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Production of xanthan gum by *Sphingomonas* bacteria carrying genes from *Xanthomonas campestris*

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Twelve genes coding for assembly, acetylation, pyruvylation, polymerization, and secretion of the polysaccharide xanthan gum are clustered together on the chromosome of the bacterium *Xanthomonas campestris*. These genes (*gumBCDEFGHIJKLM*) are sufficient for synthesis of xanthan gum when placed in bacteria from a different genus, *Sphingomonas*. The polysaccharide from the recombinant microorganism is largely indistinguishable, structurally and functionally, from native xanthan gum. These results demonstrate that a complex pathway for biosynthesis of a specific polysaccharide can be acquired by a single inter-generic transfer of genes between bacteria. This suggests the biological and commercial feasibility of synthesizing xanthan gum or other polysaccharides in non-native hosts.

Keywords: xanthan gum; *Xanthomonas campestris*; *Sphingomonas*; exopolysaccharide; complementation; foreign gene expression

Introduction

The cellular production of a typical bacterial exopolysaccharide (EPS) is a complex process involving nucleotide-sugar substrates, C_{55}-isoprenylphosphate (IP) carriers, sugar-specific glycosyl transferases, modification enzymes, polymerases, and membrane-associated proteins and cofactors [22]. This complexity and our relative ignorance of the molecular events that are involved has made it difficult to genetically manipulate the EPS-producing bacteria in order to alter culture conditions, to introduce new production microorganisms, or to change the EPS structures. In this report we describe the transfer of several genes from *Xanthomonas campestris* into a different genus, *Sphingomonas*, not only to produce xanthan gum, but also to determine which genes are essential and how the complex biosynthetic apparatus operates.

Xanthan gum (Figure 1) is an acidic EPS normally secreted by *X. campestris* [12], and is useful as an aqueous rheological control agent because it exhibits high viscosity at low concentration, pseudoplasticity, and insensitivity to a wide range of temperature, pH, and electrolyte conditions. The genes that code for its synthesis (Figure 2) are tightly clustered on the bacterial chromosome [1,7,23], and the DNA sequence revealed 14 genes, *gumA–N* [3,25]. The functions of the *gum* genes of *X. campestris* were determined by Vanderslice *et al* [25] by determining the structures of oligosaccharide intermediates accumulated by specific mutants. The *gumD, M, H, K* and *I* genes code respectively for the sequential transfer of glucose-1-phosphate, glucose, mannose, glucuronic acid, and finally mannose from nucleotide sugars to IP. Acetylation is determined by the *gumF* and *G* genes, and pyruvylation by the *gumK* gene. The *gumB, C, E* and *J* genes code for undefined steps in polymerization and secretion. Two flanking genes, *gumA* and *N*, appeared to have no role in EPS synthesis.

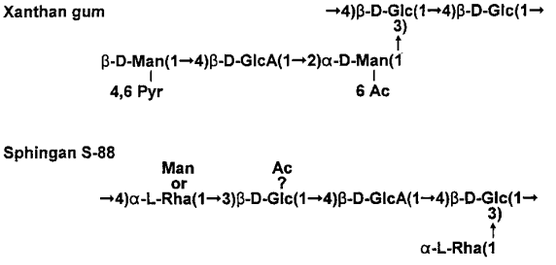

Figure 1 Structures for repeating subunits of xanthan gum and sphingan S-88 [10,11] The arrows point toward the reducing end of each repeat. For xanthan gum the IP carrier is attached at the reducing end through a phosphodiester linkage to the glucose residue which is lacking the side chain [9]. Although it is not known from structural analyses which glucose of the S-88 repeat is initially attached to the IP carrier, genetic experiments suggest the above order (Pollock *et al*, unpublished). Abbreviations: Glc, glucose; Man, mannose; GlcA, glucuronic acid; Rha, rhamnose; Ac, acetyl ester; and Pyr, acetal-linked pyruvic acid. The position and linkage of the Ac in S-88 is unknown.

A different source of commercially significant and functionally diverse biopolymers is the genus *Sphingomonas* [19]. Certain members of this genus secrete gellan, welan, rhamsan, S-88 or other strain-specific exopolysaccharides [17]. We refer to this group of polymers as 'sphingans', after the common genus, because they also have common carbohydrate-backbone structures (-x-glucose-glucuronic acid-glucose-; where x is either rhamnose or mannose) with distinct side chains. The structure for sphingan S-88 is shown in Figure 1. The organization and DNA sequence of the cluster of 23 genes (Figure 2) that direct the synthesis of sphingan S-88 have been described [26]. The functions of the *Sphingomonas* genes were determined by comparing protein sequences that were deduced from DNA sequences to analogous proteins in other EPS-secreting bacteria [26].

EPS synthesis can be restored to an EPS-negative mutant bacterium through genetic complementation, by introducing a normal copy of the defective gene into the mutant cell.

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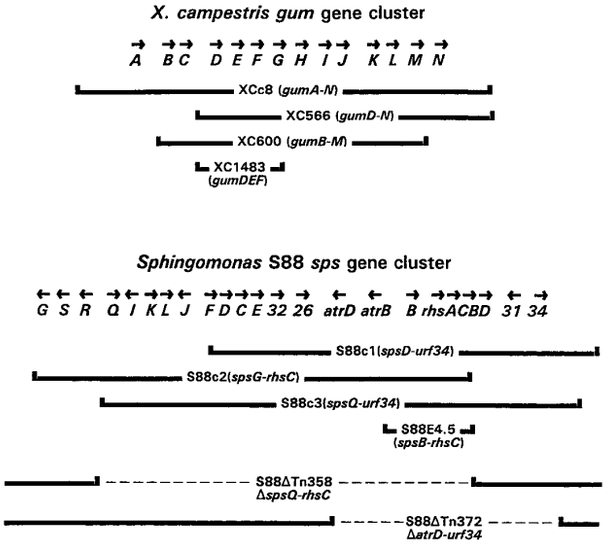

Figure 2 Maps of *X. campestris gum* and *Sphingomonas* S88 *sps* genes, boundaries of specific segments cloned in plasmids, and deletions in bacterial chromosomes. The arrows above the genes indicate the direction of transcription. The horizontal lines indicate the extent of the cloned DNA included in each plasmid with the included genes within parentheses. The dashed lines indicate the regions deleted from mutant chromosomes. The *gumB* through *M* genes (GenBank accession U22511) span about 14 kbp and the *spsG* through *urf34* genes (GenBank accession U51197) include about 29 kbp.

Reciprocal inter-generic complementation between *X. campestris* and either *Rhizobium leguminosarum* [2] or *Sphingomonas* strain S88 [20] has already been demonstrated for genes which code for glucosyl-IP transferases (*X. campestris gumD*, *R. leguminosarum pssA*, and *Sphingomonas spsB*). These enzymes recognize the same substrates and transfer glucose-1P from UDP-glucose to IP. But as expected for the transfer of this single gene, the secreted EPS is the same as the recipient's normal product [20]. By contrast, in this current work we transferred the entire *gumBCDEFGHIJKLM* cluster from *X. campestris* strain B1459 into a specifically mutated *Sphingomonas* recipient, and obtained large amounts of secreted xanthan gum which was largely indistinguishable from that produced by *X. campestris* strain B1459.

Materials and methods

Bacterial strains, recombinant DNA procedures, and growth media

Strain X59 is a spontaneous rifampicin-resistant mutant derived from wild-type *X. campestris* B1459 [23]. Cloned DNA segments and site-specific chromosomal deletions are diagrammed in Figure 2. Plasmid XCc8 is a member of a cosmid library [23] and was obtained by inserting a segment of about 24 kbp of chromosomal DNA from strain X59 into the mobilizable broad-host-range plasmid vector pRK311 [5]. The DNA segments carried by plasmids XC566(*gumD–N*) and XC600(*gumB–M*) were derived from plasmid XCc8 by standard methods of DNA isolation, digestion with restriction endonucleases, and ligation [16]. The insert in plasmid XC600 spans the *Nde*I–*Sal*I segment of XCc8, corresponding to nucleotide 919–15 400 of the *gum* sequence in GenBank accession number U22511. The left end of plasmid XC566 is at nucleotide 3460 (*Bam*HI) and the right end is the same as for XCc8. Plasmid XC1483 includes nucleotide 3460 (*Bam*HI) through 7959 (*Bam*HI) [20]. Plasmids S88c1, S88c2, and S88c3 are members of a cosmid library [26] that were derived from a partial digestion with *Sal*I enzyme of S88 chromosomal DNA, followed by ligation to the vector pRK311. The nucleotides deleted in strain S88ΔTn358 include 4485 (*Bam*HI) through 24 646 (*Eco*RI) in the S88 sequence (GenBank accession U51197). The S88ΔTn372 deletion includes nucleotides 18 812 (*Eco*RI) through about 30 600 (*Eco*RI) [26]. Strain S88m265 is defective in *spsB* [26]. DNA transformation into *E. coli*, tri-parental conjugal mating of broad-host-range plasmids into *Sphingomonas* or *X. campestris*, and selection methods were described [26]. Luria-Bertani and M9 salts are standard media [20]. M9 + YE is M9 salts supplemented with 0.05%(w/v) yeast extract. YM medium contains 3 g Bacto yeast extract, 3 g malt extract, 5 g Bacto peptone, and 10 g D-glucose per liter of deionized water. 1/4 YM-G medium is YM diluted with 3 volumes of water and with no added glucose. However, it still contains a final concentration of 0.07% carbohydrate (mostly maltose and glucose) from the diluted malt extract. The amounts of antibiotics used were as follows: rifampicin, 50 mg L^{-1}; streptomycin, 50 mg L^{-1}; kanamycin, 50 mg L^{-1}; and tetracycline, 6–15 mg L^{-1} (Sigma Chemical Co, St Louis, MO, USA). Low viscosity carboxymethylcellulose (1% w/v final, Sigma Chemical Co) was mixed with TSA blood agar base (Difco, Detroit, MI, USA), and then cultures were spotted onto the surface of the medium and grown for 4–7 days at 30°C. Zones of hydrolysis of carboxymethylcellulose were observed by gently flooding the plate with 0.1% (w/v) Congo red dye for 30 min followed by destaining with 1 M NaCl for 30 min.

Chemical and physical analyses of exopolysaccharides

Extracellular xanthan gum or sphingan S-88 was precipitated from liquid culture medium with 2–3 volumes of isopropyl alcohol, and then dried at 80°C before weighing. Hydrolysis mixtures in 0.6 ml polypropylene microcentrifuge tubes contained 0.5–5 mg of polysaccharide and 130–260 μl 2 M (v/v) trifluoroacetic acid in high performance liquid chromatography (HPLC) water, and were incubated for 16 h at 95°C. The samples were dried under vacuum, resuspended in 100 μl HPLC water, dried again, and finally resuspended in 100 μl of HPLC water. Samples and sugar standards were separated on a CarboPac PA-1 anion-exchange column and the sugar compositions were quantitated with a Dionex DX500 HPLC system as described previously [4]. Assays for acetyl [8] and pyruvyl [6] groups have been described.

Samples of polysaccharides (10 mg, powdered) were dissolved in 2 ml deionized water at 80°C for 60 min in 13×100 mm glass test tubes with an equal weight of locust bean gum and bromphenol blue dye (100 μg ml^{-1}). After cooling for 2 h, the tubes were rotated to horizontal for photography. Failure to gel resulted in horizontal movement of the mixed slurry. A sample of commercial xanthan gum (Keltrol, Kelco Company, San Diego, CA, USA) was

used for physical and chemical comparisons. For xanthan-guar mixtures, each polysaccharide was dissolved in 100 mM KCl at 0.1% w/v, and solution viscosities were measured at 20–25°C at several rpm with a Brookfield LVTDV-II viscometer and spindle 18.

Results

Inter-generic expression of genes coding for polysaccharide biosynthesis

We previously cloned the genes that direct EPS synthesis in *X. campestris* strain B1459 [23] and *Sphingomonas* strain S88 [26] by identifying chromosomal segments in cosmid libraries that could restore EPS synthesis to non-producing mutants. In the current work we transferred the original cosmid clones and specific subcloned segments (diagrammed in Figure 2) by conjugal mating into *X. campestris* and *Sphingomonas* recipients, and then determined the type of EPS secreted into the medium from the appearance of colonies and liquid cultures, and from the physical properties and carbohydrate compositions of the recovered polysaccharides.

As described in Table 1 and the legend, *X. campestris* X59 (Gum^+), *Sphingomonas* S88 (Sps^+) and the polysaccharide-negative mutant S88m265 (Sps^-), have readily distinguished colony morphologies and characteristics in liquid culture. In fact from a visual inspection alone, one can not only determine if EPS is being secreted, but also whether the EPS is sphingan S-88 or xanthan gum. For our particular conditions of sample preparation, acid hydrolysis, and quantitation of monosaccharides, the xanthan gum secreted from *X. campestris* contained the expected two neutral sugars, with glucose representing about 67% and mannose about 33% of the sum of the peak areas for the neutral sugars on the HPLC chromatograms. By contrast, sphingan S-88 contained about 18% rhamnose, 59% glucose, and 23% mannose. These two distinctive patterns of percentages are not expected to match the actual compositional values deduced from the repeat subunit structures shown in Figure 1, and are used here only to distinguish between xanthan gum and sphingan S-88 in different samples prepared by identical methods. For example, the colonial appearance (Sps^+) and composition of neutral sugars from the polysaccharides secreted by the recipient S88m265 carrying plasmid S88c1, indicated that the plasmid, which carries a normal *spsB* gene, restored sphingan S-88 synthesis to the mutant. Plasmid XC1483 with the *X. campestris gumD* gene also restored sphingan S-88 synthesis to S88m265. Both the *gumD* and *spsB* genes code for membrane-bound enzymes that transfer glucose-1P from UDP-glucose to carrier IP [25,26]. A pattern of neutral sugars that appeared to be a mixture of about one-fourth sphingan S-88 and three-fourths xanthan gum was obtained when plasmid XCc8, which carries all twelve of the necessary *gum* genes, was introduced into S88m265. This recombinant strain has all of the genes needed to make both exopolysaccharides. The apparent mixture was in striking contrast to the absence of any EPS synthesis when plasmid XCc8 was mobilized into mutants of S88 which have defects in genes required for secretion of sphingan S-88: S88m76($SpsD^-$), S88m72($SpsC^-$), or S88m41($SpsE^-$) [26]. Apparently a cell can synthesize and secrete two different polysaccharides, but each polymer requires a specific secretion apparatus.

By deleting certain *sps* genes from the S88 chromosome we obtained synthesis in *Sphingomonas* of xanthan gum alone. Although plasmid S88c3 (*sps* genes) restored synthesis of sphingan S-88 to the deletion mutant S88ΔTn358, plasmids XCc8 and XC600 (*gum* genes) caused the synthesis of a polysaccharide that matched the neutral sugar

Table 1 Sugar compositions of bacterial exopolysaccharides

Donor plasmids	Bacterial recipient and phenotype	Recombinant phenotype[a]	Percent of total neutral sugars[b]		
			Rha	Glc	Man
—	*X. campestris* X59 Gum^+		0	67	33
—	*Sphingomonas* S88 Sps^+		18	59	23
S88c1	S88m265 ($SpsB^-$)	Sps^+	19	62	19
XC1483	S88m265 ($SpsB^-$)	Sps^+	22	61	17
XCc8	S88m265 ($SpsB^-$)	Sps/Gum^+	7	64	29
XCc8	S88m76 ($SpsD^-$)	Sps^-			
XCc8	S88m72 ($SpsC^-$)	Sps^-			
XCc8	S88m41 ($SpsE^-$)	Sps^-			
S88c3	S88ΔTn358 (Sps^-)	Sps^+	19	63	19
XCc8	S88ΔTn358 (Sps^-)	Gum^+	0	64	36
XC600	S88ΔTn358 (Sps^-)	Gum^+	0	62	37
XC566	S88ΔTn372 (Sps^-)	Gum^-			
XCc8	S88m134 ($SpsB^-$ $RhsD^-$)	Gum^+			
XCc8	*S. paucimobilis* ATCC 29837	Gum^+			

[a]Gum^+ indicates a wild-type *X. campestris*-like appearance caused by the secretion of viscous xanthan gum, with large (3–5 mm in 4 days at 30°C), shiny, mucoid, light-yellow-colored colonies on solid YM medium and a viscous culture broth in liquid YM medium with non-aggregated cells. *Sphingomonas* acquires the Gum^+ appearance when expressing the *gumBCDEFGHIJKLM* gene cluster. Sps^+ indicates a wild-type appearance typical of *Sphingomonas* strains secreting a capsular sphingan polymer: colonies are opaque to transmitted light, shiny but not viscous, and produce viscous liquid culture broths containing aggregates of cells. Sps^- colonies are more flat, mat-surfaced, and translucent, and grow as single cells in liquid medium.

percentages of xanthan, and lacked rhamnose. Figure 3 is a representative HPLC chromatographic pattern of the monosaccharides obtained from EPS secreted by strain S88ΔTn358 with plasmid XCc8. Plasmid XC566, which is similar to XCc8 but is missing the *gumB* and *C* genes, did not restore xanthan gum synthesis to a similar deletion mutant S88ΔTn372.

From the appearances of recombinant colonies and liquid culture broths, we concluded that plasmid XCc8 caused the synthesis of only xanthan gum in a double mutant of *Sphingomonas* (S88m134) which has defects in glucosyl-IP transferase ($SpsB^-$) and in synthesis of the essential dTDP-rhamnose substrate ($RfbD^-$). Also based on colony and culture appearances, we observed xanthan gum synthesis in the type strain for the *S. paucimobilis* genus, ATCC 29837, which otherwise does not secrete any polysaccharide. Although sugar compositions were not determined, physical studies on this polysaccharide confirmed that it was xanthan gum.

In order to determine if the acetylase (*gumF* and *G*) and pyruvylase (*gumL*) genes of *X. campestris* were functioning in *Sphingomonas*, we measured the amounts of each component for samples of the recombinant and commercial xanthan gums. The degree of acetylation for the recombinant sample (S88ΔTn358 with plasmid XCc8) exceeded that for the commercial xanthan gum by a few percent and was similar to the degree of acetylation for xanthan gum made by *X. campestris* X59 while growing under the same conditions as the recombinant *Sphingomonas*. The recombinant samples were 4–6% by weight as pyruvate compared to 5–6% for commercial xanthan (Keltrol) and xanthan made by *X. campestris* X59.

Physical analyses of recombinant xanthan gum

Three physical properties of recombinant and commercial xanthan gum were compared. First, the viscosity synergism expected for mixtures of xanthan and guar gums was observed for the recombinant samples (Table 2). Solution viscosities were measured for samples with and without added guar gum. The viscosities of the mixtures of guar gum with either commercial xanthan gum or the recombinant samples were higher than the sum of the viscosities of the unmixed polysaccharides. Second, xanthan gum is unique in forming a rigid gel in the presence of locust bean gum. Rigid gels were formed (not shown) by mixing locust bean gum with commercial xanthan gum or with any one of three recombinant samples: plasmid XCc8 in either S88m265, S88ΔTn358, or *S. paucimobilis* ATCC 29837. Third, the viscosity of each recombinant xanthan sample was shear thinning like commercial xanthan gum (results not shown). These three physical tests confirmed that the EPS secreted by the recombinant *Sphingomonas* strains was largely indistinguishable from authentic xanthan gum. However, because we obtained lower viscosities per gram of polymer for the recombinant xanthan gums, the average polymer length for the recombinant xanthan gum may be less than for the commercial product.

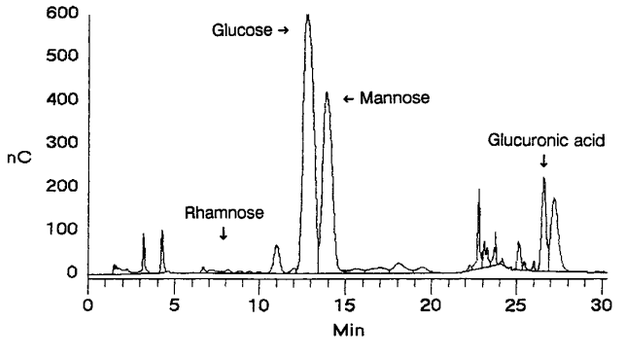

Figure 3 Chromatogram of sugars for xanthan gum made in *Sphingomonas* strain S88ΔTn358 with plasmid XCc8. Abbreviations: Rha, rhamnose; Glc, glucose; Man, mannose; and GlcA, glucuronic acid.

Table 2 Viscosity synergism for mixtures of exopolysaccharides and guar gum

EPS	Viscosity (cp)[a]	
	EPS alone	EPS with guar
None	—	4
xanthan gum	22	49
X59	27	63
S88m265/XCc8	7	18
S88ΔTn358/XCc8	9	27
ATCC 29837/XCc8	7	26

[a]Centipoise (cp) for spindle 18 at 12 rpm for final concentrations of each polymer at 0.1% in 100 mM KCl.

Alternative culture conditions

The results in Table 3 indicate that the recombinant *Sphingomonas* strains, in contrast to *X. campestris*, converted either lactose or glucose to xanthan gum to a similar extent. However, since the shaking flasks and culture media do not provide optimal growth conditions for a viscous fermentation, an assessment of relative productivity (g L^{-1} h^{-1}) is not meaningful at this time.

Production rates and yields for large-scale xanthan gum fermentations are sensitive to temperature and aeration. The highly viscous broth requires considerable stirring and cooling to achieve maximum productivity. Although *X. campestris* produces xanthan gum optimally at about 28°C, *Sphingomonas* strains are known to grow at temperatures up to about 37°C. As shown in Table 3, the recombinant *Sphingomonas* strains grew at 36°C, but less well than at 30°C. However, at 36°C xanthan gum was not secreted into the medium by either *Sphingomonas* host.

The presence of contaminating cellulase in xanthan gum may be significant in commercial applications where xanthan is mixed with or contacts cellulosic polymers. As judged by measuring zones of hydrolysis surrounding cultures spotted onto agar plates containing carboxymethylcellulose, we found that the *Sphingomonas* recombinants showed less than one-eighth of the cellulase activity observed for *X. campestris* strain X59 (not shown).

Discussion

What biosynthetic components are required to synthesize and secrete xanthan gum from *Sphingomonas*? We expected that all *Sphingomonas* strains would have the

Table 3 Cell densities and xanthan gum yields for shake flask cultures

Growth medium, temperature, and sugar substrate	X59		S88ΔTn358 with XCc8		ATCC 29837 with XCc8	
	Density (A600)	Yield (mg)	Density (A600)	Yield (mg)	Density (A600)	Yield (mg)
1/4 YM-G						
30°C glucose	1.1	43	6.0	62	2.9	37
33°C glucose	0.8	34	4.8	49	2.6	33
36°C glucose	0	0	1.2	0	2.3	0
30°C lactose	0.4	16	5.4	67	3.4	39
M9 + YE						
30°C glucose	2.4	84	2.1	30	4.9	55
30°C lactose	0.2	9	2.3	30	6.3	53

1/4 YM-G and M9 + YE were supplemented with either glucose or lactose to 2% w/v. Culture density was measured as the absorbance at 600 nm. The yield of xanthan gum (mg) is the average for samples of 10 ml taken from two separately inoculated flasks after 48 h (1/4YM-G) or 42 h (M9 + YE). The cultures were centrifuged to remove cells before precipitation of the polysaccharides with alcohol.

nucleotide-sugar precursors needed to synthesize xanthan gum since they already synthesize glycosphingolipids which contain D-mannose, D-glucuronic acid, D-*N*-acetyl-glucosamine, and D-galactose [13]. Since UDP-D-glucose is probably the immediate precursor of UDP-D-glucuronic acid, it should also be present. We also expected the amount of carrier IP to be sufficient in *Sphingomonas* since it is recycled during the polymerization of polysaccharides. Also required are enzymes for the assembly of the penta-saccharide-repeat subunit of xanthan gum on the carrier IP, modification enzymes including an acetylase and pyruvylase, and a secretory apparatus. Although we know that both bacterial genera have relatively high guanosine plus cytosine nucleotide contents, little is known about the requirements for efficient gene expression in either bacterium. Nevertheless, we found that the necessary enzymes and secretion factors were expressed from the plasmid-carried *gumBCDEFGHIJKLM* gene cluster in two different recombinant *Sphingomonas* strains, and as judged by the amounts of xanthan gum made by the recombinant *Sphingomonas*, all of the substrates and cofactors were available.

We believe that the SpsD, SpsC, SpsE, SpsJ, and SpsS proteins of *Sphingomonas* and the GumB, GumC, and GumJ proteins of *X. campestris* are essential for secretion of the respective polysaccharides. This is based on similarities in the amino acid sequences between the *Sphingomonas* and *X. campestris* proteins and putative analogues in other EPS-secreting bacteria (*Rhizobium*, *Hemophilus*, and *Escherichia*) [26]. For example, the SpsD protein is partially homologous to GumB, and likewise SpsC to GumC. It was noteworthy that the *X. campestris* secretion genes (*gumB*, *C*, and *J*) appeared to function normally in *Sphingomonas* for secretion of xanthan gum. This was despite the extensive compositional differences between the inner membrane fatty acids of *X. campestris* and *Sphingomonas* [21], and the substitution of glycosphingolipids for lipopolysaccharides in the outer membranes of *Sphingomonas* [13]. Furthermore, the secretion components were found to be specific for each EPS. The *gumB*, *C*, and *J* genes did not overcome the defects in the $SpsD^-$, $SpsC^-$, or $SpsE^-$ mutants of *Sphingomonas*. And conversely *spsD*, *C*, *E*, *J*, or *S* did not substitute for the missing *gumB* and *C* genes on plasmid XC566. The lack of complementation among the secretion proteins could be due to defective interactions or interference among the different proteins themselves, or to the inability of the proteins to interact with a foreign EPS.

We also showed that the *gumA* and *N* genes that flank the *gumB–M* cluster are not essential for xanthan synthesis in *Sphingomonas*. After we submitted the *gum* DNA sequence [3] to GenBank as accession No. U22511, we discovered by comparing deduced amino acid sequences that the GumA protein was similar to integration host factor (IHF) of *Pseudomonas aeruginosa*, but with considerable differences at the carboxyl terminus. IHF binds to specific sites on DNA and influences recombination and initiation of transcription [18]. After sequencing the DNA for this region on our clone XCc8, two frame shift errors in the original DNA sequence were apparent. The sequence corrections suggested a gene product with 78% identity to IHF and with a similar carboxyl terminus. The GumA (IHF) protein of *X. campestris* may have a role in xanthan synthesis, but this has not yet been shown. The apparent lack of a requirement for *gumA* in the recombinant *Sphingomonas* may only be due to the likely presence of an analogous IHF protein in *Sphingomonas*. The GumN protein has no similarity to any other known protein sequence and appears not to be essential. Likewise, other segments of the XCc8 clone outside the *gumB–M* region also appear to be unnecessary for abundant synthesis of xanthan gum in *Sphingomonas*.

The commercial production of highly viscous xanthan gum and other bacterial polysaccharides is a complex biosynthetic and process-engineering problem [14]. The sugar substrate is important primarily because the sugar affects productivity, but the cost of the sugar can also be significant. Currently, xanthan gum is produced by supplying *X. campestris* with corn syrup or starch. Yet, three to four typical cheese factories can provide enough low-value lactose-containing waste whey to produce all the existing xanthan gum worldwide. This assumes 100 000 gallons of whey per day per factory, 4% (w/v) lactose in whey, and

40 million pounds of xanthan produced per year. Previously, we showed that when a foreign β-galactosidase gene is inserted into the chromosome of *X. campestris*, the recombinant strain can stably convert lactose into xanthan gum in amounts equal to the conversion of glucose [24]. Here we have demonstrated an alternative genetic approach, insertion of the *gum* genes into a lactose-utilizing host, to allow the use of inexpensive waste lactose for xanthan gum production.

In addition to the choice of sugar substrate, growth temperature and secretion of enzymes from the xanthan-producing bacterium (see Results), there are two other reasons to consider alternative organisms for production of xanthan gum. First, *X. campestris* is an obligate aerobe and stops producing xanthan gum when oxygen is below a threshold. The highly viscous nature of this fermentation increases the mechanical and energetic difficulty of supply oxygen. An attempt was previously made to synthesize xanthan gum under anaerobic conditions by transferring the *gum* genes to denitrifying bacteria, but only very small yields (0.1–0.2 g L^{-1}) were obtained (MR Betlach *et al*, 1989. Recombinant DNA mediated biosynthesis of xanthan gum in denitrifying pseudomonads under anerobic conditions. Amer Inst of Chem Eng, Annual Meeting). It is possible that certain *Sphingomonas* strains will prove more tolerant of low oxygen levels since they were recovered from deep subsurface environments [15]. However, these have not yet been tested as alternative production organisms. Second, as a typical Gram-negative bacterium, *X. campestris* produces an endotoxin in membrane lipopolysaccharide. However, the cell membranes of *Sphingomonas* species contain glycosphingolipids in place of lipopolysaccharide and have no endotoxin [13]. Finally, since the existing and potential commercial applications for microbial polysaccharides are many and diverse, additional considerations which are not currently recognized may arise in the future to favor alternative production organisms.

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