10 effect in the antibiofilm macromolecules identified in this study. In addition, their
11 high intrinsic viscosity and relative loose structure could mechanically modify the
12 local conditions of adhesion and alter the perception of the surface by bacteria,
13 thereby minimizing their adhesion in the presence of the identified
14 polysaccharides (41). We therefore propose that the active antibiofilm
15 polysaccharides identified in our study could have multi-pronged activity, with
16 both short and long-range modifications of the surface-bacteria or bacteria-
17 bacteria interactions, in particular via an alteration of the local adhesion
18 conditions prevailing on the adhesion surface. The distinction between broad,
19 narrow or lack of activity would therefore depend on the specific combination of
20 biophysico-chemical properties displayed by each macromolecule.

21
22 By providing a better definition of the chemical and structural basis of the broad-
23 spectrum antibiofilm activity displayed by capsular polysaccharides, our study
24 offers the possibility to identify new surface-active polysaccharides on the basis
25 of their biophysical properties but also to design and engineer macromolecules
26 mimicking antibiofilm polysaccharide activity through total or partial synthesis.
27 These molecules could be used as non-biocidal biofilm control strategies in
28 prophylactic treatment against the initial adhesion of biofilm-forming pathogens
29 developing on medical and industrial materials.

30

1 MATERIALS AND METHODS

2

3 **Bacterial strains and growth conditions.** Bacterial strains used in this study
4 are listed in Supporting Table S3. Gram- bacteria were grown in 0.4% glucose-
5 M63B1 minimal medium (M63B1) or in lysogeny broth (LB) medium at 37°C, with
6 appropriate antibiotics when required. Gram+ strains were cultured in tryptic soy
7 broth (TSB) supplemented with 0.25% or 0.5% glucose (*E. cloacae*). Potential
8 biocidal effect of antibiofilm polysaccharides was evaluated from growth curve
9 determination in the presence of 100 µg/ml of purified polysaccharide.

10

11 **Bacterial polysaccharides.** *S. pneumoniae*, *N. meningitidis*, *S. Typhi* and *H.*
12 *influenzae* capsular polysaccharides used in this study were obtained from Sanofi,
13 Marcy l'Etoile France and Swiftwater, USA. *E. coli* polysaccharides were
14 produced at the Institut Pasteur, Paris. Teichoic acid polysaccharide (cell wall PS,
15 CWPS) from *Streptococcus pneumoniae* strain CSR SCS2 was from Statens
16 Serum Institut (Ref 3459).

17

18 **Biofilm inhibition tests.** Overnight cultures were adjusted to an OD_{600nm} of 0.05
19 before inoculating 50 µl into 96-well polyvinyl chloride (PVC) plates (Falcon;
20 Becton Dickinson Labware, Oxnard, CA) and added at a 1:1 ratio to 50 µl of filter-
21 sterilized and purified polysaccharide at the desired concentration: i) 100 µg/ml
22 for standardized antibiofilm activity experiments; ii) 3.125-100 µg/ml for test level
23 of activity of the macromolecules. Biofilms were left to grow for 16 h at 37°C
24 before quantification as described in (7).

25

26 **Polysaccharide purification.** G2cps polysaccharide was obtained from 24h
27 cultures as described in (7). Briefly, CFT073 *E. coli* strains were grown in M63B1
28 0.4%glucose for 24h at 37°C. After cold centrifugation at 7500 rpm for 10 minutes,
29 supernatants were filtered through a 0.22µm filter. Polysaccharides were
30 precipitated from cell-free supernatant using ice-cold ethanol (3:1 ratio ethanol /
31 supernatant) followed by centrifugation for 2h at 10000 rpm and 4°C. Precipitated
32 polysaccharides were resuspended and dialyzed against deionized water (10

1 kDa cassettes; Pierce, Rockford, IL). Total polysaccharide concentrations were
2 measured by Duvois colorimetric assay (42). The polysaccharides were finally
3 separated from residual lipopolysaccharides by gel filtration on Sepharose 6BCL
4 column (GE Healthcare Life Sciences) in 1% sodium deoxycholate (43) and
5 dualized against deionized water. Polysaccharide concentration was determined
6 using High-Performance Anion-Exchange Chromatography with Pulsed
7 Amperometry Detection (HPAEC-PAD) (Thermo Fischer Scientific, Dionex,
8 Sunnyval, CA) as previously described(44).

9

10

11 **Polysaccharide structure analysis by High Performance Anion Exchange** 12 **Chromatography with Pulsed Amperometric Detection (HPAEC-PAD)**

13 Polysaccharide G2cps was hydrolyzed with hydrofluoric acid (HF) (48% by mass)
14 for 16h at room temperature. HF was removed by drying under a stream of
15 nitrogen at 40°C. The sample was redissolved in water and then hydrolyzed with
16 2M trifluoroacetic acid (TFA) for 2h at 121°C. TFA was removed by drying under
17 a stream of nitrogen at 40°C. The sample was redissolved in water prior to
18 analysis. For the sake of comparison, both HF-alone and TFA-alone hydrolysis
19 were also performed on polysaccharide G2cps. Pulsed amperometric detection
20 was used incorporating a quadruple- potential waveform. Data were collected and
21 analyzed on computers equipped with Dionex Chromeleon software (Dionex).
22 Commercial monosaccharides were used as standards.

23

24 **Nuclear Magnetic Resonance (NMR) spectroscopy**

25 Approximately 5 mg of polysaccharide was lyophilized once, dissolved in 700 μ l
26 of deuterated water and 550 μ l were introduced in a 5mm tube. NMR spectra
27 were collected on a Bruker Avance 500MHz spectrometer running Topspin 2.1
28 software at an indicated probe temperature of 20°C. Spectra were measured for
29 solutions in D₂O with 0.01% DSS as an internal standard for proton (¹H) NMR (δ
30 0.00 ppm). Phosphoric acid (2%) was used as an external standard for
31 phosphorus (³¹P) NMR (δ 0.00 ppm) and TSP as an external standard for carbone
32 (¹³C) NMR (δ 0.00 ppm). The pulse programs used were those in the Bruker

1 library except for the correlation spectroscopy (COSY) and total correlation
2 spectroscopy (TOCSY) diffusion experiments. The diffusion delay was 80 ms for
3 these two-dimensional experiments and the mixing time for the TOCSY was 100
4 ms.

5 The two dimensional ^1H - ^{31}P experiment was acquired using a $J_{\text{H-P}}$ coupling value
6 of 7Hz. The ^1H - ^{13}C heteronuclear single quantum coherence (HSQC) spectra
7 were acquired using a $^nJ_{\text{H-C}}$ coupling value of 145Hz and a long range $^nJ_{\text{H-C}}$
8 coupling value of 5Hz for heteronuclear multiple bond correlation (HMBC).

9

10 **High Performance Size Exclusion Chromatography methods with triple** 11 **detection: M_w and $[\eta]$ determinations**

12 Polysaccharides were analyzed at a concentration between 600 to 1000 $\mu\text{g/ml}$ in
13 water. Analyses were performed using a TDA301 Viscotek HPSEC system
14 consisting of an automated sampler with a HPLC pump with an injection loop of
15 100 μl , an on-line degasser, a viscometer, a refractive index (RI), and a right-
16 angle laser light scattering detector (RALLS). The HPSEC analyses of
17 polysaccharide were performed using TSK G4000 PWXL (0.7 \times 30 cm) and TSK
18 G6000 PWXL analytical columns (0.7 \times 30 cm) connected in series at a flow rate
19 of 0.5 ml/min, at 30°C. Tris buffer 2.5mM, pH 7.5 or phosphate buffer 200mM pH
20 6.9 was used as mobile phase. The columns were kept at a constant temperature
21 of 30°C. Omniseq software (Malvern) was used for data collection and data
22 processing. This triple detection consisting of online RI, RALLS and viscometric
23 detector was used to determine the molecular weight (M_w) and the intrinsic
24 viscosity ($[\eta]$) of polysaccharides.

25

26 **Size reduction of G2cps polysaccharide**

27 75 μg of polysaccharide were hydrolyzed in solution with ascorbic acid, copper
28 and iron sulfate for 2 h at 30°C. Hydrolysis kinetics was followed by HPSEC
29 analysis for 15 min, 1 h and 24 h.

30

1 **Contact angle measurements.** Glass or polyester plastic microscopy coverslip
2 slides were treated with 80 μ l of distilled deionized water or with 100 μ g/ml
3 solution of purified active and inactive polysaccharides. Slides were dried under
4 a laminar flow hood and 2.5 μ l drops of water were deposited using a Kruss DSA4
5 Drop Shape Analyzer on the surface of coated and uncoated slides, and contact
6 angle was then measured 3 times.

7

8 **Atomic Force Microscopy measurements.**

9 Microscopy glass slides were treated with 100 μ g/ml solution of purified active
10 and inactive polysaccharides for 10 minutes and rinsed with ultrapure water. AFM
11 measurements were performed in ultrapure water, using a Fastscan Dimension
12 Icon with Nanoscope V controller (Bruker). Images were obtained in Peak Force
13 tapping mode, using gold-coated silicon nitride tips (NPG, Bruker), with a
14 maximum applied force of 500 pN, a scan rate of 1 Hz, a peak force amplitude of
15 300 nm and a peak fore frequency of 2 kHz. For quantifying coated-surface
16 hydrophobicity by means of chemical force microscopy, hydrophobic tips were
17 prepared by immersing gold-coated silicon nitride tips (NPG, Bruker) for 12h in 1
18 mM solution of dodecanethiol (Sigma) in ethanol, rinsed with ethanol and dried
19 with N₂. Spatial mappings were obtained by recording 32 \times 32 approach-retract
20 force-distance curves on 5 μ m \times 5 μ m areas, with a maximum applied force of
21 500 pN and an approach and retraction speed of 500 nm/s.

22

23 **Electrokinetic measurements.**

24 1 g/L stock suspensions of macromolecules were first prepared in ultrapure water
25 from corresponding frozen powders and, after 48 h, suspensions were diluted at
26 250 mg/L and adjusted to pH 5. For each macromolecule tested, batches were
27 then prepared in order to obtain a series of suspensions with NaNO₃ background
28 electrolyte concentration in the range 1 to 100 mM. Final macromolecule
29 concentration and pH conditions were adopted to optimize the measured
30 electrophoretic response after testing a range of particle concentrations from 50
31 to 250 mg/L and four pH conditions (3, 5, 7 and 9). All dispersions, stock and

1 diluted suspensions were stored at 4°C and were re-acclimatized at room
2 temperature prior to measurements.
3 24 to 48h after batches preparation, diffusion coefficients and electrophoretic
4 mobilities of the bacterial polysaccharides were measured by Dynamic Light
5 Scattering (DLS, Supporting Table S2) and Phase Analysis Light Scattering
6 (Malvern Instruments). Each reported data point in Figure 4 corresponds to three
7 measurements performed on 3 different aliquots of a given batch macromolecule
8 dispersion.

9

10 **Quantitative assessment of polysaccharide electrophoretic mobility.**

11 Electrophoretic mobility of the various macromolecules of interest were measured
12 as a function of NaNO₃ electrolyte concentration (Figure 4) and were collated with
13 the analytical theory developed by Ohshima for electrophoresis of soft colloids
14 (33) in the Hermans-Fujita limit applicable to soft polyelectrolytes (34). According
15 to the latter, μ is defined by the expression

$$\mu = \frac{\rho_o}{\eta\lambda_o^2} \left\{ 1 + \frac{1}{3} \left(\frac{\lambda_o}{\kappa} \right)^2 \left[1 + e^{-2\kappa b} - \frac{1 - e^{-2\kappa b}}{\kappa b} \right] \right. \\ \left. + \frac{1}{3} \left(\frac{\lambda_o}{\kappa} \right)^2 \frac{1 + 1/\kappa b}{(\lambda_o/\kappa)^2 - 1} \left[\left(\frac{\lambda_o}{\kappa} \right) \frac{1 + e^{-2\kappa b} - (1 - e^{-2\kappa b})/\kappa b}{(1 + e^{-2\lambda_o b})/(1 - e^{-2\lambda_o b}) - 1/\lambda_o b} \right] - (1 - e^{-2\kappa b}) \right\} \quad (1)$$

17 , where ρ_o represents the density of negative charges carried by the
18 macromolecule, κ is the reciprocal Debye layer thickness defined by

$$19 \quad \kappa = \left[2F^2 c_o / (\epsilon RT) \right]^{1/2}$$

with R the gas constant, T the absolute temperature, F the

20 Faraday number and c_o the bulk concentration of a 1:1 electrolyte (NaNO₃ in this
21 work), λ_o is the so-called softness parameter with $1/\lambda_o$ corresponding to the
22 characteristic penetration length of the electroosmotic flow within the
23 macromolecule. The quantity b in eq 1 is the radius of the polyelectrolyte
24 macromolecule. The set of $(\rho_o, 1/\lambda_o)$ couples obtained for all polysaccharidic
25 macromolecules are reported in Supporting Table S2 and the required values of

1 *b* for data fitting to eq 1 were determined by Dynamic Light Scattering and are
2 also given in Supporting Table S2.

3

4 **Statistical analysis.**

5 Two-tailed unpaired *t*-test with Welch correction analyses were performed using
6 Prism 9.0 for Mac OS X (GraphPad Software). Each experiment was performed
7 at least three times. All data are expressed as mean (\pm standard deviation, SD)
8 in figures. Differences were considered statistically significant for *P* values of
9 <0.05 ; * $p<0.05$; ** $p<0.01$; *** $p<0.001$. **** $p<0.0001$.

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11

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26

27

1 REFERENCES

- 2
- 3 1. L. Hall-Stoodley, J. W. Costerton, P. Stoodley, Bacterial biofilms: from the natural
- 4 environment to infectious diseases. *Nat Rev Microbiol* **2**, 95-108 (2004).
- 5 2. H. C. Flemming, S. Wuertz, Bacteria and archaea on Earth and their abundance in
- 6 biofilms. *Nat Rev Microbiol* **17**, 247-260 (2019).
- 7 3. P. S. Stewart, J. W. Costerton, Antibiotic resistance of bacteria in biofilms. *Lancet* **358**,
- 8 135-138 (2001).
- 9 4. D. Lebeaux, J. M. Ghigo, C. Beloin, Biofilm-related infections: bridging the gap
- 10 between clinical management and fundamental aspects of recalcitrance toward
- 11 antibiotics. *Microbiology and molecular biology reviews : MMBR* **78**, 510-543 (2014).
- 12 5. K. Lewis, A. M. Klibanov, Surpassing nature: rational design of sterile-surface
- 13 materials. *Trends Biotechnol* **23**, 343-348 (2005).
- 14 6. P. N. Danese, Antibiofilm approaches: prevention of catheter colonization. *Chem Biol*
- 15 **9**, 873-880 (2002).
- 16 7. J. Valle *et al.*, Broad-spectrum biofilm inhibition by a secreted bacterial polysaccharide.
- 17 *Proc Natl Acad Sci U S A* **103**, 12558-12563 (2006).
- 18 8. A. K. Mukherjee, K. Das, Microbial surfactants and their potential applications: an
- 19 overview. *Adv Exp Med Biol* **672**, 54-64 (2010).
- 20 9. O. Rendueles *et al.*, Screening of *Escherichia coli* species biodiversity reveals new
- 21 biofilm-associated antiadhesion polysaccharides. *MBio* **2**, e00043-00011 (2011).
- 22 10. D. Campoccia, L. Montanaro, C. R. Arciola, A review of the biomaterials technologies
- 23 for infection-resistant surfaces. *Biomaterials* **34**, 8533-8554 (2013).
- 24 11. O. Rendueles, J. B. Kaplan, J. M. Ghigo, Antibiofilm polysaccharides. *Environ*
- 25 *Microbiol* **15**, 334-346 (2013).
- 26 12. O. Rendueles, J. M. Ghigo, Mechanisms of Competition in Biofilm Communities.
- 27 *Microbiol Spectr* **3** (2015).
- 28 13. A. Asadi, S. Razavi, M. Talebi, M. Gholami, A review on anti-adhesion therapies of
- 29 bacterial diseases. *Infection* **47**, 13-23 (2019).
- 30 14. M. Sarshar *et al.*, FimH and Anti-Adhesive Therapeutics: A Disarming Strategy Against
- 31 Uropathogens. *Antibiotics (Basel)* **9** (2020).
- 32 15. B. Schachter, Slimy business--the biotechnology of biofilms. *Nat Biotechnol* **21**, 361-
- 33 365 (2003).
- 34 16. A. L. Hook *et al.*, Combinatorial discovery of polymers resistant to bacterial
- 35 attachment. *Nat Biotechnol* **30**, 868-875 (2012).
- 36 17. C. Howell, A. Grinthal, S. Sunny, M. Aizenberg, J. Aizenberg, Designing Liquid-
- 37 Infused Surfaces for Medical Applications: A Review. *Adv Mater* **30**, e1802724 (2018).
- 38 18. O. Rendueles, J. M. Ghigo, Multi-species biofilms: how to avoid unfriendly neighbors.
- 39 *FEMS Microbiol Rev* 10.1111/j.1574-6976.2012.00328.x (2012).
- 40 19. T. R. Neu, Significance of bacterial surface-active compounds in interaction of bacteria
- 41 with interfaces. *Microbiol Rev* **60**, 151-166 (1996).
- 42 20. I. M. Banat *et al.*, Microbial biosurfactants production, applications and future potential.
- 43 *Appl Microbiol Biotechnol* **87**, 427-444 (2010).
- 44 21. M. Chmit *et al.*, Antibacterial and antibiofilm activities of polysaccharides, essential oil,
- 45 and fatty oil extracted from *Laurus nobilis* growing in Lebanon. *Asian Pac J Trop Med*
- 46 **7s1**, S546-552 (2014).
- 47 22. A. Chauhan *et al.*, Preventing biofilm formation and associated occlusion by
- 48 biomimetic glycocalyxlike polymer in central venous catheters. *J Infect Dis* **210**, 1347-
- 49 1356 (2014).
- 50 23. G. A. Junter, P. Thébault, L. Lebrun, Polysaccharide-based antibiofilm surfaces. *Acta*
- 51 *Biomater* **30**, 13-25 (2016).

- 1 24. B. Costa *et al.*, Broad-Spectrum Anti-Adhesive Coating Based on an Extracellular
2 Polymer from a Marine Cyanobacterium. *Mar Drugs* **17** (2019).
- 3 25. R. M. Donlan, Preventing biofilms of clinically relevant organisms using bacteriophage.
4 *Trends Microbiol* **17**, 66-72 (2009).
- 5 26. K. Tait, I. W. Sutherland, Antagonistic interactions amongst bacteriocin-producing
6 enteric bacteria in dual species biofilms. *J Appl Microbiol* **93**, 345-352 (2002).
- 7 27. L. Travier, O. Rendueles, L. Ferrières, J. M. Herry, J. M. Ghigo, Escherichia coli
8 resistance to nonbiocidal antibiofilm polysaccharides is rare and mediated by multiple
9 mutations leading to surface physicochemical modifications. *Antimicrob Agents*
10 *Chemother* **57**, 3960-3968 (2013).
- 11 28. K. Jann, B. Jann, M. A. Schmidt, W. F. Vann, Structure of the Escherichia coli K2
12 capsular antigen, a teichoic acid-like polymer. *J Bacteriol* **143**, 1108-1115 (1980).
- 13 29. J. T. Yang, The viscosity of macromolecules in relation to molecular conformation. *Adv*
14 *Protein Chem* **16**, 323-400 (1961).
- 15 30. A. Clements *et al.*, The major surface-associated saccharides of Klebsiella pneumoniae
16 contribute to host cell association. *PLoS One* **3**, e3817 (2008).
- 17 31. J. F. Duval, V. I. Slaveykova, M. Hosse, J. Buffle, K. J. Wilkinson,
18 Electrohydrodynamic properties of succinoglycan as probed by fluorescence correlation
19 spectroscopy, potentiometric titration and capillary electrophoresis. *Biomacromolecules*
20 **7**, 2818-2826 (2006).
- 21 32. J. F. L. Duval, F. Gaboriaud, Progress in electrohydrodynamics of soft microbial
22 particle interphases. *Current Opinion in Colloid & Interface Science* **15**, 184-195
23 (2010).
- 24 33. H. Ohshima, Electrophoresis of soft particles. *Advances in Colloid and Interface*
25 *Science* doi.org/10.1016/0001-8686(95)00279-Y, 189-235 (1995).
- 26 34. J. J. Hermans, H. Fujita, *Koninkl. Ned. Akad. Wetenschap. Proc.* **B** 182 (1955).
- 27 35. F. S. Chan, J. Blachford, D. A. I. Goring, The secondary electroviscous effect in a
28 charged spherical colloid. **22**, 378-385 (1966).
- 29 36. L. Zurita, F. Carrique, A. V. Delgado, The primary electroviscous effect in silica
30 suspensions. Ionic strength and pH effects. *Colloids Surf., A* **92**, 23-28 (1994).
- 31 37. C. Berne, A. Ducret, G. G. Hardy, Y. V. Brun, Adhesins Involved in Attachment to
32 Abiotic Surfaces by Gram-Negative Bacteria. *Microbiol Spectr* **3** (2015).
- 33 38. M. A. Daeschel, J. McGuire, Interrelationships between protein surface adsorption and
34 bacterial adhesion. *Biotechnol Genet Eng Rev* **15**, 413-438 (1998).
- 35 39. S. L. Walker, J. A. Redman, M. Elimelech, Role of Cell Surface Lipopolysaccharides in
36 Escherichia coli K12 adhesion and transport. *Langmuir* **20**, 7736-7746 (2004).
- 37 40. A. Beaussart *et al.*, Supported lysozyme for improved antimicrobial surface protection.
38 *J Colloid Interface Sci* **582**, 764-772 (2021).
- 39 41. V. D. Gordon, L. Wang, Bacterial mechanosensing: the force will be with you, always.
40 *J Cell Sci* **132** (2019).
- 41 42. M. Dubois, K. Gilles, J. K. Hamilton, P. A. Rebers, F. Smith, A colorimetric method for
42 the determination of sugars. *Nature* **168**, 167 (1951).
- 43 43. X. X. Gu, C. M. Tsai, Purification of rough-type lipopolysaccharides of Neisseria
44 meningitidis from cells and outer membrane vesicles in spent media. *Anal Biochem* **196**,
45 311-318 (1991).
- 46 44. P. Talaga, M. Moreau, Quantification of C-polysaccharide in Streptococcus
47 pneumoniae polysaccharides by high-performance anion-exchange chromatography
48 with pulsed amperometric detection. *Dev Biol (Basel)* **103**, 27-34 (2000).
- 49

TABLES

Table 1: Relevant origin, and physicochemical properties of the polysaccharides used in this work. Indicated values correspond to mean values.

Bacterial origin	Name	Mw' (kDa)	Intrinsic viscosity ¹ [η] (dl/g)	Charge index <i>i</i> *
<i>Escherichia coli</i> CFT073	G2cps	780	5-10	0.5
<i>Salmonella</i> Typhi	Vi	280	10	1
<i>Haemophilus influenzae</i> serotype b	PRP	550	10	0.5
<i>Neisseria meningitidis</i> serogroup A	MenA	200	10	1
<i>Neisseria meningitidis</i> serogroup C	MenC	200	10	1
<i>Neisseria meningitidis</i> serogroup Y	MenY	600	10	0.5
<i>Neisseria meningitidis</i> serogroup W135	MenW135	500	12	0.5
<i>Streptococcus pneumoniae</i> serotype 12F	PnPS12F	1000	10	0.16
<i>Streptococcus pneumoniae</i> serotype 18C	PnPS18C	300	3	0.2
<i>Streptococcus pneumoniae</i> serotype 1	PnPS1	850	4	0.33
<i>Streptococcus pneumoniae</i> serotype 3	PnPS3	700	7	0.5
<i>Streptococcus pneumoniae</i> serotype 9V	PnPS9V,	500	2	0.2
<i>Streptococcus pneumoniae</i> serotype 8	PnPS8	300	6	0.25
<i>Streptococcus pneumoniae</i> serotype 19A	PnPS19A	250	1	0.33
<i>Streptococcus pneumoniae</i> serotype 7F	PnPS7F	1000	1	0
<i>Streptococcus pneumoniae</i> serotype 22F	PnPS22F	800	2	0.2
<i>Streptococcus pneumoniae</i> serotype 14	PnPS14	500	1	0

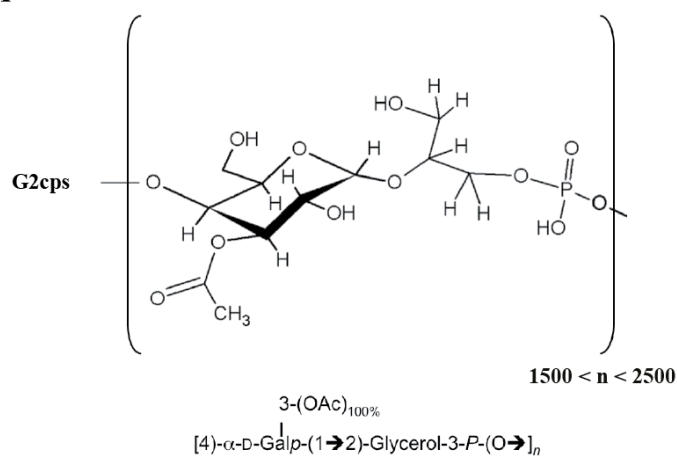
¹: The relative standard deviation of Mw and [η] determination is < 10%.

In red: active broad spectrum antibiofilm polysaccharides. In blue: active narrow spectrum antibiofilm polysaccharides. * The charge index *i* corresponds to the ratio of the number of acidic group to the number of monosaccharide residue in a repeating unit.

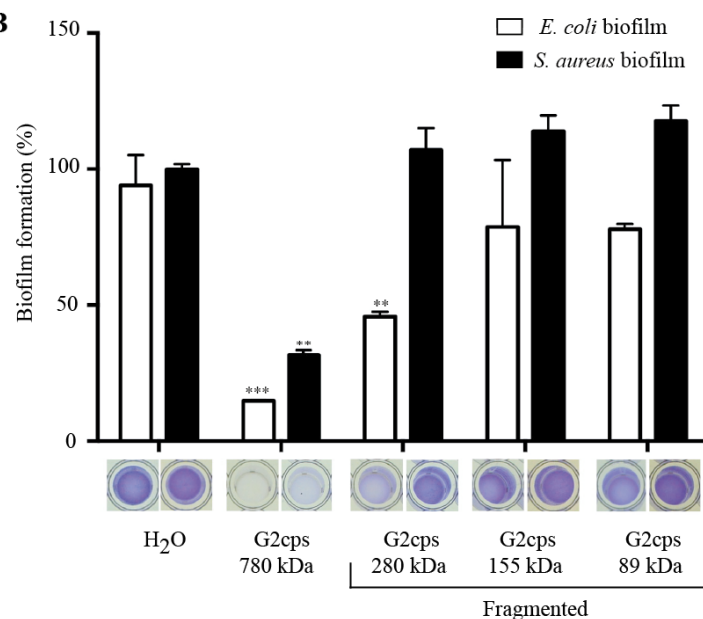
1 **FIGURES**

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A



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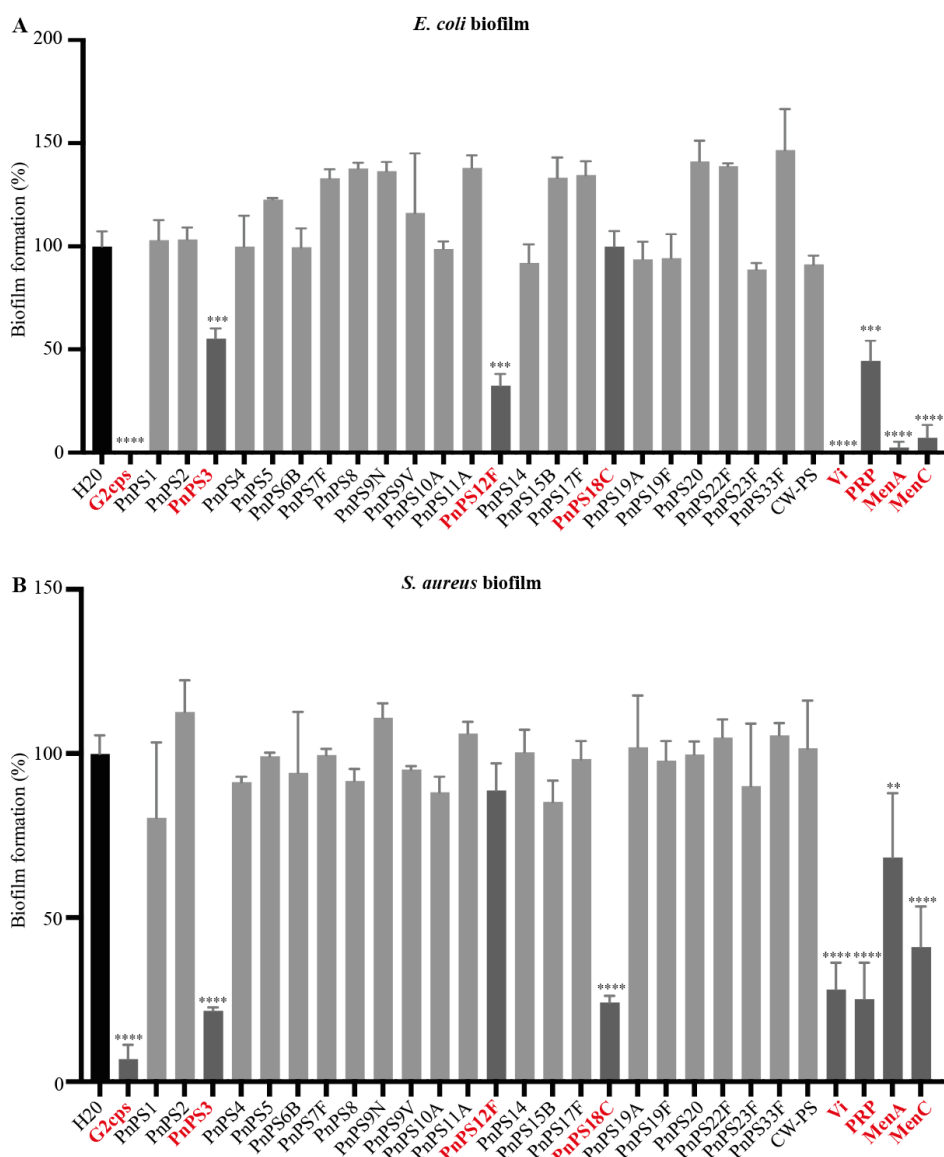
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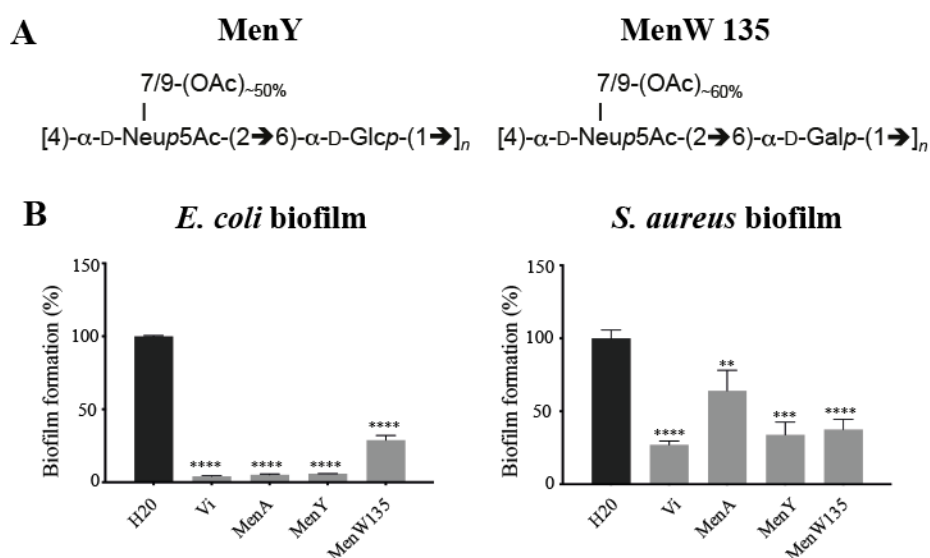
5 **Figure 1. Structure of the G2cps polysaccharide and its antibiofilm activity**
 6 **as a function of the extent of its fragmentation. A.** Structure and composition
 7 of G2cps polysaccharide. **B.** Biofilm inhibition activity against *E. coli* and *S.*
 8 *aureus* biofilm of native and fragmented G2cps polysaccharide. Biofilm assays
 9 were performed in the presence of 50 µg/ml of polysaccharide. Each experiment
 10 was performed at least 3 times. ** p<0.01; *** p<0.001.

11

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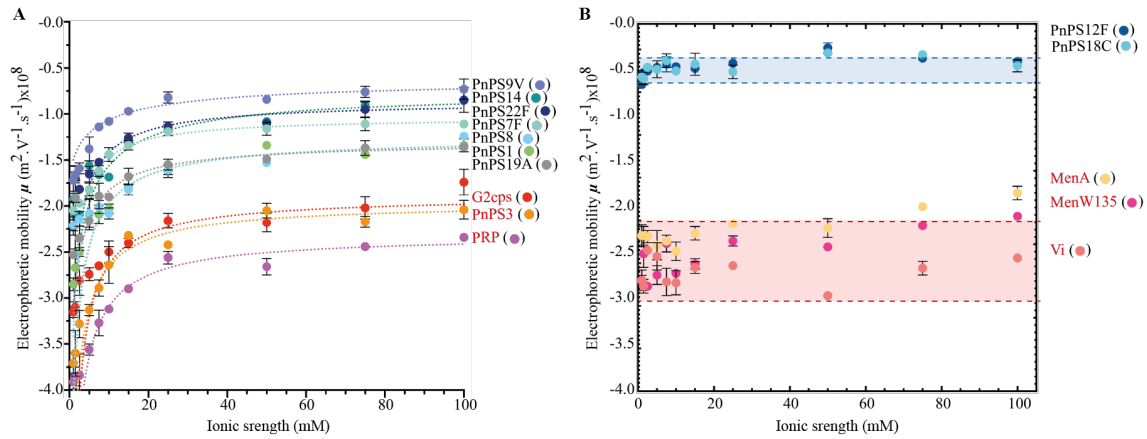


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3 **Figure 2. Antibiofilm activity of a collection of bacterial polysaccharides.** *E.*
4 *coli* (panel A) or *S. aureus* (panel B) biofilm formation assay in the presence of
5 purified bacterial polysaccharides. G2cps was included as a positive control of
6 active macromolecules and water was used as negative control. Red : active
7 polysaccharides with Vi, MenA, MenC, G2cps, PRP and PnPS3 displaying broad
8 spectrum of action, while PnPS18C is only active against Gram+ bacteria and
9 PnPS12F against Gram- bacteria. Experiments were performed twice and in
10 triplicate in presence of 100 µg/mL of each macromolecule. ** p<0.01; ***
11 p<0.001; **** p<0.0001.
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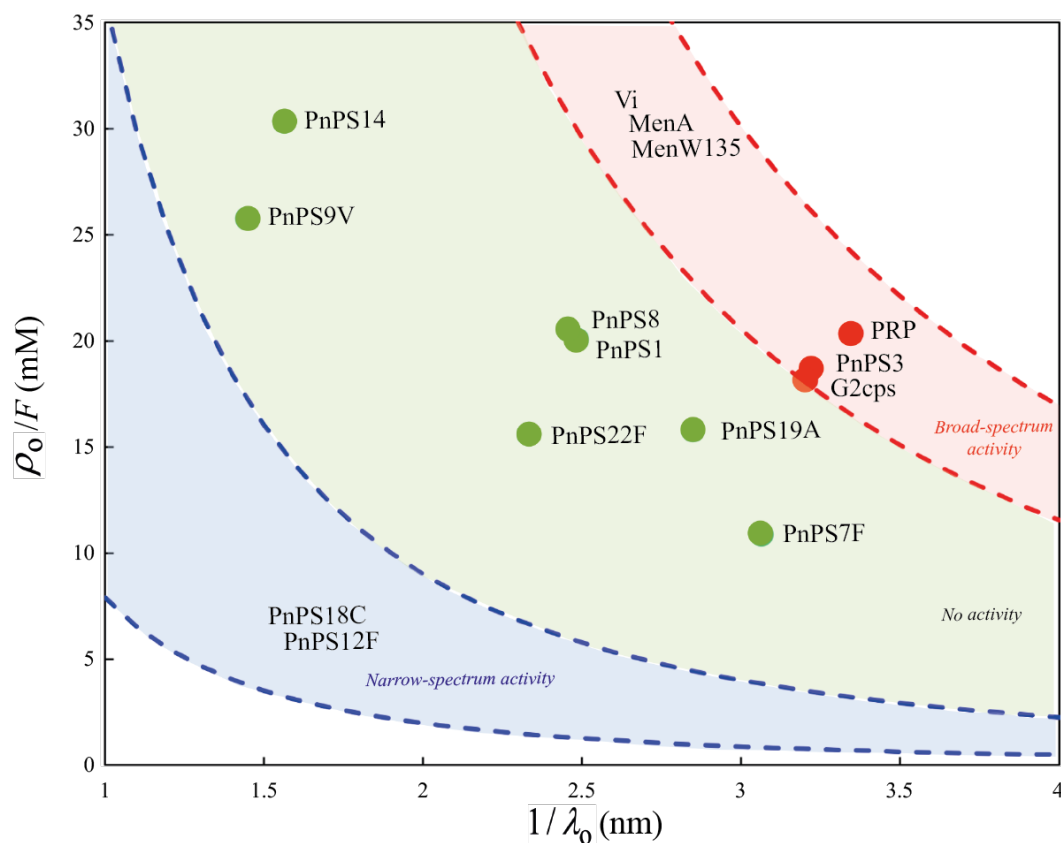
Figure 3. MenA and MenY antibiofilm activity. **A.** Chemical composition and acidic/hydrophobic group ratio of the *N. meningitidis* polysaccharides MenY and MenW135. **B.** Antibiofilm activity of MenY and MenW135 in comparison with previously identified active macromolecules. Biofilm inhibition tests were performed in presence of 50 µg/mL of polysaccharide. Distilled water was used as a negative control. Each experiment was performed at least 3 times. ** p<0.01; *** p<0.001; **** p<0.0001.



1
2

3 **Figure 4. Dependence of the electrophoretic mobility of polysaccharides on**
4 **NaNO₃ electrolyte concentration.** Points: experimental data. Dashed lines:
5 theory (eq 1). **A:** Polysaccharides whose electrophoretic mobility significantly
6 depends on solution ionic strength (fixed by NaNO₃ electrolyte concentration). **B:**
7 Bacterial polysaccharides whose electrokinetic features poorly depend on
8 electrolyte concentration. Polysaccharide with name in red display a broad-
9 spectrum antibiofilm activity. PnPS12F and PnPS18C display a narrow spectrum
10 antibiofilm activity (see Table S1). In panel B, the blue zone delimited by blue
11 dotted lines bracket the quasi-electrolyte concentration-independent
12 electrophoretic mobility values measured for the polysaccharides with narrow-
13 spectrum activity. The red zone delimited by red dotted lines bracket the quasi-
14 electrolyte concentration-independent electrophoretic mobility values measured
15 for the polysaccharides with broad spectrum activity.

16
17



1
 2 **Figure 5. Classification of the antibiofilm activity of the tested**
 3 **polysaccharides according to their charge density ρ_0 and flow penetration**
 4 **length scale $1/\lambda_0$.** The charge density ρ_0 (in C m^{-3}) is given here in the form of an
 5 equivalent concentration of anionic charges defined by ρ_0/F (in mM) with F the
 6 Faraday constant. The couple $(\rho_0, 1/\lambda_0)$ associated to each macromolecule is
 7 retrieved from the modelling of the dependence of measured electrophoretic
 8 mobility on the electrolyte concentration in solution according to eq 1. The dotted
 9 lines correspond to the set of solutions $(\rho_0, 1/\lambda_0)$ to the equation $\mu = \mu^*$ obtained for
 10 the macromolecules PnPS18C and PnPS12F and for the macromolecules Vi,
 11 MenW135 and MenA (see colored parts in Figure 4B) whose mobility μ over the
 12 whole range of electrolyte concentrations does not significantly deviate from the
 13 mobility value μ^* measured at large electrolyte concentrations (100 mM). For
 14 these macromolecules, the poor dependence of μ on electrolyte concentration
 15 renders difficult any accurate evaluation of both ρ_0 , and $1/\lambda_0$ as electrokinetic data
 16 fitting then reduces to solve one equation (eq 1) with two unknowns (ρ_0 and $1/\lambda_0$).
 17 The space of solutions $(\rho_0, 1/\lambda_0)$ associated with Vi, Men135, MenA and
 18 PnPS18C, PnPS12F correspond to the red and blue zones, respectively. They
 19 are delimited by dotted lines that correspond to the $(\rho_0, 1/\lambda_0)$ solutions of the
 20 equation $\mu = \mu^*$ where μ^* identifies with the polysaccharide mobilities marked by
 21 the dotted lines represented in Figure 4B.
 22