Dispersal and Inhibitory Roles of Mannose, 2-Deoxy-*D*-Glucose, and *N*-Acetylgalactosaminidase on the Biofilm of *Desulfovibrio vulgaris*

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SIGNIFICANCE

Annual global corrosion costs are around 3.4% of the global gross domestic product ($2.5 trillion), and biocorrosion is responsible for up to 30% of all corrosion losses. These losses are especially important in oil production, drinking water systems, and pipelines. Sulfate-reducing bacteria (SRB) are responsible for much of the biocorrosion, and *Desulfovibrio vulgaris* is the model organism for studying SRB. However, little is known about its biofilm matrix or how to control its biofilm formation. Although previously reported to consist primarily of protein, here, we determine that the biofilm matrix of *D. vulgaris* also consists of the polysaccharides mannose, fucose, and *N*-acetylgalactosamine (GalNAc). Based on this understanding of its biofilm matrix, we discover two ways to disperse *D. vulgaris* biofilms, by adding the mannose analog 2-deoxy-*D*-glucose and by adding *N*-acetylgalactosaminidase, which degrades GalNAc. Hence, we have found means to control SRB biofilm formation.

ABSTRACT

Biofilms of sulfate-reducing bacteria (SRB) are often the major cause of microbiologically influenced corrosion. The representative SRB *Desulfovibrio vulgaris* has previously been shown to have a biofilm that consists primarily of protein. In this study, by utilizing lectin staining, we identified that the biofilm of *D. vulgaris* also consists of the matrix components mannose, fucose, and *N*-acetylgalactosamine (GalNAc), with mannose predominating. Based on these results, we found that the addition of mannose and the non-metabolizable mannose analog 2-deoxy-*D*-glucose inhibits the biofilm formation of *D. vulgaris* as well as that of *D. desulfuricans*; both compounds also dispersed the SRB biofilms. In addition, the enzyme *N*-acetylgalactosaminidase, which degrades GalNAc, was effective in dispersing *D. vulgaris* biofilms. Therefore, by determining composition of the SRB biofilm, effective biofilm control methods may be devised.

INTRODUCTION

Desulfovibrio vulgaris Hildenborough and *D. desulfuricans* are anaerobic chemoorganotrophic Gram-negative sulfate-reducing bacteria (SRB)(Gao et al., 2016) that colonize many anaerobic environments including oil reservoirs and metal pipelines; hence, they are major culprits in microbiologically influenced corrosion (MIC) (Santana and Crasnier-Mednansky, 2006; Clark et al., 2007; Gao et al., 2016). MIC of copper and steel allows by SRB costs about $5 billion annually(Jayaraman et al., 1999).

The majority of the research on *Desulfovibrio* species focuses on the reduction of heavy metals with little study on their biofilm structure and function. Biofilms of *D. vulgaris* have been reported to consist primarily of protein(Clark et al., 2007), while that of *D. desulfuricans* have been reported to consist of mannose, glucose, and galactose in the extracellular matrix(Beech et al., 1991).

Lectin stains are highly specific and are beginning to be used to identify polysaccharides present in bacterial biofilms; for example, the extracellular matrix polysaccharide composition of *Burkholderia thailandensis* was identified using lectin stains such as peanut agglutinin (PNA) which detects galactose, *Ricinus communis* agglutinin I (RCA I) which detects *N*-acetylgalactosamine (GalNAc), and *Ulex europaeus* agglutinin (UEA I) which detects fucose(Tseng et al., 2016). Similarly, the lectin stain concanavalin A (Con A) has been used to demonstrate staphylococcal biofilms consist of mannose, and soybean agglutinin I (SBA) and *Ricinus communis* agglutinin I stains have been used to show these biofilms contain GalNAc (Frank and Patel, 2007). Furthermore, SBA lectin was used to show *Neisseria gonorrhoeae* biofilms contain GalNAc, and the lectin stain wheat germ agglutinin (WGA) demonstrated the presence of *N*-acetylglucosamine (GlcNAc) (Greiner et al., 2005).

In this work, we utilized seven lectin stains to determine that the biofilm of *D. vulgaris* consists of not only proteins, but polysaccharide polymers of fucose, mannose, and GalNAc. Based on its composition, we then utilized *N*-acetylgalactosaminidase to disperse *D. vulgaris* biofilms as well as utilized mannose and the non-metabolizable 2-deoxy-*D*-glucose (2DG) to inhibit as well as disperse SRB biofilms.

RESULTS

***D. vulgaris* biofilms include polysaccharides.** To test for the presence of polysaccharides in *D. vulgaris* biofilms, we utilized a variety of polysaccharide-degrading enzymes such as a commercial cellulase from *Trichoderma reesei*, *T. reesei* exocellulases (Cel7A, and Cel6A), *T. reesei* endocellulase (Cel7B)(Cho et al., 2015), *T. reesei* xylanases (Xyn 1 and Xyn 2), and xylanase 1 with the carbohydrate binding module of exocellulase Cel7A (Xyn 1C) (V. G. Poosarla and B. T. Nixon, unpublished work). Since *D. vulgaris* biofilms are dispersed by proteases such as proteinase K, trypsin and chymotrypsin (Clark et al., 2007), we utilized the proteases protease 1 (Savinase) and protease 2 (everlase) (Molobela et al., 2010), which cleaves peptide bonds in proteins specifically amino-ester bonds, as positive controls. As expected, both the proteases (1 and 2) dispersed greater than 90% corroborating the results with proteases tested earlier (**Fig. 1**)(Clark et al., 2007). Critically, cellulase, and some of the purified matrix-degrading enzymes from *T. reesei* (Cel6A, Cel7B, Xyn 1, Xyn 1C, and Xyn 2) also dispersed *D. vulgaris* biofilms up to 70% **(Fig. 1)**. Therefore, these results suggest there are matrix polysaccharides in the *D. vulgaris* biofilm which consist of β-linked *D*-glucose (the building block of cellulose)(Cho et al., 2015), and xylose linked possibly to L-arabinofuranosyl, acetyl, *D*-glucuronosyl and 4-O-methylglucuronosyl residues (the building blocks of xylan)(Giridhar and Chandra, 2010).

To corroborate our results with the matrix-degrading enzymes, we extracted and purified carbohydrates from the *D. vulgaris* biofilm. We found 0.11 mg of matrix polysaccharide per 1 L of culture from two independent cultures. Together, these results demonstrate the existence of polysaccharide-based components in the matrix of *D. vulgaris* which has not been reported for this strain.

***D. vulgaris* biofilms include mannose, fucose, and GalNAc**. To discern the chemical composition of the SRB biofilm matrix, we used lectin stains specific for the polysaccharides GalNAc (*Dolichos biflorus* agglutinin, DBA and SBA), galactose (PNA), galactose/GalNAc (RCA I), fucose (UEA I), mannose (Con A), and GlcNAc (WGA)(Greiner et al., 2005; Frank and Patel, 2007; Tseng et al., 2016). For this, we developed a biofilm assay for *D. vulgaris* in which we incubated glass slides in culture medium and probed for the presence of the various polysaccharides. We identified that the SRB biofilm includes three

polysaccharide building blocks: GalNAc (through lectin stains SBA, RCA 1, and DBA), fucose (through lectin stain UEA 1), and mannose (through lectin stain Con A) **(Fig. 2a-j).** For the lectin staining, we show both bright-field images **(Fig. 2a, c, e, g, i)** and the rhodamine-fluorescence images **(Fig. 2b, d, f, h, j)**; the bright-field images demonstrate significant biofilm formed on the slides, and the fluorescence images demonstrate that the lectin fluoresces only for that respective sugar (e.g., GalNAc, fucose, and mannose). Since mannose is a C-2 epimer of glucose, this result corroborates the results seen with cellulase. Galactose (via lectin stain PNA) and *N*-acetylglucosamine (via lectin stain WGA) did not exhibit rhodamine-fluorescence in the biofilms of *D. vulgaris* **(Supplementary Fig. 1)**.

To support our findings for the rhodamine-conjugated lectin for the biofilms of *D. vulgaris*, we used both positive and negative controls for GalNAc, mannose, and fucose. Specifically, in agreement with the literature, we found *Pseudomonas aeruginosa* PA14 is a positive control for GalNAc and mannose **(Supplementary Fig. 2)**, and negative control for fucose **(Supplementary Fig. 2)**, because the PA14 biofilm matrix contains Pel, a polymer of GalNAc and GlcNAc(Jennings et al., 2015). *V. harveyi* BB120 was selected as negative control for GalNAc,(Kierek and Watnick, 2003), and we found that it indeed stains negative with the lectins DBA, RCA 1, and SBA that are specific for GalNAc **(Supplementary Fig. 3)**. To identify positive control for fucose, *E. coli* MG1655 Δ*yncC* was selected because it overproduces a large amount of colanic acid (Zhang et al., 2008) in its biofilm matrix, and it stained positive for fucose with lectin stain UEA 1 **(Supplementary Fig. 4a)**. We selected *Streptomyces lividans* TK24 as a negative control for mannose (Manteca et al., 2008; Shao et al., 2012), and found the absence of mannose when stained with Con A, as expected **(Supplementary Fig. 4b)**. Based on the staining results and from the controls, we conclude lectin results for extracellular polymeric substances (EPS) of *D. vulgaris* comprise mannose, fucose and GalNAc.

To confirm the polysaccharides are specific for *D. vulgaris* biofilms and are not present on the exterior of planktonic cells, we also stained the planktonic cells of *D. vulgaris* by fixing cells with a paraformaldehyde-glutaraldehyde solution (Moloney et al., 2004; Manteca et al., 2008; Chao and Zhang, 2011) and found no rhodamine-fluorescence with all seven lectins (DBA, PNA, RCA 1, SBA, UEA 1,

Con A, and WGA) **(Supplementary Fig. 5a)**. Hence, the lectin fluorescence seen previously was from the biofilm matrix of *D. vulgaris* and was not from polysaccharides present on the planktonic cells. Furthermore, we also fixed the biofilms of *D. vulgaris* to slides with paraformaldehyde and stained with Con A (mannose); since the biofilms showed strong rhodamine-fluorescence, the fixation process did not alter lectin staining **(Supplementary Fig. 5b)**.

***D. vulgaris* biofilms are inhibited and dispersed by mannose and mannose analogs.** Based on the lectin staining results, we hypothesized that adding mannose, GalNAc, and *L*-fucose may inhibit (or disperse) the *D. vulgaris* biofilms. We initiated the experiment by adding sugars (mannose, GalNAc, and fucose) at 30 mM and found mannose caused 50% inhibition, whereas GalNAc and fucose had no effect on inhibiting biofilm formation by *D. vulgaris* **(Supplementary Fig. 6)**. Since mannose (30 mM) inhibited SRB biofilm formation by 2-fold, we tested higher concentrations and found that 100 to 500 mM inhibited SRB biofilm formation by up to 90% **(Supplementary Fig. 7a)**. Similar behavior has been reported with *Salmonella typhimurium* DT104 biofilms inhibited by 100 mM mannose(Ngwai et al., 2006).

Given the success of inhibiting SRB biofilm formation with the matrix compound mannose and since mannose is a possible carbon source of for bacteria in mixed-species biofilms, we tested three non-metabolizable mannose analogs for biofilm inhibition, 2-deoxy D-glucose (2DG), methyl α-D-mannopyranoside (αMM), and methyl α-D-glucopyranoside (αMG) (Sutrina et al., 2016). Among these three analogs, 2DG inhibited *D. vulgaris* biofilm formation in a dose dependent manner with 500 mM inhibiting biofilm formation by 94% **(Supplementary Fig. 7b)**. Biofilm growth was inhibited even at very low concentrations of 2DG **(Supplementary Fig. 8a)** with greater than 70% biofilm inhibition at 5, 10, and 20 mM, and even 50% biofilm inhibition at 1 mM 2DG. Addition of αMM (500 mM) and αMG (500 mM) also inhibited SRB biofilms by more than 60% **(Supplementary Fig. 9a, b**). The biofilm formation of another representative SRB, *D. desulfuricans*, was inhibited in a similar manner with mannose, 2DG, αMM, and αMG **(Supplementary Fig. 8b, Supplementary Fig. 10)**; hence, these compounds are general in their inhibition of SRB biofilm formation. Planktonic cell growth of SRB was

not inhibited with mannose or 2DG (a 3-day lag was seen with 2DG but cells achieved the same final $OD600_{nm}$); hence, these compounds inhibit biofilm formation of SRB, not growth.

Mannose and 2DG also dispersed *D. vulgaris* and *D. desulfuricans* biofilm formation by 50% in our 2 h dispersal assay **(Supplementary Fig. 7c, d, Supplementary Fig. 11)**. αMM and αMG dispersed *D. vulgaris* and *D. desulfuricans* biofilms moderately (10 to 25%) **(Supplementary Fig. 9c, d, Supplementary Fig. 11)**. Based on all the results, 2DG and mannose are promising compounds with potential to inhibit/disperse the biofilms of *D. vulgaris* and *D. desulfuricans*, whereas αMM and αMG are only moderately effective in inhibiting/dispersing SRB biofilms (25%).

Confocal microscopy was also used to investigate the effect of 2DG and mannose on SRB biofilm formation. For this experiment, *D. vulgaris* was labeled with GFP via pMRP9-1 **(Supplementary Fig. 12)**. As with the 96-well microtiter plate assays, the confocal microscopy results with inhibitory compounds 2DG and mannose **(Fig. 3)** corroborate that mannose and 2DG (500 mM) inhibit SRB biofilm formation dramatically.

***D. vulgaris* biofilms are dispersed by *N*-acetylgalactosaminidase.** Since we identified the SRB matrix consists of polymers of mannose, GalNAc, and fucose, we tested enzymes such as mannosidases (α1-2,3 mannosidase and α1-6 mannosidase), *N*-acetylgalactosaminidase, and α1-2 fucosidase to see if they could degrade the SRB matrix and cause biofilm dispersal. We found that *N*-acetylgalactosaminidase was effective for both the dispersal and inhibition of *D. vulgaris* biofilms (2-fold) **(Supplementary Fig. 13a, d)**. In contrast, fucosidase and the two mannosidases did not show much effect **(Supplementary Fig. 13a)**. Different concentrations of *N*-acetylgalactosaminidase were also investigated, and we found that addition of 60 U of the enzyme to the biofilms of *D. vulgaris* showed the maximum dispersal (2-fold), and higher concentrations did not show any effect further **(Supplementary Fig. 13b)**. Since the *D. vulgaris* matrix does not contain the matrix component GlcNAc, we also performed the negative control to disperse the *D. vulgaris* biofilm with β-*N*-acetylglucosaminidase and found this enzyme had no effect (1%) on the SRB biofilm dispersal corroborating with the lectin staining results **(Supplementary Fig. 13c)**.

Potential exopolysaccharide synthesis loci in the *D. vulgaris* genome. Based on the results with the lectin stains, we investigated the genome to identify putative exopolysaccharides biosynthesis gene loci in *D. vulgaris* related to mannose, GalNAc and fucose. For possible mannose synthesis, 14 genes located (from dvu0685 to dvu0698) in a 14 kb region were identified that include three key polysaccharide biosynthetic genes, dvu0685, dvu0697 and dvu0698 that appear related to the synthesis of a mannose-containing EPS (**Fig. 4**). DVU0685, annotated as a phosphomannomutase by the KEGG GENES database, has phosphoglucomutase/phosphomannomutase I-IV motifs, which catalyze the reversible conversion between 1-phospho and 6-phospho-sugars. BLAST analysis shows it has 46% amino acid identity to the phosphomannomutase AlgC of *P. aeruginosa* PAO1, which provides precursor Man-1-P for the synthesis of exopolysaccharide alginate and Psl (Franklin et al., 2011; Ma et al., 2012). This suggests that DVU0685 is responsible for mannose-related EPS precursor synthesis. Also, protein DVU0697, annotated as a mannose-1-phosphate guanylyltransferase/mannose-6-phosphate isomerase, has a nucleotidyl transferase motif and a mannose-6-phosphate isomerase motif. It has 45% identity with the alginate biosynthesis protein AlgA of *P. aeruginosa* PAO1, which is involved in alginate precursor synthesis(Hay et al., 2010). Protein DVU0698 has a dTDP-4-dehydrorhamnose 3,5-epimerase motif and showed 45% identity with epimerase RmlC of *P. aeruginosa* PAO1, which catalyzes the isomerization of dTDP-4-dehydro-6-deoxy-D-glucose with dTDP-4-dehydro-6-deoxy-L-mannose(Dong et al., 2007). DVU0690 has 33% amino acid identity to the Alg44 of *P. aeruginosa* PAO1, which is cyclic diguanylate binding protein and involved in sugar precursor transport and polymerization of alginate (Hay et al., 2010) (**Fig. 4**).

Similarly, a biosynthetic locus that possibly synthesizes a GalNAc-containing polymer was identified which contains 13 genes located on a 15 kb region (from dvu0319 to dvu0331, **Fig. 4**). Within this locus, protein DVU0319 has motifs as a UDP-N-acetylglucosamine 4-epimerase, which catalyzes the conversion between UDP-GlcNAc and UDP-GalNAc and supplies the GalNAc related EPS precursor. Hence, this indicates that DVU0319 is the key enzyme for GalNAc-related EPS precursor synthesis. DVU0319 has 45% identity with saccharide epimerase WbpP of *P. aeruginosa* PAO1(Ishiyama et al.,

2004). Also, DVU0322, annotated as a phosphopyruvate hydratase, has N-terminal and C-terminal domains of enolase, and has 61% identity with enolase of *P. aeruginosa* PAO1. DVU0327 was annotated as a putative exopolysaccharide biosynthesis protein with a bacterial sugar transferase motif. The sugar transferase motif region has 50% identify with Alg8, and 44% identify with PslA of *P. aeruginosa* PAO1, which is responsible to sugar precursor transport and polymerization (Hay et al., 2010) (**Fig. 4**). It DVU0328 has two glycosyl transferase motifs, and has 38% identity with glycosyl transferase RfaB of *P. aeruginosa* PAO1.

Similarly, a biosynthetic locus that possibly synthesizes a fucose-containing polymer was identified which contains 27 genes located on a 33 kb region (from dvu0072 to dvu0093, **Fig. 4**), including GDP-fucose synthetase gene (dvu0090), which encodes a protein with an epimerase/dehydratase motif that may catalyzed the NADP-dependent synthesis of GDP-fucose from GDP-mannose and supplying the fucose related EPS precursor; DVU0090 has 27% amino acid identity with putative epimerase of *P. aeruginosa* PAO1, and other novel potential polysaccharides biosynthesis genes. Based on a protein motif analysis, DVU0077 and DVU0078 are probably the sugar transporters (**Fig. 4**). Therefore, there is a plausible genetic basis for the three EPS building blocks identified by the lectin staining.

DISCUSSION

We demonstrate here for the first time the presence of three polymers (mannose, GalNAc, and fucose) in the EPS of *D. vulgaris* based on lectin staining results. We also demonstrated total carbohydrates are present in the EPS of *D. vulgaris*. Further efforts are needed to isolate the EPS of *D. vulgaris* in high yield to determine their exact composition, but it is clear from our results that the biofilm matrix of *D. vulgaris* contains these polysaccharides. We also provide bioinformatics data showing where the loci for the enzymes responsible for synthesizing the three polymers are located in the genome.

Based on this determination for the composition of the EPS, we hypothesized that the addition of any of the three building blocks (mannose, GalNAc, and fucose) of *D. vulgaris* to the biofilms of SRB would inhibit or disperse the extracellular matrix; we found mannose inhibited biofilm formation up to 90%

suggesting that mannose is one of the main polysaccharide building blocks of the SRB biofilm. Since the non-metabolizable mannose analog, 2DG, inhibited the biofilm formation of *E. coli* (Sutrina et al., 2016), we focused on testing 2DG on the biofilms of SRB (*D. vulgaris* and *D. desulfuricans*), and found a dramatic inhibition (greater than 90%), which was similar to the effect of mannose addition. Hence, this compound shows great promise for controlling SRB biofilm formation. Note that the toxicity (LD50) for 2DG is 8000 mg/Kg (Vijayaraghavan et al., 2006) which is equivalent to 50 mM, and this compound does not have any toxicity to humans. The inhibitory/dispersal role of mannose on the biofilms could be due to mannose being very effective in preventing bacterial adhesion, thereby preventing cells from forming biofilms, as has been seen for *E. coli* in the urinary tract (Kranjčec et al., 2014). Since 2DG is a non-metabolizable mannose analog, we could expect a similar mechanism with SRB for this compound.

Based on the determining mannose, fucose, and GalNAc are present in the *D. vulgaris* biofilm, we tested mannosidases, fucosidases, and *N*-acetylgalactosaminidase to see if they could disperse these biofilms. Only *N*-acetylgalactosaminidase showed 2-fold dispersal and inhibition of the *D. vulgaris* biofilm, which confirms the presence and importance of the building block GalNAc and suggests that GalNAc is a major component in the *D. vulgaris* biofilm matrix.

The inactivity of the fucosidases and mannosidases we tried suggests the specific polysaccharide linkages they degrade are not present in *D. vulgaris* biofilms. α1-2,3 Mannosidase catalyzes the hydrolysis of linear α1-2 and α1-3 linked *D*-mannopyranosyl residues from oligosaccharides, α1-6 mannosidase catalyzes the hydrolysis of α1-6 linked *D*-mannopyranosyl residues from oligosaccharides, and α1-2 fucosidase catalyzes the hydrolysis of linear α1-2 linked *L*-fucopyranosyl residues from oligosaccharides; hence, these bonds may not be present in *D. vulgaris* biofilms (although the polymer building blocks mannose and fucose are clearly present). Also, these bonds may not be available for cleavage in the *D. vulgaris* biofilm.

CONCLUSIONS

In this study, we discovered that the biofilm of *D. vulgaris* contains polymers of mannose, fucose, and GalNAc and that the addition of mannose, the non-metabolizable and relatively non-toxic 2-deoxy-D-glucose, and the enzyme *N*-acetylgalactosaminidase inhibit biofilm formation. Therefore, the potential inhibitors (mannose and 2-deoxy-D-glucose) may be prepared at high concentrations (up to 50 mM) and applied easily to limit SRB biofilm formation.

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FIGURE LEGENDS

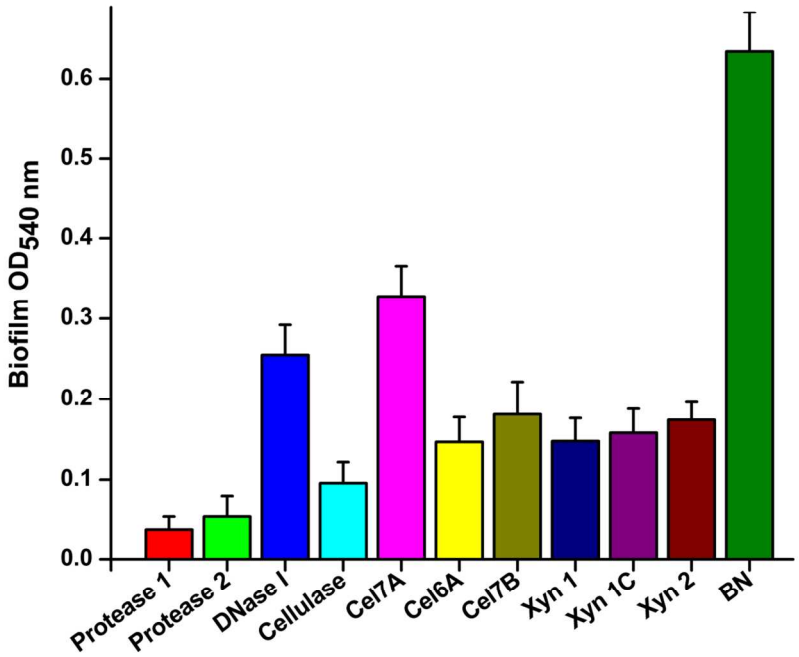
Fig. 1. Dispersal of SRB biofilms with protease, DNase I, cellulase, and xylanase. Remaining *D. vulgaris* biofilm formed at 30°C for 48 h (OD_{540nm}) in modified Baar's medium after 24 h of treatment with protease 1 (Savinase) at 0.048 U, protease 2 (everlase) at 0.048 U, DNase 1 at 0.006 U, cellulase from *T. reesei* at 2 U, exocellulase from *T. reesei* at 0.01 U (Cel7A), exocellulase from *T. reesei* at 0.01 U (Cel6A), endocellulase from *T. reesei* at 0.03 U (Cel7B), xylanase from *T. reesei* (Xyn 1) at 0.006 U, xylanase with carbohydrate binding module of Cel7A (Xyn 1C) at 0.03 U, xylanase of from *T. reesei* at 0.03 U (Xyn 2), and buffer negative control (BN).

Fig. 2. *D. vulgaris* matrix composition as identified through lectin staining. *Dolichos biflorus* agglutinin (DBA) detects *N*-acetylgalactosamine (GalNAc), *Ricinus communis* agglutinin I (RCA 1) detects galactose and GalNAc, soybean agglutinin I (SBA) detects GalNAc, *Ulex europaeus* agglutinin I (UEA I) detects fucose, and concanavalin A (Con A) detects mannose. Each image represents staining with a specific lectin linked to rhodamine, and each image represents the results from two independent cultures. The left and right images of each panel correspond to bright field images (a ,c, e, g, i) and rhodamine fluorescent images (b, d, f, h, j), respectively. Scale bar = 20 µm.

Fig. 3. Confocal microscopy of the biofilms of *D. vulgaris* (GFP-tagged) after inhibition with 2DG and mannose. Representative IMARIS (Bitplane, Zurich, Switzerland) images of biofilms cultured in modified Baar's medium on Nunc glass bottom dishes after 24 h with the addition of mannose (500 mM) and 2DG (500 mM) at the time of inoculation (Scale bars, 10 µm). Top panel shows the SRB biofilm formation (without mannose and 2DG), middle panel shows SRB biofilm with 2DG added, and the bottom panel shows SRB biofilm formation with mannose added. Biomass was obtained using COMSTAT image-processing software(Heydorn et al., 2000).

Fig. 4. Schematic of the putative exopolysaccharide synthesis loci related to mannose, GalNAc, and fucose from the annotated *D. vulgaris* Hildenborough genome. The mannose-related gene cluster contains 14 putative ORFs (~14 kb), annotated DVU0685 to DVU0698. The GalNAc-related gene cluster contains 13 putative ORFs (~15 kb), annotated DVU0319 to DVU0331. The fucose-

related gene cluster contains 27 putative ORFs (~33 kb), annotated DVU0072 to DVU0093. Green (polysaccharide synthesis), red (EPS transport), blue (other EPS synthesis) and yellow (regulation) colored arrows indicate ORFs that share homology with proteins involved in EPS synthesis. Gene numbering is based on *D. vulgaris* genome annotation from the KEGG GENES database.



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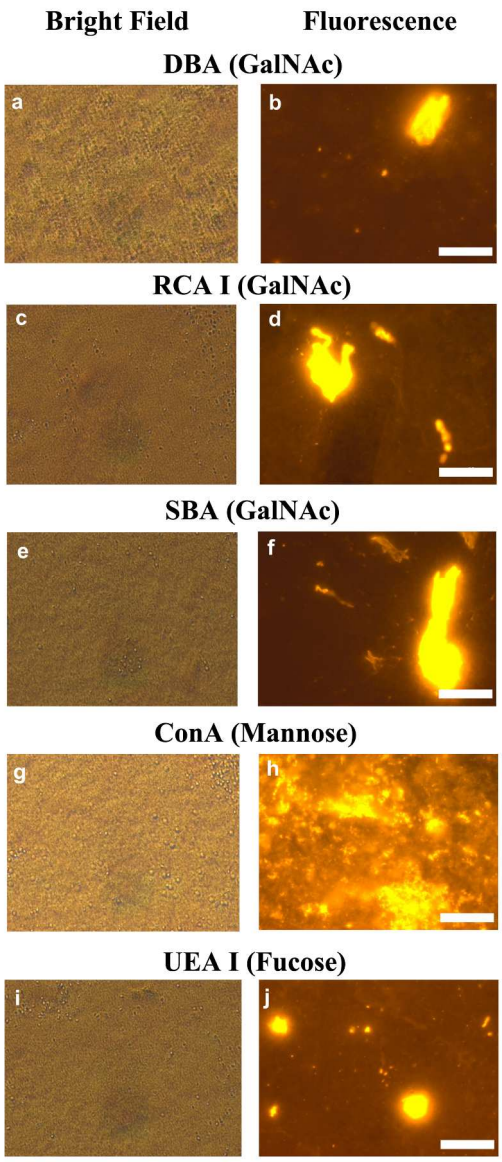

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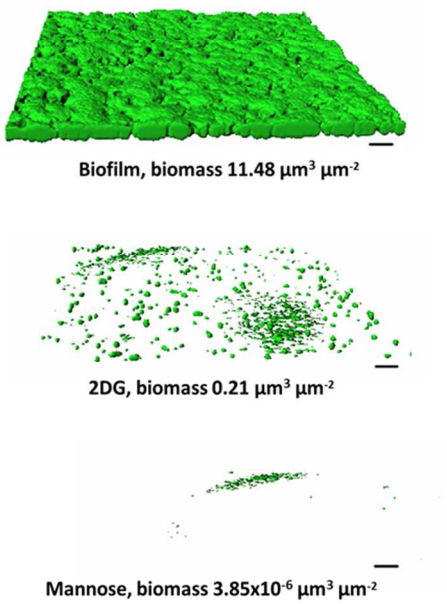

Confocal microscopy of the biofilms of D. vulgaris (GFP-tagged) after inhibition with 2DG and mannose. Representative IMARIS (Bitplane, Zurich, Switzerland) images of biofilms cultured in modified Baar's medium on Nunc glass bottom dishes after 24 h with the addition of mannose (500 mM) and 2DG (500 mM) at the time of inoculation (Scale bars, 10 µm). Top panel shows the SRB biofilm formation (without mannose and 2DG), middle panel shows SRB biofilm with 2DG added, and the bottom panel shows SRB biofilm formation with mannose added. Biomass was obtained using COMSTAT image-processing software (Heydorn et al., 2000).

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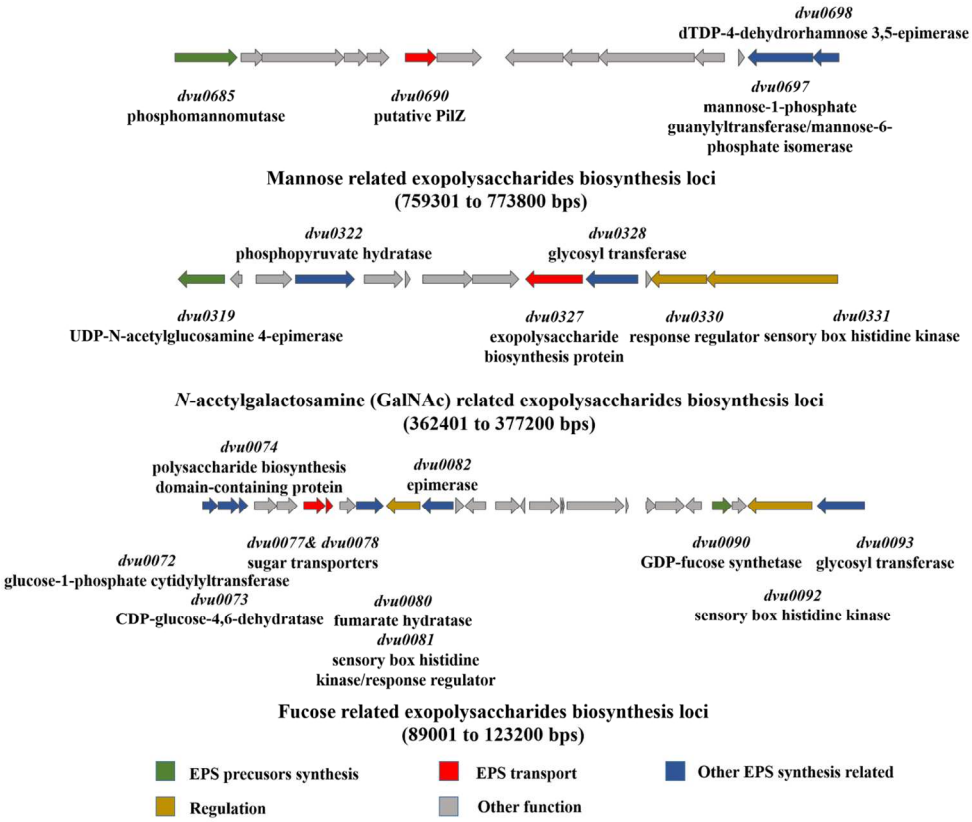

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164x141mm (220 x 220 DPI)