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Extracellular polysaccharides from *Desulfovibrio desulfuricans* and *Pseudomonas fluorescens* in the presence of mild and stainless steel

I. B. Beech[1], C. C. Gaylarde[2], J. J. Smith[3], and G. G. Geesey[3]

[1] Lancashire Polytechnic, Department of Applied Biology, Preston PR1 2TQ, UK
[2] City of London Polytechnic, Department of Biological Sciences, Old Castle Street, London, E1 7NT, UK
[3] California State University, Long Beach, Department of Microbiology, 1250 Bellflower Boulevard, Long Beach, CA 90840-4101, USA

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Summary. This communication reports the presence of polysaccharides in biofilms formed by pure and mixed cultures of *Desulfovibrio desulfuricans* and *Pseudomonas fluorescens* on mild and stainless steel surfaces. The results of colorimetric assays, indicating significant differences between the amounts of neutral sugars present in these biofilms, were supported by gas chromatographic (GC)-mass spectrophotometric and GC-flame ionisation detection analyses. Neutral sugars in biofilms grown on mild steel surfaces were identified and quantified, revealing glucose as a major carbohydrate followed by mannose and galactose in all types of biofilm. Extracellular polymeric substances (EPS) precipitated from bacterial cultures grown with and without steel surfaces were also analysed for their carbohydrate content. The influence of the surfaces present in the cultures on the amount and type of sugars released into the bulk phase was established. There was significantly more carbohydrate in EPS harvested from pure and mixed cultures of *D. desulfuricans* incubated mild and stainless steel coupons than in EPS obtained from coupon-free cultures. No significant difference in sugar quantities was observed in EPS precipitated from cultures of *P. fluorescens* grown under different conditions (absence or presence of steel surfaces). The main carbohydrates identified in all types of EPS samples were mannose, glucose and galactose in order of prevalence.

Introduction

Many microorganisms produce exopolymers that promote adhesion to other cells or to inert surfaces (Costerton 1987). These exopolymers are frequently acidic polysaccharides (Geesey et al. 1988), which can compose a major proportion of biofilms including accumulations of microbial cells, extracellular polymeric substances (EPS) and trapped debris on an immersed surface. The EPS produced by some bacteria have been shown to bind metal ions and hence can promote the corrosion of metals such as copper alloys (Jolley et al. 1988; Geesey et al. 1988) and steels (White et al. 1986; Ford et al. 1988). In addition to their innate aggressive activity towards metals, EPS also enhance corrosion by providing a matrix for the attachment of microbial cells to a metal surface. Close contact between bacteria and metal has been shown to be an important factor in determining the rate of corrosion (Gaylarde and Johnston 1980; Gaylarde and Videla 1987).

One of the most important groups of corrosion-causing organisms is the sulphate-reducing bacteria (SRB), a heterogeneous collection of anaerobic heterotrophs the economic influence of which has been particularly important in the oil industry (Hamilton 1985). These bacteria are known to cause corrosion by a number of mechanisms (Tiller 1983; Iverson 1987), but their ability, or otherwise, to produce EPS has not been recorded.

Members of the genus *Pseudomonas* have frequently been reported to produce exopolymers (White et al. 1985; Read and Costerton 1987; Jolley et al. 1988; Geesey et al. 1988) and these bacteria have been suggested to be primary colonisers of surfaces in aqueous environments (Corpe 1970). *P. fluorescens* has the ability to attach rapidly to the surfaces of mild and stainless steel (Beech and Gaylarde 1989) and in the presence of *Desulfovibrio desulfuricans*, a thick biofilm containing both species of bacteria builds up (Gaylarde and Beech 1989). Scanning electron microscopy suggests that EPS are produced by the bacteria in this biofilm (Moreno et al. 1990), but no work has been reported on the isolation and characterisation of these compounds.

This project sets out to isolate and analyse the EPS excreted into the environment by *D. desulfuricans* and *P. fluorescens* and to compare these with EPS within biofilms formed on mild and stainless steel coupons in the presence of these bacteria.

Offprint requests to: I. B. Beech

Materials and methods

Organisms. D. desulfuricans subsp. *desulfuricans* New Jersey (NCIMB 8313) was grown as batch cultures in medium C of Postgate (1984) at 37° C. Five-day-old cultures were used to provide the inoculum. *P. fluorescens* was isolated from a contaminated metal working fluid and identified by the Analytical Profile Index (API) 20 NE STRIP (API System S.A., Montalieu Varcieu, France). The organism was grown on nutrient agar plates at 24° C and 48-h cultures were used as inoculum.

Metal surfaces. Mild steel (British Standard 970) and stainless steel (British Standard 02134) coupons (1.5 cm × 10 cm × 0.16 cm) were sterilised dry by autoclaving in watertight containers. Before inoculation with bacterial cultures the coupons were immersed in 70% alcohol, flamed and placed vertically inside glass screw-capped flasks containing 135 ml sterile medium C (five coupons per flask). The coupons were positioned to encourage biofilm growth on both sides.

Growth conditions. Cells of *D. desulfuricans* were harvested from broth cultures by centrifugation (500 *g* for 30 min) and resuspended in sterile medium C. Cells of *P. fluorescens* were washed from the surface of the nutrient agar plate with sterile medium C. Cell suspensions of pure and mixed cultures of *D. desulfuricans* and *P. fluorescens* were adjusted by counting with an improved Neubauer haemacytometer to give a final concentration of 10^5 cells/ml in coupon-containing flasks and control coupon-free flasks. Flasks were set up in triplicate. Each set of three contained growth medium and either five stainless steel or five mild steel coupons or no coupons (control). Flasks were incubated for 7 days at 30° C. The whole procedure was repeated three times.

EPS Samples. EPS were obtained from the bulk phase of pure and mixed batch cultures of *D. desulfuricans* and *P. fluorescens* grown aseptically for 7 days at 30° C in medium C containing mild steel and stainless steel coupons (fifteen coupons of each type per assay). Coupons were removed aseptically and cultures (500 ml per trial) were centrifuged for 30 min at 10,000 *g* to remove bacterial cells. The EPS were recovered from the supernatant by precipitating with 3 vol isopropanol for 48 h at 4° C. The precipitated polymer was redissolved in double distilled water (ddH_2O), dialysed against ddH_2O overnight at 4° C, lyophilised to dryness and stored at −80° C. EPS were also harvested from bacterial cultures (500 ml) incubated without coupons and from precipitate obtained from 500 ml of 7-day-old sterile medium C.

Biofilm samples. The mild steel and stainless steel coupons removed from the above cultures were immediately submerged in liquid nitrogen (to avoid formation of oxides) and freeze-dried. Lyophilised biofilms were removed from the coupon surfaces with a razor blade and the total biofilm from each set of fifteen coupons combined and stored at −80° C.

Scanning electron microscopy (SEM). Freshly withdrawn mild steel coupons were rinsed in 0.01 M cacodylate buffer, pH 7.4, and fixed in 0.5% (v/v) glutaraldehyde at 4° C for 24 h. They were then rinsed in cacodylate buffer and fixed for 3 h in 2.5% glutaraldehyde at 4° C. After washing three times in cacodylate buffer, the coupons were dehydrated by passing through a graded series of reagent grade acetone (30–100%) and frozen rapidly in liquid nitrogen prior to freeze-drying overnight. Dried coupons were cut into segments (1.5 cm × 3 cm × 0.16 cm), mounted on aluminium stubs, sputter-coated with gold and examined under a Hitachi (Tokyo, Japan) S450 scanning electron microscope at an accelerating voltage of 20 kV.

Removal of corrosion products from biofilm samples. Biofilms were treated to remove inorganic corrosion products by resuspending in 10 ml ddH_2O, heating at 40° C for 10 min, vortexing for 10 min and centrifuging at 500 *g* for 30 min. The supernatants were collected and the pellets extracted two more times. All washes were combined, centrifuged at 10,000 *g* for 30 min and the supernatants, now essentially free from metal oxides and sulphides, were lyophilised to dryness. Purified biofilms were stored at −40° C prior to analysis.

Determination of sugars in EPS and in biofilm samples. Crude EPS and crude and treated biofilm samples were assayed for the presence of neutral hexoses (Dubois et al. 1956) and uronic acids (Blumenkrantz and Asboe-Hansen 1973). Reduction of sugars was performed by the method of York et al. (1985) using sodium borodeuteride and hydrolysis was conducted according to the procedure of Fazio et al. (1982). Derivatisation of the monomeric sugars using hydroxylamine hydrochloride and acetic anhydride followed the method of Quintero et al. (1990). Sugar standards (15) were prepared as 0.02 M solutions and derivatised in the same way as samples. All procedures were performed using acid-washed glassware.

Gas chromatography (GC). This was performed with a Varian (Sunnyvale, Calif., USA) model 3700 gas chromatograph with a flame ionisation detector (FID) and Varian CSD111 data system. Samples and sugar standards (1 µl) were injected in triplicate into a polar 30 m fused silica capillary column, 0.25 mm ID (SP-2330, Supelco, Bellefonte, Pa., USA) using splitless injection. The temperature was programmed to rise from 160 to 210° C at a rate of 5° C/min, after which an isothermal period was held for 10 min, followed by a temperature ramp to 225° C at a rate of 5° C/min, and this temperature held for 3 min. Hydrogen was used as the carrier gas at a flow rate of 30 cm^3/min. The temperature of the injection port and the detector were controlled at 250° C. The run was completed in 32 min.

GC-mass spectrometry. GC-mass spectrometry was performed with a Hewlett-Packard (Palo Alto, Calif., USA) 5890 gas chromatograph and 5970 mass selective detector (MSD). Temperature programmes, MSD parameters and data analysis were controlled with a Hewlett-Packard 59970 MS Chemstation. Samples and sugar standards were injected as 1 µl aliquots into a polar 30 m fused silica capillary column DB225-30N (J & W Scientific, Rancho Cordova, Calif., USA), with splitless injection and 0.75-min venting time. The temperature was set to rise from 160 to 210° C at 2° C/min, followed by an 18-min isothermal period. The temperature was raised to 225° C/min and held for 4 min. The helium carrier gas was operated at a head pressure of 34.5 kPa and a column flow rate of 30 cm/s. The injection port was held at 250° C and the detector at 280° C. The mass spectrometer was autotuned with perfluorotributylamine and the electron multiplier voltage was 2000 V. The instrument was used in the selective ion monitoring mode for highest sensitivity.

Results

Characterisation of biofilms formed in steel coupons

Figure 1a–c shows biofilms formed on mild steel coupons after 7 days of incubation with pure and mixed bacteria in medium C. Biofilms developed in the presence of *P. fluorescens* are scanty, few cells being visible above the cracked surface deposit (Fig. 1a). In contrast, biofilms of *D. desulfuricans* are thicker and comprise high densities of cells with abundant EPS, visible as fibres extending from the cells (Fig. 1b). Mixed culture biofilms appear equally if not more voluminous than those formed from pure cultures of *D. desulfuricans*. Cells and EPS fibres are to some extent obscured by amorphous corrosion products (Fig. 1c).

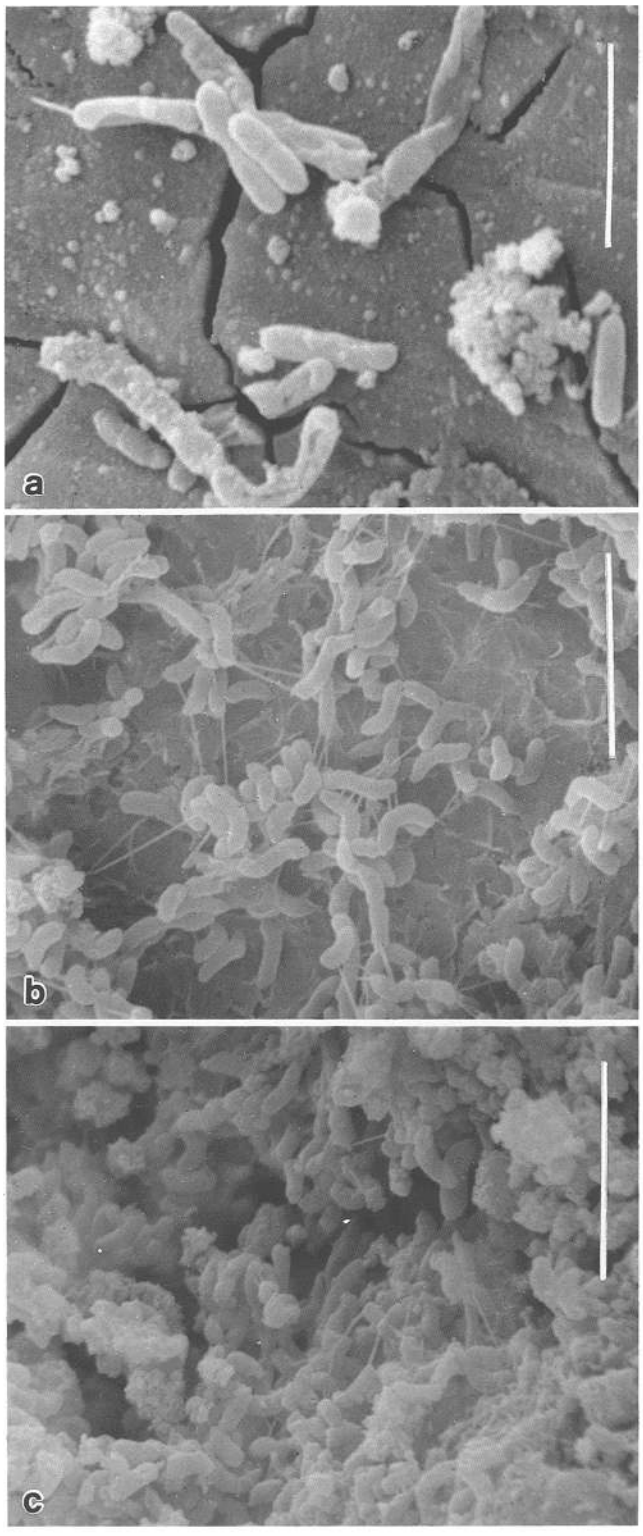

Fig. 1. Scanning electron micrographs of biofilms formed on mild steel coupons after 7 days incubation in medium C (Postgate 1984) inoculated with **a** *Pseudomonas fluorescens*, **b** *Desulfovibrio desulfuricans* and **c** *P. fluorescens* and *D. desulfuricans*. *Bar* represents 5 μ

Table 1. Dry weight of biofilms formed on fifteen mild steel (MS) and fifteen stainless steel (SS) coupons incubated for 7 days with bacterial cultures in medium C (Postgate 1984)

Inoculum	Dry weight (mg + SD)	
	MS	SS
Pseudomonas fluorescens	103.7 ± 13.0	7.9 ± 3.0
Desulfovibrio desulfuricans	118.7 ± 20.0	10.1 ± 1.0
P. fluorescens and *D. desulfuricans*	102.0 ± 20.0	7.2 ± 1.0

Table 2. Colorimetric estimation of neutral hexose content in crude and treated biofilms

Inoculum	Neutral hexose content (% of dry weight)		
	MS		SS
	Crude biofilm	Treated biofilm	Crude biofilm
P. fluorescens	3.48	3.42	2.8
D. desulfuricans	7.55	5.87	3.0
P. fluorescens and *D. desulfuricans*	8.77	6.18	4.8

Table 1 shows the dry weights of biofilms removed from mild steel (MS) and stainless steel (SS) coupons. There was no significant difference between the dry weights of 7-day-old biofilms formed by pure and mixed bacterial cultures on either MS or SS surfaces. However, the amount of biofilm recovered from SS coupons was significantly lower than that obtained from MS coupons in all cultures.

Table 2 shows the amount of neutral hexoses expressed as a percentage of the dry weight detected colorimetrically in crude and treated biofilm samples. Analysis of variance shows that there was a significantly greater amount of neutral hexoses in crude and treated biofilms recovered from pure and mixed cultures of *D. desulfuricans* compared to that in biofilms formed by pure cultures of *P. fluorescens* on MS coupons. There were significantly more neutral hexoses present in crude biofilm formed by *Desulfovibrio* on MS coupons than in that removed from SS coupons.

The amounts of uronic acid detected colorimetrically in biofilms formed on MS surfaces by pure and mixed bacterial cultures are listed in Table 3. Biofilm treatment did not influence the efficiency of uronic acid recovery. The amounts of uronic acids present in treated biofilms were similar to those detected in crude biofilms.

Analysis of carbohydrates present in biofilms grown on MS coupons by GC-FID

Neutral sugar composition of 7-day-old biofilms formed on MS coupons by pure and mixed cultures of *P. fluorescens* and *D. desulfuricans* is summarised in Ta-

Table 3. Uronic acid content in crude and treated biofilms formed on MS coupons

Inoculum	Uronic acid content (μg/mg)	
	Crude biofilm	Treated biofilm
P. fluorescens	3,74	3.04
D. desulfuricans	5.03	4.69
P. fluorescens and *D. desulfuricans*	5.89	4.98

Table 4. Neutral carbohydrates present in biofilms recovered from fifteen MS coupons after 7 days of incubation with bacterial cultures

Type of sugar	Neutral carbohydrates (μg/mg ± SD)		
	P. fluorescens	*D. desulfuricans*	*P. fluorescens* *D. desulfuricans*
Rhamnose	—	0.372 ± 0.070	—
Mannose	0.475 ± 0.009	0.630 ± 0.004	1.190 ± 0.070
Glucose	0.973 ± 0.060	0.927 ± 0.020	1.102 ± 0.059
Galactose	0.130 ± 0.020	0.602 ± 0.040	0.487 ± 0.040
Xylose	0.096 ± 0.014	0.180 ± 0.002	—
Allose	—	0.175 ± 0.037	—
Gulose	0.053 ± 0.020	—	—
Ribose	0.094 ± 0.070	0.250 ± 0.001	0.296 + 0.020
Total sugar recovered (mg ± SD)	0.199 ± 0.020	0.372 ± 0.070	0.313 ± 0.070

— not detected

Table 5. Molar ratios of the main neutral sugars present in biofilms formed on MS coupons

Organism	Glucose : Galactose : Mannose		
P. fluorescens	1.0	0.1	0.5
D. desulfuricans	1.0	0.4	1.1
Mixed cultures	1.0	0.65	0.7

ble 4. The total amount of neutral sugar present in biofilms removed from MS coupons was calculated from the values obtained for individual sugars.

Table 5 shows the molar ratios of glucose, mannose and galactose, the main sugars detected in all three types of biofilms. Glucose contributed 53% (w/w) of the total sugars detected in biofilms of *P. fluorescens*, 29.5% (w/w) for *D. desulfuricans* and 36% (w/w) in biofilms from mixed populations. Mannose contributed 26%, 20% and 36% (w/w) respectively. Rhamnose was found only in biofilms of *D. desulfuricans* and gulose was detectable only in biofilms of *P. fluorescens*.

Biofilms formed on MS surfaces by pure and mixed cultures of *D. desulfuricans* contained significantly more neutral sugar than biofilms developed on MS coupons in the presence of pure cultures of *P. fluorescens*. There was no significant difference in the amount of neutral sugars detected chromatographically between biofilms from pure and mixed *Desulfovibrio* cultures. The quantities of biomass removed from the SS coupons were too small to detect individual sugars by gas chromatographic analysis.

Analysis of bacterial EPS released into the aqueous phase

The weight of crude EPS harvested from the bulk liquid phase of pure and mixed bacterial cultures incubated for 7 days in the presence and absence of MS or SS coupons is shown in Table 6. Analysis of variance indicates that there was no significant difference in the amount of polymer released from the respective bacteria into the bulk aqueous phase when SS or MS coupons were present or absent.

The amount of polymer obtained from *Pseudomonas* cultures was significantly greater than that recovered from the respective pure and mixed *Desulfovibrio* cultures when the organisms were grown in the absence of steel surfaces, or when SS surfaces were present. No significant difference in polymer levels was observed between the cultures containing MS coupons.

Results of GC-mass spectrometric and GC-FID analysis showing the composition of carbohydrates and molar ratios of the main sugars detected in EPS harvested from 7-day-old pure and mixed cultures of *D. desulfuricans* and *P. fluorescens* incubated with MS and SS coupons are presented in Tables 7 and 8, respectively. The quantification of neutral hexoses was performed only for peaks of certain height. This corresponded to the concentration of 0.05 μg carbohydrate in 1 mg sample. Peaks below the arbitrary height were not integrated and it was agreed to refer to these carbohydrates as trace amounts.

Analysis of neutral carbohydrates by GC-FID showed that mannose was the dominant sugar in all types of EPS samples, being especially prevalent in *D. desulfuricans*-containing cultures. The relative abundance of this sugar was, however, greatly decreased in

Table 6. Extracellular polymeric substances (EPS) recovered from culture medium

Inoculum	Sample type	Weight of EPS (mg ± SD)
P. fluorescens	MS	95.9 ± 26.6
	SS	79.2 ± 16.6
	Coupon-free	73.6 ± 6.7
D. desulfuricans	MS	66.5 ± 14.4
	SS	68.0 ± 26.1
	Coupon-free	48.6 ± 7.1
P. fluorescens and *D. desulfuricans*	MS	60.6 ± 12.2
	SS	55.1 ± 3.4
	Coupon-free	42.6 ± 5.7

Table 7. Neutral sugars detected in free EPS recovered from pure and mixed bacterial cultures incubated for 7 days with and without steel coupons

Type of sugar	*P. fluorescens*			*D. desulfuricans*			*P. fluorescens* *D. desulfuricans*		
	MS	SS	Coupon free	MS	SS	Coupon free	MS	SS	Coupon free
Rhamnose	tr	+	+	+	tr	+	–	–	–
Mannose	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	tr	+	+
Xylose	–	–	+	–	–	+	–	–	–
Arabinose	+	–	+	–	–	–	tr	+	tr
Altrose	–	–	–	–	+	–	–	tr	–
Ribose	+	+	+	tr	tr	+	tr	tr	+

+, detected amount (greater than, or equal to 0.05 µg/mg); tr, trace amount (less than 0.05 µg/mg); –, not detected

Table 8. Molar ratios of the main neutral sugars present in free EPS samples

Cultures grown with MS coupons			
	Glucose :	Mannose :	Galactose
P. fluorescens	1.0	2.5	0.1
D. desulfuricans	1.0	12.0	0.2
P. fluorescens and *D. desulfuricans*	1.0	10.0	tr
Cultures grown with SS coupons			
	Glucose :	Mannose :	Galactose
P. fluorescens	1.0	3.0	0.03
D. desulfuricans	1.0	5.0	0.20
P. fluorescens and *D. desulfuricans*	1.0	3.5	0.10
Cultures grown with no coupons			
	Glucose :	Mannose :	Galactose
P. fluorescens	1.0	3.0	0.02
D. desulfuricans	1.0	11.5	0.40
P. fluorescens and *D. desulfuricans*	1.0	11.0	0.10

Table 9. Neutral sugar content of EPS obtained from bacterial cultures incubated for 7 days with and without steel coupons

Organism	Sample type	Neutral sugar content of EPS (mg ± SD)
P. fluorescens	MS	6.65 ± 1.85
	SS	4.86 ± 1.02
	Coupon-free	5.64 ± 0.52
D. desulfuricans	MS	1.24 ± 0.27
	SS	0.84 ± 0.28
	Coupon-free	0.39 – 0.04
P. fluorescens and *D. desulfuricans*	MS	2.74 ± 0.55
	SS	2.28 ± 0.14
	Coupon-free	1.63 ± 0.30

EPS from SS-containing cultures when compared with the other two samples.

The total amount of neutral sugars detected chromatographically in EPS recovered from bacterial cultures is given in Table 9. Analysis of variance shows that significantly more neutral sugar was present in EPS recovered from cultures of *D. desulfuricans* incubated with MS or SS coupons compared with that from coupon-free cultures. This observation is also true for EPS samples obtained from mixed bacterial cultures. There was no significant difference in the neutral sugar content of EPS precipitated from *P. fluorescens* cultures incubated with or without steel coupons. The quantity of neutral sugars in EPS from *P. fluorescens* was significantly higher than the level of sugars in EPS of either pure or mixed cultures of *D. desulfuricans* regardless of the culture conditions. The content of neutral sugars in all types of EPS harvested from *D. desulfuricans* cultures was significantly lower than that obtained from mixed cultures.

Analysis of carbohydrates in precipitate from sterile medium C

The percentage of neutral hexoses detected by colorimetric assay in 6.5 mg dry weight of precipitate collected from 500 ml of 7-day-old, sterile medium C was 40% w/w. The types and quantities of sugars detected by GC-FID are presented in Table 10. Mannose and ribose were the main components of the medium C precipitate and they contributed 48% and 34% respectively to the total sugar amount. Glucose (7% of total sugar) and galactose (11% of total sugar) were also present.

The possible contribution of sugars detected in sterile medium C towards the total amount of sugar present in EPS recovered from bacterial cultures incubated with and without steel coupons is shown in Table 11. The possible contribution of sugar present in the precipitate from sterile medium C towards the total level of sugar detected in free EPS samples varied from 1.8% w/w to 30% w/w.

Table 10. Carbohydrates detected in precipitate from sterile medium C

Type of sugar	Quantity (μg/mg ± SD)
Mannose	8.845 ± 3.700
Glucose	1.275 ± 0.110
Galactose	2.048 ± 0.314
Ribose	6.244 ± 1.120
Total	18.312 ± 5.244

Table 11. Possible contribution of sugar detected in precipitate from sterile medium C towards the total amount of sugar present in EPS recovered from bacterial cultures incubated for 7 days with and without steel coupons

Organism	Sample type	Weight of sugar from C (%)
P. fluorescens	MS	1.77
	SS	2.44
	Coupon-free	2.10
D. desulfuricans	MS	9.59
	SS	14.16
	Coupon-free	30.50
P. fluorescens and *D. desulfuricans*	MS	4.34
	SS	5.21
	Coupon-free	7.30

Discussion

These results demonstrate unequivocally the ability of *D. desulfuricans* to produce extracellular polysaccharides. Furthermore, the excretion of polysaccharides is shown to be stimulated by the presence of steel surfaces (Table 9). The analysis of uninoculated medium C (Table 10) indicates that a variable amount of sugars, especially mannose and ribose, can be derived directly from the medium constituents. It is not possible to distinguish unmetabolised medium components from cell products. However, some comparisons may still be made. In previous work on exopolymer production by a *Desulfovibrio* sp. (Ochynski and Postgate 1963), paper chromatography was employed to identify mannose as the only neutral sugar in free EPS. For the SRB strain used in our study mannose also appears to be a dominating, but not the only, sugar in the free polymer. In the presence of MS coupons the exopolymer was particularly enriched in mannose when compared with that from SS cultures (Table 8). This difference in composition of polysaccharide stimulated by different types of metals has been noted previously by Ford et al. (1988). Although EPS of *P. fluorescens* is also rich in mannose, the proportion of this carbohydrate was not altered by the presence of steel surfaces (Table 8), and neither did *P. fluorescens* produce greater amounts of EPS in response to incubation with steel coupons (Table 9).

It has been suggested that the sugar composition of bacterial surface exopolymers is identical to that of polymers found in the liquid phase of the incubating medium (Kennedy and Sutherland 1987). The results of our investigation clearly show that under the experimental conditions chosen the composition of neutral carbohydrates from biofilms and from bulk phases differed (Tables 4 and 7). Whilst mannose was apparently the major sugar component of free EPS (Table 8), biofilm extracts revealed an abundance of glucose (Table 5). The ribose detected in these samples is likely to have originated from cell components, both the bacterial cells and the yeast extract that was a part of the culture medium.

No significant differences were detected in the amount of biofilm produced on any particular coupon recovered from 7-day-old pure cultures of *P. fluorescens* or *D. desulfuricans*, or from mixed cultures of the two species (Table 1). However, the amount of biofilm recovered from SS coupons was always significantly less than that from MS coupons from each of the 7-day-old cultures. The lack of a significant difference between the dry weights of biofilms from *P. fluorescens* and weights of biofilms from pure and mixed cultures of *D. desulfuricans*, in spite of the appearance of SEM micrographs, may be explained by the presence of inorganic material removed from the surface of the coupons during recovery of the biofilms.

The neutral sugar content of biofilms recovered from MS varied depending on species composition and was lower than the amounts obtained from SS coupons (Table 2). Mixed culture biofilms generally had a higher neutral hexose content than biofilms formed by each of the species grown as pure cultures. The biofilms developing in co-cultures of the two bacteria yielded a glucose:galactose ratio that was in between the ratios obtained from the biofilms of each of the pure cultures, indicating a contribution from both species.

Biofilms of both pure and mixed cultures of the two species on MS coupons contained traces of uronic acids when evaluated by colorimetric assay (Table 3). Biofilms of *P. fluorescens*, *D. desulfuricans* and co-cultures of the two species contained 3.7, 5.0 and 5.9 μg uronic acid/mg dry weight, respectively. Uronic acids were not detected in free EPS, emphasising the differences in chemical composition between free and biofilm-bound polysaccharides.

In future studies it is hoped to determine the influence of these bacterial EPS on the corrosion of steel. Geesey et al. (1988) have shown that some bacterial EPS, but not others, can facilitate the corrosion of copper surfaces because of a high binding affinity for cupric ions. In early studies on microbial corrosion Corpe (1975) implicated microbial polysaccharides in the dissolution of metals. Exopolymers from *P. atlantica* have been shown to be aggressive to SS (White et al. 1985). However, apart from a direct effect, bacterial EPS could also influence corrosion indirectly. The formation of biofilm mediated by EPS production may lead to a high acid concentration at the surface thus promoting deterioration of metal. The demonstration of poly-

saccharide production by a member of the sulphate reducers offers the possibility of determining yet one more mechanism by which this group of bacteria may induce corrosion of metal.

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