Gellan-related polysaccharides and the genus *Sphingomonas*

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The biochemical and physiological characteristics of several different existing bacterial isolates which secrete gellan-related polysaccharides were compared. Although they were originally classified into diverse genera, these bacteria are shown here to be closely related to each other and to members of the genus *Sphingomonas*.

Introduction

Several bacteria isolated during the past two decades are known to secrete acidic heteropolysaccharides that contain a common repeating backbone structure related to gellan (Moorhouse, 1987). The common repeating tetrasaccharide is [→3)Glc(β1→4)GlcA(β1→4)Glc-(β1→4)L-Rha or L-Man(β1→], where Glc is glucose, GlcA is glucuronic acid, Rha is rhamnose, and Man is mannose. In gellan (also called PS-60) the fourth sugar is L-Rha, while in polysaccharide NW11 it is L-Man. In polysaccharides PS-88 and PS-198 the fourth sugar is either L-Man or L-Rha. In addition to the common backbone, the gellan-related polysaccharides contain different sidegroups consisting of L-glycerate, *O*-acetyl groups, monosaccharides or disaccharides (Jansson *et al.*, 1983, 1985, 1986; Kuo *et al.*, 1986; Chowdhury *et al.*, 1987*a*, *b*; O'Neill *et al.*, 1990). These related polysaccharides and their bacterial origins are listed as the first ten entries in Table 1.

The bacteria producing gellan and related polysaccharides were originally relegated to diverse genera, including *Pseudomonas*, *Alcaligenes*, *Azotobacter* and *Xanthobacter*. Recently, another bacterium was isolated and shown to secrete a gellan-related polysaccharide with a composition similar to gellan and with the capacity to form gels after deacetylation (Anson *et al.*, 1987). This isolate was tentatively classified as *Pseudomonas paucimobilis*, within a group of bacteria described by Holmes *et al.* (1977). Yabuuchi *et al.* (1990) proposed a new name for this group, *Sphingomonas paucimobilis* gen. nov. and comb. nov., based on sequence similarities of rRNA and the common presence of a rare sphingoglycolipid (Yamamoto *et al.*, 1978; Kawahara *et al.*, 1991). Similarity between the general characteristics of the members of the gellan group of bacteria and of *Sphingomonas* was apparent. For example, all were yellow-pigmented, nonfermentative, Gram-negative rod-shaped bacteria. A re-evaluation of the biochemical and phyisological properties of these bacteria showed that all the producers of gellan-related polysaccharides were closely related and probably members of the genus *Sphingomonas*. In this work the gellan-related polysaccharides are referred to as 'sphingans' after the genus.

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Methods

Bacterial strains and culture media. The original names, polysaccharides produced, sources and references for the bacteria used in this work are listed in Table 1. Luria–Bertani medium (LB) contained 10 g Bacto tryptone, 5 g Bacto yeast extract and 10 g NaCl per litre of water. All Bacto products were from Difco. YM medium contained 3 g Bacto yeast extract, 3 g Bacto malt extract, 5 g Bacto peptone and 10 g Bacto dextrose per litre of water. M9 medium contained 6 g Na_2HPO_4, 3 g KH_2PO_4, 0·5 g NaCl and 1 g NH_4Cl per litre of water, with 10 ml 10 mM-$CaCl_2$ and 1 ml 1 M-$MgSO_4.7H_2O$ added after sterilization by autoclaving. Oxidation–fermentation medium (OF) contained 10 g Bacto peptone, 5 g NaCl and 18 mg phenol red per litre of water and was adjusted to pH 7·2. The water was deionized and 1·5% Difco agar was added for solid medium. Other nutrient plates were Bacto nutrient broth, Bacto TSA (trypticase soy agar) blood agar base (used without blood), Bacto Kligler iron agar (KIA) and Bacto MacConkey agar base.

Physiological tests for strain identification. Utilization of carbon sources was detected by the MicroLog1 system (release 3.00 of Biolog, Inc.); this measures an increase in respiration of cells which in turn causes an irreversible reduction of tetrazolium dye. Bacteria were grown from single colonies on TSA plates for 48 h at 28 °C. Some of the strains studied formed strongly adherent aggregates on plates or rafts of thousands of cells in liquid culture. Cells were partially disaggregated by grinding with a mini-pestle in a 1·5 ml conical microtube containing 0·85% NaCl. The remaining large aggregates were allowed to settle for a few minutes and then separated from the suspended cells. The cell concentration was adjusted to match the turbidity standards supplied by Biolog and then the dispersed cells and small aggregates (< 100 cells per aggregate by microscopy) were

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Table 1. *Source of bacterial strains and exopolysaccharides*

Strain designation*	Original classification	Polysaccharide produced	Reference
ATCC 31461	*Pseudomonas elodea*	PS-60	Kang & Veeder (1982*a*)
ATCC 31554	*Pseudomonas* sp.	PS-88	Kang & Veeder (1985)
ATCC 31554V	*Pseudomonas* sp.	PS-88	This work
ATCC 31853	*Alcaligenes* sp.	PS-198	Peik *et al.* (1985)
ATCC 31853V	*Alcaligenes* sp.	PS-198	This work
ATCC 31555	*Alcaligenes* sp.	PS-130	Kang & Veeder (1982*b*)
ATCC 31961	*Alcaligenes* sp.	PS-194	Peik *et al.* (1983)
ATCC 31961V	*Alcaligenes* sp.	PS-194	This work
ATCC 21423	*Azotobacter indicus*	PS-7	Kang & McNeely (1976)
ATCC 53272	*Xanthobacter* sp.	NW11	Robison & Stipanovic (1989)
ATCC 29837^T	*Sphingomonas paucimobilis*		Holmes *et al.* (1977)
Escherichia coli DH5α			Raleigh *et al.* (1989)
Xanthomonas campestris X55		Xanthan	Cadmus *et al.* (1976)
Pseudomonas aeruginosa 8853			Darzins & Chakrabarty (1984)
Xanthobacter autotrophicus DSM432			Tunail & Schlegel (1974)

* Strains designated ATCC were from the American Type Culture Collection, Rockville, MD, USA; other strains were from the referenced laboratories. V indicates a variant described in the text. ATCC 29837 is the type strain.

transferred to the wells of the GN MicroPlates (Biolog). Each of 95 wells contains a different carbon source and one well lacks a carbon source and is a negative control. The colour for each test well was compared by eye to the control well after 4–6 h and 24 h at 30 °C, and recorded as either ' − ' for no difference, ' + ' for a blue-purple colour darker than the control, or '\' for a borderline reaction. The pattern was compared with a Gram-negative data base including 569 isolates distributed in 82 genera. The program identifies a closest species and calculates a 'similarity index' which quantifies the acceptability of the identification.

Cytochrome oxidase was detected with Kovac's reagent (1 %, w/v, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride from Sigma) on filter paper. A blue colour within 10 s indicated positive oxidase activity. A positive catalase reaction was observed as gas bubbles evolving from a bacterial colony immersed in an equal volume of 3 % (v/v) hydrogen peroxide. Indole was detected with 1 % (w/v) *p*-dimethylaminocinnamaldehyde (Sigma) dissolved in 10 % (v/v) HCl. A positive indole result was a blue-green colour. Hydrolysis of aesculin was shown by a reddish-black halo around colonies on agar plates containing LB medium, 0·1 % aesculin and 0·05 % $FeSO_4$. In addition to these tests, strains were cultured on MacConkey agar plates and Kligler iron agar slants (Koneman *et al.*, 1988). Resistance to antibiotics was tested in liquid cultures of M9 medium containing 1 % (w/v) D-mannose with the antibiotic concentration at 20 μg ml^{-1}. ATCC strains 31461, 31554, 31853, 31555, 31961 and 21423 were cultured overnight and then scored as resistant (full growth), partly resistant (slightly turbid) and sensitive (no growth) compared to controls without antibiotics. Each strain was tested for its ability to grow on various sugars as sole carbon source. The strains were cultured overnight in M9 medium with 0·3 % glucose and then sets of growth tubes containing M9 medium with or without 1 % (w/v) sugar were inoculated with one drop of the overnight cultures for a 1 % (v/v) inoculum.

Fatty acids and pigments. Fatty acids were analysed by the Microcheck Company (Northfield, VT, USA). Bacteria were grown on trypticase soy broth agar, total cellular lipids were saponified, fatty acids were methylated, and the fatty acid methyl esters were separated by gas chromatography and detected by flame ionization.

For pigment analysis each strain was grown at 30 °C for 3–5 d on YM plates. About 100 μl of aggregated cells were scraped from the agar surface and placed in 1·5 ml capped microcentrifuge tubes. Methanol (0·5 ml) was added to extract methanol-soluble pigments at 50 °C for 5 min. After centrifugation at 10000 *g* at 25 °C for 5 min the supernatant was scanned for absorbance with a Beckman DU640 spectrophotometer using a methanol blank.

Results

Metabolic fingerprints

Oxidation of individual carbon sources was detected indirectly by observing the reduction of tetrazolium dye using the MicroLog1 system. Table 2 lists representaive results for each available strain of bacteria known to secrete a sphingan polysaccharide and *Sphingomonas paucimobilis* ATCC 29837, the type strain. The following compounds gave positive results for each strain: dextrin, glycogen, L-arabinose, cellobiose, D-fructose, D-galactose, gentobiose, D-glucose, maltose, D-mannose and methyl pyruvate. These compounds gave negative results for each strain: *N*-acetyl-D-galactosamine, adonitol, D-arabitol, erythritol, *myo*-inositol, D-mannitol, D-sorbitol, formic acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, malonic acid, sebacic acid, L-histidine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D-serine, DL-carnitine, γ-aminobutyric

Table 2. *Oxidation of carbon sources*

For strain ATCC 21423 a 4 h assay was used; for the other strains the assay time was 24 h. +, Positive reaction; \, borderline; −, negative.

ATCC strain no.								Well no. and carbon source	
31461	31554	31853	31555	31961	21423	53272	29837		
−	−	−	−	−	−	−	−	A1	None
+	+	+	−	+	+	−	+	A2	α-Cyclodextrin
\	+	−	+	\	−	\	+	A5	Tween 40
+	+	−	+	\	−	\	+	A6	Tween 80
+	+	+	+	+	−	−	+	A8	*N*-Acetyl-D-glucosamine
\	−	\	−	−	−	−	−	B3	L-Fucose
+	+	+	+	+	\	−	+	B8	D-Lactose
+	+	+	+	+	\	−	+	B9	Lactulose
+	+	\	+	+	+	+	+	C1	D-Melibiose
+	+	+	+	\	+	−	+	C2	Methyl β-D-glucoside
\	+	+	\	\	+	−	+	C3	D-Psicose
+	+	\	−	+	+	−	+	C4	D-Raffinose
+	+	−	−	\	−	+	−	C5	L-Rhamnose
+	+	+	\	+	+	−	+	C7	Sucrose
\	+	+	\	\	+	−	+	C8	D-Trehalose
\	+	+	\	\	+	−	+	C9	Turanose
−	−	−	\	−	−	−	−	C10	Xylitol
−	\	+	\	−	−	+	+	C12	Monomethyl succinate
−	+	−	+	−	+	\	+	D1	Acetic acid
−	−	−	\	−	−	−	+	D2	*cis*-Aconitic acid
−	−	−	\	−	−	−	−	D3	Citric acid
−	−	\	\	−	−	−	−	D5	D-Galactonic acid lactone
−	−	\	+	\	−	−	−	D6	D-Galacturonic acid
−	−	−	\	−	−	−	−	D7	D-Gluconic acid
−	−	−	\	−	−	−	−	D8	D-Glucosaminic acid
+	+	+	+	+	−	−	−	D9	D-Glucuronic acid
−	−	−	\	−	−	+	+	D10	α-Hydroxybutyric acid
+	+	+	+	+	−	+	+	D11	β-Hydroxybutyric acid
+	+	+	+	\	−	+	+	E3	α-Ketobutyric acid
\	−	+	\	−	−	−	−	E4	α-Ketoglutaric acid
\	−	−	+	−	−	+	+	E5	α-Ketovaleric acid
−	−	\	\	\	−	+	−	E6	DL-Lactic acid
−	\	−	+	−	−	+	−	E8	Propionic acid
−	−	\	−	−	−	−	−	E9	Quinic acid
−	−	\	−	−	−	−	−	E10	D-Saccharic acid
+	+	+	+	\	−	−	+	E12	Succinic acid
+	+	+	+	\	−	−	+	F1	Bromosuccinic acid
−	−	−	−	\	−	−	−	F2	Succinamic acid
−	−	−	−	\	−	−	−	F3	Glucuronamide
+	+	+	+	+	+	−	+	F4	Alaninamide
−	\	−	−	\	−	−	−	F5	D-Alanine
+	+	+	+	+	+	−	+	F6	L-Alanine
+	+	+	+	+	+	−	+	F7	L-Alanylglycine
−	−	−	−	\	−	+	+	F8	L-Asparagine
−	−	−	+	\	−	−	+	F9	L-Aspartic acid
+	\	\	+	+	+	\	+	F10	L-Glutamic acid
−	−	\	−	\	−	+	−	F11	Glycyl-L-aspartic acid
\	\	+	+	\	+	+	+	F12	Glycyl-L-glutamic acid
\	−	+	\	+	\	−	+	G2	Hydroxy-L-proline
\	−	−	\	+	−	−	+	G3	L-Leucine
\	\	\	\	+	\	−	+	G6	L-Proline
\	\	−	−	+	+	−	+	G9	L-Serine
\	−	−	−	−	−	−	+	G10	L-Threonine
−	−	−	\	−	−	−	−	H9	Glycerol
−	−	\	−	−	−	−	−	H11	Glucose 1-phosphate
−	−	\	−	−	−	−	−	H12	Glucose 6-phosphate

acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol and DL-α-glycerol phosphate. All the strains except ATCC 53272 were identified by the MicroLog1 program as the species *Pseudomonas* (Sphingomonas) *paucimobilis* A, with similarity indices ranging from 0·75 to 0·91,

Table 3. *Fatty acids of S. paucimobilis and sphingan-producing bacteria*

Strain	Percentage in profile*										
	14:0	14:0 2-OH	15:0	16:0	16:1 *cis*-9	16:1 C	17:0	17:1 C	18:0	18:1 B	18:1 *cis*-11
Yabuuchi†	1·1	24·3	0	10·7	3·0	–	0	1·0	0	0	58·0
	(0·4)	(5·2)	–	(1·1)	(1·5)	–	–	(0·1)	–	–	(3·6)
Stead‡	0·6	7·7	0	14·0	8·2	1·0	0	1·0	0·8	1·1	65·2
	(0·2)	(1·3)	–	(1·8)	(1·3)	(0·1)	–	(0·7)	(0·2)	(0·2)	(1·0)
Microcheck§	1	5	0	9	4	1	–	2	–	2	76
ATCC 31554	0	7	1	18	4	2	0	6	0	0	62
ATCC 31554V	0	8	0	24	2	0	0	3	0	0	63
ATCC 31853	1	14	0	22	0	0	0	0	0	0	61
ATCC 31853V	0	12	0	24	0	0	0	0	0	0	64
ATCC 31961	0	13	0	16	3	1	0	0	0	0	66
ATCC 31961V	0	13	0	15	3	1	0	1	0	0	66
ATCC 21423	0	16	0	12	3	0	0	3	0	0	66

*Shorthand names of fatty acids: 14:0, tetradecanoic (myristic) acid; 14:0 2-OH, 2-hydroxytetradecanoic acid; 15:0, pentadecanoic acid; 16:0, hexadecanoic (palmitic) acid; 16:1 *cis*-9, *cis*-9-hexadecenoic (palmitoleic) acid; 16:1 C, hexadecenoic acid isomer C; 17:0, heptadecanoic (margaric) acid; 17:1 C, heptadecenoic acid isomer C; 18:0, octadecanoic (stearic) acid; 18:1 B, octadecenoic acid isomer B; and 18:1 *cis*-11, *cis*-11-octadecenoic (*cis*-vaccenic) acid.

† Means and (standard deviations) for seven different strains of *S. paucimobilis* (Yabuuchi *et al.*, 1990). Dashes indicate no measurement.

‡ Means and (standard deviations) for four different strains of *S. paucimobilis* (Stead, 1992).

§ Type strain ATCC 29837^T.

where > 0·75 indicates a close fit. Strain ATCC 53272 was identified as *P. paucimobilis* B with a similarity index of 0·60. The subgroups A and B are unofficial designations of Biolog used to indicate that some of their strains of *P. paucimobilis* do not match closely the characteristics of the type strain ATCC 29837, which is in subgroup A.

Fatty acids

As received from ATCC, there were some slight differences in the translucence of colonies among the progeny derived from three strains: ATCC 31554, 31853 and 31961. In order to distinguish between variation of a strain and contamination, these were sent to the Microcheck Corporation for strain identification based on fatty acid analysis. In Table 3 the percentage compositions of fatty acids extracted from these strains are compared with similarly treated standards of *S. paucimobilis*. Each strain was tested once. All the strains showed the major fatty acid as *cis*-11-octadecenoic acid (18:1 *cis*-11; *cis*-vaccenic acid). The second most prevalent fatty acid was hexadecanoic acid (palmitic acid; 16:0). The presence of 2-hydroxymyristic acid (2-OH 14:0) as the only hydroxylated fatty acid is typical of all *Sphingomonas* members. However, the presence of sphingolipid was not investigated in this study. The similarity of the fatty acid profiles for the pairs of strains (ATCC 31554 and 31554V; 31853 and 31853V; 31961 and 31961V) suggested that they were closely related variants. According to the similarity analysis by Microcheck, ATCC strains 31554, 31853, 31961 and 21423 were related to each other at the genus level, and nearly to the species level with *S. paucimobilis*.

Absorbance spectra of pigments

Methanol-soluble pigments extracted from whole cells of each of the sphingan-polysaccharide-secreting isolates showed absorbance spectra as described previously for *P. paucimobilis*, but distinct from *Xanthomonas campestris* (Holmes *et al.*, 1977; Jenkins *et al.*, 1979), which is also yellow in pigmentation. A representative spectrum for each included a major peak centred at 449 to 451 nm, a secondary peak at 475 to 478 nm, and a slight inflection between about 415 and 430 nm. By contrast the methanol-soluble pigments from *X. campestris* showed a major peak at 446 nm, and two slight inflections at about 465 to 480 nm and 420 to 425 nm.

Other characteristics

The seven members of the sphingan group and four control bacterial strains were analysed for other biochemical properties. As shown by the representative

Table 4. *Characteristics of the gellan group of strains and bacterial standards*

Characteristic	Strain*										
	1	2	3	4	5	6	7	Xc	Ec	Pa	Xa
Aesculin hydrolysis	+	+	+	+	+	+	+	+	−	−	−
Growth on MacConkey	−	−	−	−	−	−	−	−	+	−	−
Indole production	−	−	−	−	−	−	−	−	+	−	−
Oxidase (Kovac's)	−	−	−	−	−	−	−	−	−	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+
KIA (slant/butt)											
Growth	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/+	+/−	+/+
pH†	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/A	K/K	K/A
Sugar source‡:											
L-Arabinose	yo	yo	yo	or	yo	yo	y	or	y	r	NT
Fructose	or	o	y	y	r	yo	r	or	y	r	NT
Galactose	y	yo	yo	or	y	y	o	or	y	r	NT
Glucose	yo	yo	y	y	yo	yo	or	or	y	r	NT
Lactose	yo	yo	or	or	yo	r	r	r	y	r	NT
Maltose	yo	yo	yo	y	o	yo	or	or	y	r	NT
Mannose	yo	yo	yo	y	yo	yo	r	or	y	r	NT
Raffinose	o	o	or	r	or	or	r	r	r	r	NT
L-Rhamnose	o	yo	r	o	r	r	or	r	yo	r	NT
Salicin	o	yo	y	y	o	or	r	r	r	r	NT
Sucrose	yo	o	y	yo	o	yo	r	or	r	r	NT
None	r	or	r	r	or	r	r	r	r	r	NT

* Strain abbreviations: 1, ATCC 31461; 2, ATCC 31554; 3, ATCC 31853; 4, ATCC 31555; 5, ATCC 31961; 6, ATCC 21423; 7, ATCC 53272; Xc, *Xanthomonas campestris* X55; Ec, *E. coli* DH5α; Pa, *P. aeruginosa* 8853; Xa, *Xanthobacter autotrophicus* DSM432.

† K, alkaline; A, acidic.

‡ Strains were cultured in OF medium with the indicated sugar (D-form unless noted) at 1 % (w/v) for 24 h at 32 °C. Symbols (colour) and pH: y (yellow) < 6·5; yo (yellow-orange) 6·8; o (orange) 7·1; or (orange-red) 7·4; r (red) > 7·7; NT, not tested.

results in Table 4, all members of the sphingan group hydrolysed aesculin, failed to grow on MacConkey agar, were negative for tryptophanase (indole production) and cytochrome oxidase, and were positive for catalase. Each grew only on the slant on Kligler iron agar tubes and were alkaline throughout. The results in Table 4 also show the pH-indicating colours of cultures grown with various sugar sources. The pattern can be used to distinguish these closely related isolates.

All of the sphingan-producing strains were resistant to 20 μg ml^{-1} of ampicillin, streptomycin, kasugamycin or hygromycin B, and partly resistant to kanamycin. They were sensitive to rifampicin and vancomycin at 20 μg ml^{-1}. ATCC strains 31461, 31554, 31853 and 31961 were partly resistant to both tetracycline and chloramphenicol, while 31555 and 21423 were sensitive to both drugs. Additionally, all the strains were resistant to streptomycin in agar plates at 60 μg ml^{-1}. Lastly, all the strains grew in liquid minimal medium (M9) in the absence of NaCl, and including 0·2 % D-mannose, and ampicillin, streptomycin, kasugamycin and hygromycin B each at 20 μg ml^{-1}. However ATCC 31961 grew less densely.

ATCC strains 31853 and 31555 could grow on any of the following sugars as the sole carbon source: L-arabinose, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannose, D-raffinose, L-rhamnose, salicin, and D-sucrose. However, from this list of sugars ATCC strain 31461 failed to use D-raffinose; 31554 failed to use D-raffinose, L-rhamnose and D-sucrose; 31961 failed to use D-raffinose and L-rhamnose; 21423 failed to use L-rhamnose; and 53272 failed to use D-lactose, D-mannose and salicin, and used D-fructose and D-raffinose only marginally. Only for L-arabinose were the cultures uniformly dense. The patterns of sugar utilization, substrate metabolism and respiration can be used to distinguish between the members of the gellan group.

Discussion

Several bacterial isolates that synthesize structurally related exopolysaccharides were originally classified into diverse genera. A re-examination of the biochemical and physiological characteristics of those bacteria indicates that they are actually closely related to each other and to *Sphingomonas paucimobilis*, previously referred to as *Pseudomonas paucimobilis*. They share with *S. paucimobilis* a specific pattern of oxidization of carbon compounds. In addition their fatty acid profiles and absorption spectra for methanol-soluble pigments are indistinguishable from *Sphingomonas*, and they share several other characteristics. From a biotechnological

standpoint the most important common characteristic is the secretion of sphingan polysaccharides, including gellan, welan and rhamsan. These and other members of the group are the result of a successful screening effort by the Kelco Company for polysaccharides produced by bacteria that might have food and industrial applications (Moorhouse, 1987). At this time we do not know if these isolates belong to one or more of the previously identified species of *Sphingomonas* (Yabuuchi *et al.*, 1990). An additional bacterial isolate that also secretes a sphingan polysaccharide was recently assigned to the genus *Xanthomonas* (Peik *et al.*, 1987). However because it also secretes a sphingan polysaccharide, it may be necessary to look more closely at this assignment when the strain is available for study.

The relatedness between the sphingan-producers and *Sphingomonas* species may have implications for human health. The sphingan-secreting bacteria were isolated from soil and water samples from diverse environmental locations. A few other strains of *P. paucimobilis* were isolated from soil: two with the ability to degrade the biphenyl structures in lignin compounds (Katayama *et al.*, 1988) and polychlorinated biphenyls (Taira *et al.*, 1988); one that degrades the herbicide phosphinothricin (Tebbe & Reber, 1988); and one that may be a root pathogen of lettuce (van Bruggen *et al.*, 1989). However, most of the strains of *S. paucimobilis* were isolated from human clinical specimens and samples of water taken from hospital environments (Holmes *et al.*, 1977; Yabuuchi *et al.*, 1990) and a possible pathogenic role for *Sphingomonas* in humans is emerging (Reina *et al.*, 1991; Decker *et al.*, 1992; Casadevall *et al.*, 1992), but not yet well established. By analogy with alginic acid, the polysaccharide secreted by pathogenic *Pseudomonas aeruginosa*, it is conceivable that acidic sphingan heteropolysaccharides might play a similar role in pathogenesis for *S. paucimobilis*. Also, it may be necessary to modify some industrial methods for producing the useful polysaccharides by fermentation and handling of the dry polysaccharide powders to reduce exposure of operators to airborne contaminants.

Based on this work the capacity for polysaccharide production appears to be a more common characteristic of *S. paucimobilis* than was previously recognized. Recently, two new isolates of *P. paucimobilis* were shown to produce polysaccharides that seem to be related to gellan in sugar composition (Balandreau *et al.*, 1990; Then *et al.*, 1989). A different earlier isolate of *Sphingomonas capsulata* was initially reported to have a capsule but the trait disappeared from the only remaining progeny (Yabuuchi *et al.*, 1990). Exopolysaccharide production is usually most obvious when bacteria are supplied with abundant sugar and with minimal nitrogen. Thus it is possible that other *Sphingomonas* species whose colonies are not obviously mucoid in appearance might also have the capacity to secrete polysaccharides when supplied with the right conditions. Since the existing members of the sphingan group secrete polysaccharides with special and potentially useful commercial properties, it is reasonable to expect that new isolates of *Sphingomonas* might also make commercially useful products with different rheological properties based on slightly different structures.

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