Evidence for a role for the *gumB* and *gumC* gene products in the formation of xanthan from its pentasaccharide repeating unit by *Xanthomonas campestris*

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The biosynthesis of the extracellular polysaccharide xanthan in *Xanthomonas campestris* pv. *campestris* is directed by a cluster of 12 genes, *gumB–gumM*. Several xanthan-deficient mutants of the wild-type strain 8004 have previously been described which carry Tn5 insertions in this region of the chromosome. Here it is shown that the transposon insertion in one of these mutants, strain 8397, is located 15 bp upstream of the translational start site of the *gumB* gene. EDTA-treated cells of strain 8397 were able to synthesize the lipid-linked pentasaccharide repeating unit of xanthan from the three nucleotide sugar donors (UDP-glucose, GDP-mannose and UDP-glucuronic acid) but were unable to polymerize the pentasaccharide into mature xanthan. A subclone of the *gum* gene cluster carrying *gumB* and *gumC* restored xanthan production to strain 8397 to levels approximately 28% of the wild-type. In contrast, subclones carrying *gumB* or *gumC* alone were not effective. These results are discussed with reference to previous speculations, based on computer analysis, that *gumB* and *gumC* are both involved in the translocation of xanthan across the bacterial membranes.

Keywords: xanthan biosynthesis, *Xanthomonas campestris*, *gumB* and *gumC* genes, pentasaccharide precursor

INTRODUCTION

The bacterial plant pathogen *Xanthomonas campestris* produces an extracellular polysaccharide (EPS) called xanthan, which is of great industrial importance. Xanthan is a polymer comprising repeating pentasaccharide units with the structure mannose-1,4- β -glucuronic acid-1,2- β -mannose-1,3- α -cellobiose (Jansson *et al.*, 1975). The pentasaccharide units are derivatized with acetyl and pyruvyl moieties (Cadmus *et al.*, 1976; Stankowski *et al.*, 1993). *In vitro* studies have indicated that the biosynthesis of xanthan gum occurs in at least two stages (Ielpi *et al.*, 1993). In the first, the repeating unit is sequentially assembled linked to a polyprenol through a diphosphate bridge. In the second

stage, the repeating units are polymerized and the polymer liberated into the growth medium.

The genes that encode the enzymes involved in the transfer of the sugars and of the non-glycosidic substituents are located in a cluster (Barrère *et al.*, 1986; Harding *et al.*, 1987; Thorne *et al.*, 1987) which comprises 12 predicted ORFs, *gumB–gumM* (Capage *et al.*, 1987; Vanderslice *et al.*, 1990). Transcriptional analysis has shown that the *gum* genes are mainly expressed as an operon from a promoter upstream of the first gene, *gumB* (Katzen *et al.*, 1996). Secondary (weak) promoters may exist upstream of *gumK* (Katzen *et al.*, 1996) and *gumD* (Vanderslice *et al.*, 1990; Pollock *et al.*, 1994). The function of some of the gene products has been established. GumD, GumH, GumI, GumK and GumM are known to be involved in the assembly of the pentasaccharide lipid intermediate. GumL is the pyruvate ketal transferase (Marzocca *et al.*, 1991). GumB, GumC, GumE and GumJ are speculated to have a role in the polymerization and translocation of xanthan (Van-

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Abbreviation: EPS, extracellular polysaccharide.

derslice *et al.*, 1990; Paulsen *et al.*, 1997). Direct evidence for the role of these latter gene products is lacking, perhaps in part due to the apparent lethal effects of transposon insertions in some of these genes (Capage *et al.*, 1987; Vanderslice *et al.*, 1990; Katzen *et al.*, 1996).

Xanthan-deficient mutants of *X. campestris* pv. *campestris* have been derived from the wild-type strain 8004 by Tn5 mutagenesis (Barrère *et al.*, 1986). In this paper we report further genetic and biochemical analyses of one of these mutants, strain 8397, which provide evidence for a role for *gumB* and *gumC* in the formation of high-molecular-mass xanthan from its penta-saccharide repeating unit.

METHODS

Strains and microbiological techniques. The *X. campestris* wild-type strain 8004 (Turner *et al.*, 1984) and xanthan-deficient mutant 8397 (Barrère *et al.*, 1986) were grown in PYM medium as reported by Cadmus *et al.* (1976). The *Escherichia coli* strain used in transformation was DH5 α (Sambrook *et al.*, 1989) and was grown at 37 °C in Luria-Bertani (LB) medium (Maniatis *et al.*, 1982). Mating procedures were as described by Turner *et al.* (1984, 1985). When necessary, appropriate antibiotics were added: kanamycin (50 μ g ml⁻¹), rifampicin (50 μ g ml⁻¹) and tetracycline (5 and 10 μ g ml⁻¹ to *X. campestris* and *E. coli* cultures, respectively). To detect DNA inserts cloned for β -galactosidase interruption of α -complementation on plates, X-Gal and IPTG were added to solid medium.

DNA manipulations. Preparation of plasmid DNA from *E. coli*, agarose gel electrophoresis, cloning procedures and Southern hybridization were carried out following established protocols (Maniatis *et al.*, 1982). Total DNA from *X. campestris* was isolated according to Hull *et al.* (1981) and *X. campestris* plasmid DNA was isolated as reported by Harding *et al.* (1987).

Subclones of the *gum* gene cluster. Subclones of the *gum* gene cluster carrying *gumB* alone or *gumB* and *gumC* together were obtained by PCR using as template pIZD15-261 which carries the entire *gum* cluster cloned in cosmid pRK311 (Katzen *et al.*, 1996). The primers used to amplify *gumB* alone were P1 (5' CCCCAAGCTTCCAACGCCGTCAAAA 3') and P2 (5' GAAGATCTGCGACGAAGAGGAACGA 3'). To amplify the *gumBC* genes the primers were P1 and P3 (5' GAAGATCTGCGGTATCGACGATGGT 3'). Primer sequences were derived from the sequence of the *gum* gene cluster determined by Capage *et al.* (1987). The conditions of PCR (30 cycles) were: 1 min at 94 °C, 2 min at 55 °C and 1 min at 72 °C, followed by 15 min at 72 °C. The PCR fragments were cloned into pGEM-T (Promega). The insert DNA was excised by digestion with *Hind*III and *Bgl*II and ligated into pRK404 (Ditta *et al.*, 1985) cut with *Hind*III and *Bam*HI to produce pAV1 (*gumB*) and pAV2 (*gumBC*). To obtain *gumC* alone, a deletion was created in the *gumB* gene in the pGEM-T derivative carrying *gumB* and *gumC*. This plasmid was cut with *Bam*HI and *Eco*RI (which are both unique sites), the ends were filled and the plasmid religated. The insert DNA was excised by digestion with *Hind*III and *Bgl*II and ligated into pRK404 cut with *Hind*III and *Bam*HI to produce pAV3 (*gumC*). The construction of this deletion derivative was checked by sequencing.

DNA sequencing to determine the site of Tn5 insertion in strain 8397. The 1.9 kb *Eco*RI fragment containing the Tn5

insert was cloned in pUC19 and sequenced with a Sequencing Kit from Pharmacia-LKB using primers based on the sequence of *gumB*.

Chemicals. UDP-D-[¹⁴C]glucose (UDP-[¹⁴C]Glc, 300 Ci mol⁻¹, 11.1 TBq mol⁻¹), UDP-D-[¹⁴C]glucuronic acid (UDP-[¹⁴C]-GlcA, 300 Ci mol⁻¹, 11.1 TBq mol⁻¹) and GDP-D-[¹⁴C]-mannose (GDP-[¹⁴C]Man, 238.9 Ci mol⁻¹, 8.8 TBq mol⁻¹) were prepared as described previously (Couso *et al.*, 1980; García *et al.*, 1974). UDP-glucose, GDP-mannose and UDP-glucuronic acid were purchased from Sigma. Restriction enzymes and T4 DNA ligase were obtained from New England Biolabs. Enzymes were used under the conditions specified by the suppliers.

In vitro analysis of biosynthesis of lipid intermediates and xanthan. *In vitro* analysis of xanthan biosynthetic steps was done radiochemically using bacterial cells treated with EDTA (Ielpi *et al.*, 1981a). Cells were grown as described by Cadmus *et al.* (1976) and harvested by centrifugation at late-exponential phase. The cell pellet was resuspended in 0.01 M EDTA/Tris buffer (pH 8.2), and frozen and thawed several times before use.

Assay procedure. The standard incubation mixture contained 70 mM Tris/HCl buffer (pH 8.2), 5 mM MgCl₂, EDTA-treated cells (about 0.6–0.8 mg protein), and UDP-[¹⁴C]Glc (17 μ M) or UDP-[¹⁴C]GlcA (17 μ M) or GDP-[¹⁴C]Man (17 μ M), as indicated in each case. The unlabelled sugar nucleotides, UDP-Glc, UDP-GlcA and GDP-Man (357 μ M each) were added where appropriate. The total reaction volume was 70 μ l. The reactions were performed for 30 min at 20 °C and were stopped by adding 0.5 ml 70 mM Tris/HCl buffer (pH 8.2) containing 30 mM EDTA. The mixtures were centrifuged in an Eppendorf centrifuge at 14000 *g* for 2 min and the pellets were resuspended and washed twice with Tris/HCl buffer without EDTA.

For analysis of xanthan production, the combined supernatants were hydrolysed at pH 2 (see below) to destroy the excess of sugar nucleotide donors, and dialysed twice against 70 mM Tris/HCl (2 l) for 2 h and then overnight against distilled water. The dialysed samples were concentrated under reduced pressure before gel filtration analysis on BioGel A 5m (Bio-Rad) to determine polymer formation. The column (110 $\times$ 1.2 cm) was equilibrated and eluted with 0.1 M pyridinium acetate buffer (pH 5.0), 0.5 ml fractions were collected at a flow rate of 0.25 ml min⁻¹ and 0.1 ml aliquots were taken for counting of radioactivity. Material in the exclusion volume for high-molecular-mass materials has been previously characterized and shown to be xanthan (Ielpi *et al.*, 1993).

For analysis of lipid intermediates, the washed cell pellets were extracted three times with 0.2 ml chloroform/methanol/water (1:2:0.3, by vol.) This extract, which contains the polyprenol-linked [¹⁴C]oligosaccharides (Ielpi *et al.*, 1981) will be referred to as the organic solvent extract.

Chemical treatments. Mild acid hydrolysis was carried out at pH 2 (0.01 M HCl) and 100 °C for 10 min (Couso *et al.*, 1982). Cyclic phosphoric esters and diphosphoric esters were cleaved by treatment at pH 1 (0.1 M HCl) and 100 °C for 10 min. Total hydrolysis was performed with 1 M HCl at 100 °C for 16 h in sealed tubes.

Chromatography and electrophoresis. Paper chromatography and electrophoresis techniques, as well as the location procedures for the different compounds, were as described previously (Couso *et al.*, 1980; García *et al.*, 1974). The following solvents were used: solvent A, pyridine/acetic

acid/water (1:0.04:9, by vol.), pH 6.5; solvent B, 2-propanol/acetic acid/water (27:4:9, by vol.); solvent C, 0.1 M sodium molybdate, pH 5.0. Radioactivity was detected with a Packard radiochromatogram scanner, model 7201 (Packard).

DEAE-cellulose column chromatography was on a column (1 × 60 cm) equilibrated with methanol/water (99:1, v/v) and was eluted with a linear gradient of ammonium acetate (0–2 M) in the same solvent in a total volume of 400 ml, as reported previously (García *et al.*, 1974). The radioactivity in 0.25 ml aliquots of each fraction (3 ml) were counted as described by Bray (1960) with a 1214 Rackbeta Wallac liquid scintillation counter.

Estimation of xanthan production in liquid cultures. To estimate xanthan production, strains were cultured at 28 °C in 50 ml PYM liquid medium supplemented with 2% (w/v) glucose in 250 ml flasks, using an orbital shaker rotating at 200 r.p.m. The cells were removed by centrifugation (25 000 *g* for 30 min), the supernatant fluids were supplemented with KCl at 1% (w/v) final concentration and 2 vols ethanol were added. The precipitated crude xanthan was collected, dried and weighed. The cells were washed by centrifugation in distilled water. The weight of cells after removal of all excess water was taken as wet weight. Duplicate measurements of xanthan production and wet weight were made.

RESULTS

Location of the Tn5 insertion in the xanthan-deficient mutant strain 8397

The xanthan-deficient *X. campestris* strain 8397 is a derivative of wild-type strain 8004 which carries a Tn5 insertion in the *gum* cluster of genes, specifically within a 1.9 kb *Eco*RI fragment (Barrère *et al.*, 1986). Analysis of the restriction fragment pattern of the *gum* gene cluster suggested that the insertion was in the 1.9 kb *Eco*RI DNA fragment that carries the *gumB* gene. This

location was confirmed by Southern blotting (data not shown). To determine the exact site of insertion, the 7.7 kb *Eco*RI fragment from the mutant 8397, comprising the original 1.9 kb fragment and the Tn5 transposon (5.8 kb), was isolated, cloned into pUC19 and subjected to sequencing using primers based on the sequence of *gumB*. This sequence analysis located the Tn5 insertion 15 bp upstream of the translation initiation site of the *gumB* gene (Fig. 1). Although an insertion at this location might be expected to have polar effects on the expression of all of the genes of the *gum* operon (Katzen *et al.*, 1996), subsequent examination of the various steps in xanthan biosynthesis by *in vitro* radiochemical methods suggested that this was not the case.

Radiochemical analysis of xanthan biosynthetic steps in the wild-type strain 8004 and mutant strain 8397

EDTA-treated cells of the wild-type strain 8004 showed substantial incorporation of radioactivity from UDP-[¹⁴C]Glc, UDP-[¹⁴C]GlcA or GDP-[¹⁴C]Man into both the organic solvent extract (lipid-linked intermediates) and polymer fractions (Table 1). In contrast to strain 8004, the mutant did not incorporate any radioactivity into the polymeric fraction, although levels of incorporation into the organic solvent fraction were comparable to those obtained with the wild-type strain (Table 1).

The radiolabelled materials in the organic solvent extract of strain 8397 labelled from UDP-[¹⁴C]Glc were subjected to paper electrophoresis in solvent A both before and after mild acid hydrolysis (pH 2, 10 min, 100 °C). Although the unhydrolysed material remained at the origin of the electrophoretogram (Fig. 2a i), after mild acid hydrolysis five labelled components were resolved (Fig. 2b i). The major component behaved as a neutral compound on paper electrophoresis. On paper chromatography in solvent B, this material had the mobility of monomeric glucose. The two fastest moving components on paper electrophoresis had the mobilities of glucose phosphate and glucose cyclical phosphate. All three of these components are likely to have been released by mild acid hydrolysis from a lipid-diphosphate glucose (Ielpi *et al.*, 1981a, b, 1983). The component with mobility relative to UMP (R_{UMP}) of 0.6 was subjected to paper chromatography in solvent B (Fig. 2c i). Two components with the expected mobilities of the pentasaccharide repeating unit and its acetylated derivative (Ielpi *et al.*, 1981a, 1983) were resolved. The compound with R_{UMP} of 1 was treated at pH 2 for 90 min at 100 °C and then submitted to electrophoresis in solvent A. The major product behaved as the pentasaccharide (R_{UMP} of 0.6). All these properties of the component with R_{UMP} of 1 are consistent with it being the pyruvylated pentasaccharide derivative which has been characterized previously (Ielpi *et al.*, 1981b).

The analysis of radiolabelled components derived from UDP-[¹⁴C]GlcA and GDP-[¹⁴C]Man provided further support for the notion that strain 8397 can synthesize a

Fig. 1. Restriction map of the *gum* gene cluster of *X. campestris* and construction of the subclones used for complementation studies. The restriction sites for *Eco*RI (E) and *Bam*HI (B) and the organization of the genes in the *gum* cluster are shown in the upper part of the figure. The lower part shows the annealing positions of the primers P1, P2 and P3 used to generate the subclones pAV1 and pAV2 by PCR and the position of the *Eco*RI (E) and *Bam*HI (B) sites used to create the deletion derivative pAV3.

Table 1. Incorporation of radioactive sugars into lipid-linked sugars and polysaccharides by strains of *X. campestris*

EDTA-treated cells of wild-type strain 8004 and mutant strain 8397 were incubated with labelled nucleotide sugar donors and incorporation of radiolabel into the organic solvent phase (representing potential lipid-linked intermediates) and into the polymeric fraction (EPS) was measured as described in Methods. Incorporation of label is expressed as c.p.m. (mg protein)⁻¹ and also in parentheses as pmol sugar (mg protein)⁻¹. Values given are the means of three determinations which differed from the mean by <10%.

Radioactive precursor	Strain 8004		Strain 8397	
	Organic phase	EPS	Organic phase	EPS
UDP-[¹⁴ C]Glc	52000 (84)	43400 (70)	38053 (62)	<100 (<0.2)
GDP-[¹⁴ C]Man	62000 (125)	48000 (97)	39200 (79)	<100 (<0.2)
UDP-[¹⁴ C]GlcA	54000 (87)	28200 (46)	42173 (68)	<100 (<0.2)

Fig. 2. Radiochemical analysis of lipid-linked intermediates of xanthan biosynthesis in *X. campestris* strain 8397 using UDP-[¹⁴C]glucose (i), UDP-[¹⁴C]glucuronic acid (ii) or GDP-[¹⁴C]mannose (iii) as labelled sugar donor. EDTA-treated cells were incubated with the labelled sugar donor and unlabelled GDP-mannose and UDP-glucuronic acid (i), UDP-glucose and GDP-mannose (ii) or UDP-glucose and UDP-glucuronic acid (iii). Labelled material incorporated into chloroform/methanol/water (1:2:0.3, by vol.) was analysed by paper electrophoresis in solvent A both before (a) and after (b) mild acid hydrolysis. The material with R_{UMP} of 0.6 released by mild acid hydrolysis was further analysed by paper chromatography in solvent B (c). The positions of markers for adenosine monophosphate (AMP), uridine monophosphate (UMP), glucose (Glc), glucuronic acid (GlcA) and malto-oligosaccharides of degree of polymerization 2–6 (M_2 – M_6) are indicated.

pentasaccharide-lipid intermediate. [¹⁴C]GlcA-labelled materials in the organic solvent phase remained at the origin on paper electrophoresis in solvent A (Fig. 2a ii) but after mild acid hydrolysis most of the material migrated as a pentasaccharide repeating unit (R_{UMP} of 0.6) (Fig. 2b ii). This material analysed by paper chromatography in solvent B indicated the presence of

the pentasaccharide and its acetylated derivatives (Fig. 2c ii). The pyruvylated pentasaccharide derivative (R_{UMP} of 1) was also detected (Fig. 2b ii). A third compound with the mobility of glucuronic acid was also obtained. This compound had the same behaviour as galacturonic acid but not glucuronic acid on paper electrophoresis in solvent C. This galacturonic acid was

Fig. 3. Ion-exchange chromatography on DEAE-cellulose of lipid-linked intermediates in mutant strain 8397 (a) and wild-type strain 8004 (b). EDTA-treated cells were incubated with UDP-[^{14}C]glucose and unlabelled UDP-glucuronic acid and GDP-mannose as described in the legend to Fig. 2. The column was eluted with a gradient of ammonium acetate in methanol/water (99:1, v/v).

probably liberated from a lipid-bound galacturonide as has already been described (Baldessari *et al.*, 1990). Organic-solvent-phase materials labelled with ^{14}C -man-

nose from GDP-[^{14}C]Man also remained at the origin on paper electrophoresis in solvent A (Fig. 2a iii). However, after mild acid hydrolysis, labelled components with the mobility on paper electrophoresis of the pentasaccharide and pyruvylated pentasaccharide were obtained (Fig. 2b iii). On paper chromatography in solvent B, the major component appeared to be the pentasaccharide (Fig. 2c iii).

The ^{14}C -glucose-labelled components in the organic solvent phase were also analysed by DEAE chromatography. Three compounds eluting at 0.5, 0.8–0.9 and 1.1 M ammonium acetate were observed (Fig. 3a). These components were separately hydrolysed with mild acid and were analysed by paper electrophoresis and paper chromatography as described above. This analysis showed that the compounds eluting at 0.5, 0.8–0.9 and 1.1 M ammonium acetate were lipid diphosphate glucose, lipid diphosphate pentasaccharide and lipid pyruvylated pentasaccharide, respectively (data not shown). These three components were also seen in chromatograms of ^{14}C -glucose-labelled components of the wild-type (Fig. 3b), although the components were present in different proportions.

Restoration of xanthan biosynthesis by subclones of the *gum* gene cluster

The biochemical analysis of strain 8397 indicated that although the mutant was capable of synthesis of the pentasaccharide lipid intermediate it could not synthesize xanthan. Synthesis of the pentasaccharide-lipid intermediate requires the action of genes downstream of *gumB* and *gumC*, which suggests that the effects of the Tn5 insertion 15 bp upstream of the translation initiation site of *gumB* are not polar. We reasoned that this was likely to be due to the action of secondary promoters internal to the *gum* gene cluster, in particular that suggested to lie upstream of *gumD*, which however was not detected by other workers (Katzen *et al.*, 1996).

Table 2. Production of EPS by strains of *X. campestris*

The *in vivo* production of EPS by different strains was determined gravimetrically after ethanol precipitation from the culture supernatant and is expressed both in terms of the volume of culture and of the wet weight of cells. Production of EPS was also measured *in vitro* by following the incorporation of [^{14}C]GlcA from UDP-[^{14}C]GlcA by EDTA-treated cells. Values given are the means of two determinations which differed from the mean by <5%. The experiment was repeated twice with essentially the same results. NA, Not available.

Strain	Relevant genes carried on plasmid	EPS production <i>in vivo</i>		Incorporation of GlcA [pmol (mg protein) $^{-1}$]
		g (l culture) $^{-1}$	mg (g wet wt cells) $^{-1}$	
8004		6.46	420.0	42.0
8397		0.08	4.8	<0.2
8397/pAV1	<i>gumB</i>	0.10	6.6	<0.2
8397/pAV2	<i>gumB</i> , <i>gumC</i>	1.76	119.0	14.8
8397/pAV3	<i>gumC</i>	0.03	4.2	NA
8397/pIZD15-261	<i>gumB</i> –M	7.40	480.0	47.5

These phenotypes of the mutant suggested that we could use strain 8397 as a tool to test the proposed roles of the *gumB* and *gumC* gene products in the later steps of xanthan biosynthesis by providing these genes *in trans* either together or separately. Synthesis of xanthan was estimated both by precipitation of the polymer from cultures and radiometrically by following the incorporation of label from UDP-[¹⁴C]GlcA into high-molecular-mass material by EDTA-treated cells. As an initial control, the recombinant plasmid pIZD15-261, which carries the whole *gum* gene cluster (Katzen *et al.*, 1996), was introduced into the mutant. The synthesis of xanthan as estimated by both methods was restored; the levels of xanthan were slightly higher than in the wild-type (Table 2). Although provision of *gumB* or *gumC* genes separately (as the recombinant plasmids pAV1 and pAV3) had no effect on xanthan production, provision of the two genes together on the plasmid pAV2 allowed restoration of xanthan production to levels approximately 28% of the wild-type (Table 2). The inability of *gumB* alone to complement strain 8397 for xanthan production was not due to errors induced by PCR amplification; sequencing of the amplified DNA showed that it would encode an unaltered GumB protein. These results clearly suggest that GumB and GumC operate together to synthesize xanthan from the pentasaccharide subunit.

DISCUSSION

The mutant strain 8397, which carries a Tn5 insertion immediately upstream of *gumB*, is unable to synthesize xanthan although it can still synthesize the lipid-linked pentasaccharide repeating unit of xanthan. Provision of *gumB* and *gumC* together restores xanthan synthesis to strain 8397 to approximately 28% of wild-type levels. The simplest interpretation of these results is that Tn5 insertion has a polar effect on the expression of *gumB* and *gumC* but that expression of the rest of the *gum* cluster (which is required for the synthesis of the pentasaccharide) is directed from a promoter upstream of *gumD*. The incomplete restoration of xanthan production by complementation with *gumB* and *gumC* together may be a result of lower *in vivo* levels of the pentasaccharide-lipid intermediate in strain 8397 compared to the wild-type. This is suggested by the lower incorporation of radiolabelled sugars into this fraction by EDTA-treated cells of strain 8397 compared to the wild-type.

Polymerization and export of bacterial extracellular polysaccharides are in general poorly understood processes. Although our data implicate GumB and GumC in the processing of the lipid-linked pentasaccharide into mature extracellular xanthan, we cannot define precisely which steps these two proteins mediate. These processing steps may include 'flipping' of the lipid-pentasaccharide intermediate across the cytoplasmic membrane, pore formation, transport through one or both membranes and the polymerization step which incorporates the pentasaccharide into the growing xanthan

chain. A similar situation exists in studies of the biosynthesis of succinoglycan in *Rhizobium* (now *Sinorhizobium*) *meliloti* which appears to have many parallels with the biosynthesis of xanthan in *X. campestris* (Reuber & Walker, 1991; Glucksmann *et al.*, 1993a, b). Nineteen *exo* genes are required for the synthesis of succinoglycan, which is a polymer of octasaccharide units comprising one galactose and seven glucose residues with acetyl, pyruvyl and succinyl modifications. A combination of genetic and biochemical analysis has established precise roles for 15 of the *exo* genes in the biosynthesis of the octasaccharide-lipid intermediate, which is believed to be assembled in the cytoplasm. However precise roles for products of the *exoP*, *exoQ* and *exoT* genes, which are required to process the octasaccharide subunits into the mature polysaccharide, are yet to be established, although all three genes encode membrane-spanning proteins (Müller *et al.*, 1993; Glucksmann *et al.*, 1993b).

On the basis of computer analysis, Paulsen *et al.* (1997) suggested that GumB and GumC are involved in translocation of xanthan across the cytoplasmic and outer membranes. GumB was suggested to be an outer-membrane protein belonging to a small family which includes ExoF of *S. meliloti* and KpsD, a protein known to be involved in translocation of the capsular polysaccharide of *E. coli*. GumC was suggested to be localized in the cytoplasmic membrane and to have both cytoplasmic and periplasmic domains. The cytoplasmic domain bears an ATP-binding motif. It is tempting to speculate from our own data and the work of Paulsen *et al.* (1997) that the polymerization of xanthan is tightly coupled to its translocation across the two membranes such that, in the absence of translocation, synthesis is halted.

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