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Location and Cloning of the Ketal Pyruvate Transferase Gene of *Xanthomonas campestris*

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Genes required for xanthan polysaccharide synthesis (*xps*) are clustered in a DNA region of 13.5 kb in the chromosome of *Xanthomonas campestris*. Plasmid pCHC3 containing a 12.4-kb insert of *xps* genes has been suggested to include a gene involved in the pyruvylation of xanthan gum (N. E. Harding, J. M. Cleary, D. K. Cabañas, I. G. Rosen, and K. S. Kang, J. Bacteriol. 169:2854–2861, 1987). An essential step toward understanding the biosynthesis of xanthan gum and to enable genetic manipulation of xanthan structure is the determination of the biochemical function encoded by the *xps* genes. On the basis of biochemical characterization of an *X. campestris* mutant which produces pyruvate-free xanthan gum, complementation studies, and heterologous expression, we have identified the gene coding for the ketal pyruvate transferase (*kpt*) enzyme. This gene was located on a 1.4-kb *Bam*HI fragment of pCHC3 and cloned in the broad-host-range cloning vector pRK404. An *X. campestris kpt* mutant was constructed by mini-Mu(Tet^r) mutagenesis of the cloned gene and then by recombination of the mutation into the chromosome of the wild-type strain.

Xanthomonas campestris produces an acidic exopolysaccharide, xanthan gum, composed of D-glucosyl, D-mannosyl, and D-glucuronyl acid residues in a molar ratio of 2:2:1 and variable proportions of *O*-acetyl and pyruvyl groups. Its primary structure has been elucidated (24, 29) and comprises a cellulose backbone with trisaccharide side chains, the repeating unit being a pentasaccharide (Fig. 1A). The actual distribution pattern of acetyl and pyruvyl substituents is unknown.

Several genetic loci are involved in the biosynthesis of xanthan gum (2, 16, 34). Harding et al. have shown that mutations affecting xanthan polysaccharide synthesis (*xps*) are clustered in a 13.5-kb region and fall into at least five complementation groups (16). Of particular interest are the functions of the gene products of this *xps* region. It has been reported that pCHC3, a recombinant plasmid derivative of this *xps* region, significantly increases the pyruvate content of the xanthan gum in the wild-type strain. In addition, an *X. campestris* mutant, strain ATCC 31313, which produces essentially nonpyruvylated xanthan gum (Fig. 1B) (32, 35) was complemented by plasmid pCHC3 (16).

Previous studies utilizing an in vitro system demonstrated that the β-mannose-(1,4)-β-glucuronic acid-(1,2)-α-mannose-(1,3)-β-glucose-(1,4)-α-glucose diphosphate lipid (pentasaccharide diphosphate lipid [ps-P-P-lipid]) and its pyruvylated derivative (pyruvyl-pentasaccharide diphosphate lipid [pyr-ps-P-P-lipid]) are intermediates in xanthan gum biosynthesis (21, 22). pyr-ps-P-P-lipid is produced in a reaction that involves the transfer of the pyruvyl residue from phosphoenolpyruvate (PEP) to the external mannose of ps-P-P-lipid, catalyzed by the enzyme ketal pyruvate transferase (KPT):

$$\text{ps-P-P-lipid} + \text{PEP} \xrightarrow{\text{KPT}} \text{pyr-ps-P-P-lipid} + P_i \quad [1]$$

As a consequence of the formation of pyr-ps-P-P-lipid, the pyruvyl residues become incorporated into the xanthan gum (22).

In this article we show that EDTA-treated cells obtained from strain ATCC 31313 synthesize ps-P-P-lipid. However, this mutant is unable to carry out the enzymatic incorporation of pyruvyl substituents into ps-P-P-lipid according to reaction 1 shown above. The results presented here demonstrate that the mutation on strain 31313 resides within the structural gene for the KPT, which we propose to call *X. campestris kpt* gene. We have also studied the expression of the cloned gene in the heterologous hosts *Acetobacter xylinum* and *Escherichia coli* and subcloned a portion of pCHC3 containing the *kpt* gene.

MATERIALS AND METHODS

Bacterial strains and media. The wild-type *X. campestris* strain used was NRRL B-1459 (25). ATCC 31313 is a nitrosoguanidine *X. campestris* mutant that produces essentially pyruvate-free xanthan gum (32, 35). *A. xylinum* PEA-1 is a cellulose-deficient mutant derived from the wild-type strain NRRL B-42 after mutagenesis with nitrosoguanidine. It produces similar amounts of the acidic exopolysaccharide acetan gum as strain NRRL B-42 (8) and will be fully described elsewhere. *E. coli* strains and their relevant characteristics were JZ279 (*recA56 lacY galK2 galT22 metB1 trpR55 supE44 supF58 hsdR514*) (17) and TB1 [*ara thi* (*lac proAB*) ϕ80d*lacZ*ΔM15 *hsdR rpsL* (1)].

X. campestris cells were grown at 28°C in YM medium (16), with kanamycin, rifampin, or tetracycline when required (50, 100, or 10 μg/ml, respectively). *A. xylinum* cells were grown at 28°C in the medium described by Hestrin and Schramm (19), with rifampin or kanamycin when required (100 μg/ml). *E. coli* strains were grown at 37°C in LB medium, with ampicillin, kanamycin, or tetracycline when required (250, 50, or 15 μg/ml, respectively). Cultures were grown in a gyratory shaker at 200 rpm.

Plasmids and DNA manipulations. Plasmid pCHC3 (Km^r)

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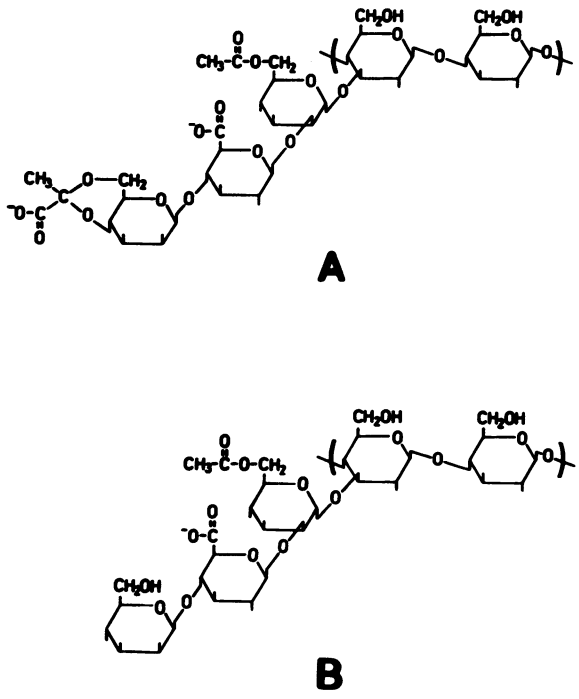

FIG. 1. Structures of the pentasaccharide repeating unit of xanthan gum (A) (24, 29) and of the depyruvylated xanthan gum produced by mutant strain 31313 (B) (32). From right to left, the monosaccharide residues of the pentasaccharide repeating unit are glucose, glucose, mannose, glucuronic acid, and mannose.

is based on broad-host-range pRK293 and carries 12.4 kb of genes essential for xanthan polysaccharide synthesis (16). The plasmid vector used for cloning was pRK404 (Tc^r Tra^-, RK2 replicon) (13). *E. coli* plasmid isolations were performed essentially by the method of Birnboim and Doly (4). Procedures for restriction analysis and ligation have been described elsewhere (28). Subclones were made from CsCl gradient-purified pCHC3, which was digested with *Bam*HI. The 1.4-kb *Bam*HI fragment was gel purified and ligated into the unique *Bam*HI site of the vector pRK404 to yield subclones pBXP3 and pBXP4. Construction of pNH229 and pNH236 was described previously (16). Transformation of *E. coli* cells was performed as described previously (15). Recombinant IncP group plasmids were transferred from *E. coli* to *Acetobacter* and *Xanthomonas* species by triparental matings with the helper plasmid pRK2073 (12). For selection of transconjugants, rifampin-resistant derivatives of *X. campestris* and *A. xylinum* strains were isolated as described by Miller (30).

Transposon and replacement mutagenesis. Insertional mutagenesis of *xps* genes in pCHC3 was done in *E. coli* with the transposon mini-Mu(Tet^r) (3) and was previously described (16). To select for transfer of the transposons to the chromosome of *X. campestris* by homologous recombination, pPH1JI, a self-transmissible IncP plasmid (20), was mated into a rifampin-resistant derivative of B-1459 harboring pCHC3::mini-Mu(Tet^r). Recombinants were selected in YM medium containing rifampin to counterselect *E. coli*, gentamicin (10 μg/ml) and spectinomycin (25 μg/ml) to select pPH1JI, and tetracycline (3 μg/ml) to select for retention of mini-Mu(Tet^r). Strains were verified to be sensitive to kanamycin, indicating the loss of plasmid pCHC3.

Preparation of cell extracts, reaction mixtures, and assay procedures. The enzyme preparations consisted of EDTA-treated cells from either *E. coli*, *A. xylinum*, or *X. campestris*, obtained as previously described (21). The standard reaction mixtures for the synthesis of ps-P-P-lipid or pyr-ps-P-P-lipid contained 70 mM Tris-HCl buffer (pH 8.2), 8 mM $MgCl_2$, EDTA-treated cells (400 to 800 μg of protein), 357 μM UDP-glucose, 142 μM GDP-mannose, 121 nM UDP-[U-^{14}C]glucuronic acid (UDP-[^{14}C]GlcA), and, where indicated, 4.3 mM PEP. The reactions were performed in a total volume of 70 μl at 20°C for 30 min and stopped by adding 0.3 ml of 70 mM Tris-HCl buffer (pH 8.2). The mixtures were vortexed and centrifuged at 6,000 × *g* for 5 min, and the pellets were resuspended and washed two times with the same buffer. The washed pellets were each extracted three times with 150 μl of chloroform-methanol-water (1:2:0.3, vol/vol/vol). This extract, which contains the glycolipids, will be referred to as the 1203 extract.

Analysis of the radioactive components extracted in chloroform-methanol-water. The amount of radioactive label incorporated in a portion of the 1203 extract was determined. The remainder of this fraction was analyzed by ascending chromatography on a solvent system of ethanol (96%)-ammonium hydroxide (7:3, vol/vol), which is equivalent to a mild alkali treatment (9) and converts aldoses joined to a diphosphate group and with a *cis*-hydroxyl in position 2 into their cyclic phosphate esters. In this system, the cyclic-phosphate-ester derivatives of pentasaccharide and pyruvyl-pentasaccharide have similar mobilities (R_f = 0.23 to 0.25) (21, 22). Radioactive compounds on chromatograms were detected by using a model 7201 radiochromatogram scanner (Packard Instruments Co., Rockville, Md.), and those with mobilities of R_f values of 0.23 to 0.25 were eluted from the paper strip with water and subjected to paper electrophoresis in a solvent system of pyridine-acetic acid-water (1:0.04:9, vol/vol; pH 6.5) (9) after UMP was added as the internal standard. The radioactive compounds on electropherograms were located, after observing the UMP standard under UV light, by cutting the paper strip into 1-cm sections and analyzed by liquid scintillation.

Assay for the synthesis of pyr-ps-P-P-lipid from exogenously supplied ps-P-P-lipid. The 1203 extract containing [^{14}C-GlcA]ps-P-P-lipid was obtained as described above by using EDTA-treated cells from either strain B-1459 or 31313 as indicated. The organic solvent was reduced to about a third of its original volume at room temperature under a stream of nitrogen, water was added, and the solvent was again reduced. This procedure was repeated once more, and Triton X-100 was added to the lipid emulsion to give a concentration of 0.01% in the final incubation mixture. The extract containing 8,000 to 11,000 dpm of [^{14}C-GlcA]ps-P-P-lipid was incubated, in the presence or absence of 4.3 mM PEP, for 2 h at 20°C after the addition of Tris-HCl (70 mM, pH 8.2), $MgCl_2$ (8 mM), and EDTA-treated cells from either B-1459 or 31313 (250 to 500 μg of protein). Reaction mixtures were terminated by the addition of 0.7 ml of chloroform-methanol (1:2, vol/vol) and then by two further extractions of chloroform-methanol-water (1:2:0.3). Radioactive glycolipids extracted into the organic phase were subsequently analyzed by ascending chromatography and paper electrophoresis as described above.

Assay for xanthan gum pyruvate. Xanthan gum pyruvate was determined by high-pressure liquid chromatography on an Aminex HPX-87H ion-exclusion column (Bio-Rad Laboratories, Richmond, Calif.) after acid hydrolysis (0.25 M H_2SO_4, 96°C, 4 h) as described by Tait and Sutherland (33).

Radiochemicals and biochemicals. UDP-D-[U-^{14}C]gluc-

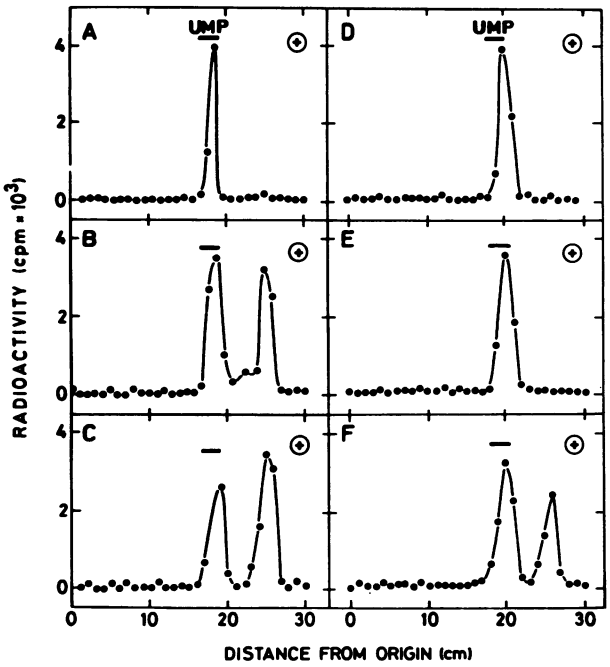

FIG. 2. Comparative analysis of the radioactive glycolipids synthesized in vitro. Standard incubations were performed as described in Materials and Methods by employing EDTA-treated cells of the following strains plus PEP where indicated: B-1459 (A); B-1459 and PEP (B); B-1459(pCHC3) and PEP (C); 31313 (D); 31313 and PEP (E); 31313(pCHC3) and PEP (F). After incubation, the glycolipids were extracted and aliquots were analyzed as described in Materials and Methods. UMP was added as the internal standard.

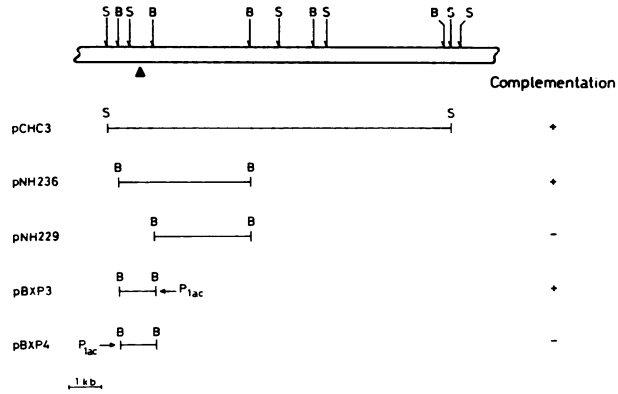

FIG. 3. Cloned inserts used in this study. A portion of the chromosomal *xps* region is shown (empty bar) (16). Inserts of plasmids are shown aligned with the corresponding sites in the genome. The directions of transcription from the vector *lac* promoter are indicated by arrows. The insertion site of mini-Mu(Tet^r) recombined from pCHC3::mini-Mu(Tet^r) into the *X. campestris* genome to construct strain 3972 is marked (▲). The restriction sites relevant to the construction of plasmids are also indicated. S, *Sal*I; B, *Bam*HI. To the right of each plasmid construction are indicated the results of complementation analysis of strain 31313, carried out by high-pressure liquid chromatography determination of pyruvate residues on the respective polysaccharides.

uronic acid, specific activity 285 Ci/mol, was synthesized enzymatically from UDP-[U-^{14}C]glucose by using partially purified UDP-glucose dehydrogenase from calf liver (36). UDP-glucose, GDP-mannose, and PEP (monopotassium salt) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Restriction enzymes and T4 DNA ligase were obtained from New England BioLabs, Inc. (Beverly, Mass.). Enzymes were used under the conditions specified by the suppliers.

RESULTS

In vitro synthesis of lipid intermediates by EDTA-treated cells obtained from wild-type and mutant strains. We investigated the ability of *X. campestris* B-1459 and 31313 to synthesize ps-P-P-lipid and pyr-ps-P-P-lipid by using previously developed in vitro assays (21, 22). Incubation of EDTA-treated cells from strain B-1459 with UDP-glucose, GDP-mannose, and UDP-[^{14}C]glucuronic acid resulted in the incorporation of radioactivity into a product previously characterized as ps-P-P-lipid by partial acid hydrolysis (21) and also by column chromatography and permethylation assays (23) (Fig. 2A). When PEP was added to the incubation mixture, a second radioactive product characterized as pyr-ps-P-P-lipid was recovered from the 1203 extracts in addition to ps-P-P-lipid (22) (Fig. 2B). Parallel experiments with EDTA-treated cells prepared from strain 31313 revealed the incorporation of [^{14}C]glucuronic acid into ps-P-P-lipid (Fig. 2D). However, in contrast to the results observed with extracts obtained from strain B-1459, no incorporation of [^{14}C]glucuronic acid into pyr-ps-P-P-lipid was detected when PEP was added to the incubation mixture (Fig. 2E). These data clearly demonstrate that the synthesis of pyr-ps-P-P-lipid is impaired in the mutant strain. The results also suggest that the defect in pyr-ps-P-P-lipid synthesis is not due to a partial defect in the synthesis of PEP.

Plasmid pCHC3, which harbors a portion of the *X. campestris xps* region as a 12.4-kb *Sal*I insert (16) (Fig. 3), was introduced by conjugation into both strain 31313 and strain B-1459. Analysis of standard incubations resulted in the appearance of KPT activity when EDTA-treated cells of 31313(pCHC3) were used (Fig. 2F). A significant increase in the ratio of pyruvylated to nonpyruvylated ps-P-P-lipid was observed in the case of B-1459(pCHC3) EDTA-treated cells (Fig. 2B and C).

In vitro synthesis of pyr-ps-P-P-lipid by EDTA-treated cells and exogenously supplied radioactive ps-P-P-lipid. We tested the possibility that the inability of strain 31313 to synthesize pyr-ps-P-P-lipid is not due to defective KPT activity but rather that the ps-P-P-lipid synthesized by the mutant might be structurally altered such that it is unable to function as an acceptor of pyruvyl residues. To solve this problem, we developed assay conditions (described in Materials and Methods) that allowed us to study the in vitro activity of the KPT enzyme by using exogenously added radioactive ps-P-P-lipid. [^{14}C-GlcA]ps-P-P-lipid was synthesized in vitro by using EDTA-treated cells of strain 31313 (ps-P-P-lipid^{31313}). After removing the organic solvents from the 1203 extracts, the lipid-linked intermediate was used as an exogenously supplied acceptor of pyruvyl substituents. Incorporation of pyruvyl residues into [^{14}C-GlcA]ps-P-P-lipid^{31313} was observed in reaction mixtures containing EDTA-treated cells prepared from strain B-1459. In contrast, formation of pyr-ps-P-P-lipid was not detectable when [^{14}C-GlcA]ps-P-P-lipid prepared from a wild-type strain and EDTA-treated cells of strain 31313 were used (Table 1). These results demonstrate that ps-P-P-lipid synthesized by strain 31313 is able to function as an acceptor of pyruvyl residues.

TABLE 1. Incorporation of pyruvyl substituents into exogenously supplied [^{14}C-GlcA]ps-P-P-lipid

Enzyme source	Additions[a]	Radioactivity (dpm)[b]
B-1459	[^{14}C-GlcA]ps-P-P-lipid^{31313} + PEP	2,260
B-1459	[^{14}C-GlcA]ps-P-P-lipid^{1459} + PEP	1,680
B-1459	[^{14}C-GlcA]ps-P-P-lipid^{1459}	ND
None	[^{14}C-GlcA]ps-P-P-lipid^{31313} + PEP	ND
31313	[^{14}C-GlcA]ps-P-P-lipid^{1459} + PEP	ND

[a] [^{14}C-GlcA]ps-P-P-lipid was obtained from either strain 31313 or B-1459 and incubated in the presence of EDTA-treated cells and PEP as indicated.
[b] Values indicate the amount of cumulative radioactivity of [^{14}C-GlcA]pyr-ps-P-P-lipid isolated after paper electrophoresis assays as shown in Fig. 2. ND, not detected (less than 50 dpm).

Cloning and expression of *X. campestris kpt* gene in different hosts. The biochemical analysis of strain 31313 indicated a deficiency of the KPT enzyme. The mutation would therefore either be located in the structural gene for this enzyme or in some other gene which affected the activity of the enzyme. To distinguish between these possibilities, we introduced the plasmid pCHC3 into *E. coli* JZ279 and *A. xylinum* PEA-1. Both *E. coli* and *A. xylinum* cell extracts were assayed for KPT activity, by employing PEP and radioactive ps-P-P-lipid as substrates. The results (Table 2) revealed that, in contrast to EDTA-treated cells of *E. coli* JZ279(pCHC3), EDTA-treated cells of *A. xylinum* PEA-1(pCHC3) were able to synthesize pyr-ps-P-P-lipid in vitro. The extent of [^{14}C-GlcA]pyr-ps-P-P-lipid formed was similar to that of *X. campestris* B-1459. These data clearly support the conclusion that the mutation on strain 31313 is located in the KPT coding region.

Further characterization of this region was achieved by subcloning pCHC3. Complementation tests showed that plasmid pNH236, but not plasmid pNH229, complemented mutant 31313 (Fig. 3), so we examined whether the 1.4-kb *Bam*HI fragment contained the entire *kpt* gene. To facilitate the biochemical characterization of the *kpt* gene in hosts other than *X. campestris*, we cloned the 1.4-kb restriction fragment into pRK404, a broad-host-range pRK290-derived plasmid which contains the *lac* promoter. Plasmids pBXP3 and pBXP4 (Fig. 3) were constructed by digestion of pCHC3 with *Bam*HI, isolation of the 1.4-kb restriction fragment, and ligation into the *Bam*HI site of pRK404. Clone pBXP3 complemented the mutant strain 31313, as determined by the in vitro assay of pyr-ps-P-P-lipid formation (Table 3) and the pyruvic acid content of the xanthan produced (Fig. 4C). The high background of pyruvylated intermediate in the absence of PEP (Table 3) is likely due to the presence of endogenous PEP in the preparation of permeabilized cells. We have frequently observed similar results when wild-type cells were used (23). The insertion of a 1.4-kb fragment in the reverse orientation on pBXP4 resulted in the absence of KPT activity in the absence or presence of PEP (Table 3). Accordingly, no pyruvic acid was detected on the xanthan produced (Fig. 4D). The vector alone conferred no KPT activity (Table 3). The *kpt* gene was also expressed in EDTA-treated cells of *E. coli* TB1 containing pBXP3, but not in *E. coli* TB1(pBXP4) (Table 2), indicating that the cloned gene was expressed from the vector promoter. We

TABLE 2. Heterologous expression of the *X. campestris kpt* gene

Enzyme source	Distribution of radioactivity[a]	
	ps-P-P-lipid	pyr-ps-P-P-lipid
A. xylinum PEA-1	4,820 (100)	ND (0)
A. xylinum PEA-1(pCHC3)	3,140 (68)	1,480 (32)
E. coli JZ279(pCHC3)	4,470 (98)	80 (2)
E. coli TB1(pBXP3)	3,510 (75)	1,170 (25)
E. coli TB1(pBXP4)	4,650 (99)	55 (1)
X. campestris B-1459	2,130 (53)	1,890 (47)

[a] Exogenous [^{14}C-GlcA]ps-P-P-lipids obtained from strain B-1459 and PEP were incubated and analyzed as described in Materials and Methods. Values are expressed in disintegrations per minute (percentages of total radioactivity recovered shown in parentheses) of pentasaccharide and pyruvyl-pentasaccharide derivatives isolated after paper electrophoresis. ND, not detected (less than 50 dpm).

TABLE 3. Isolation of *X. campestris kpt* gene

Enzyme source	Addition	[^{14}C]GlcA incorporated (dpm) into:[a]	
		ps-P-P-lipid	pyr-ps-P-P-lipid
31313(pRK404)	PEP	11,205	ND
31313(pBXP3)		14,900	1,870
31313(pBXP3)	PEP	9,320	8,680
31313(pBXP4)		15,660	90
31313(pBXP4)	PEP	12,760	120

[a] Standard incubations were performed and analyzed as described in Materials and Methods. ND, not detected (less than 50 dpm).

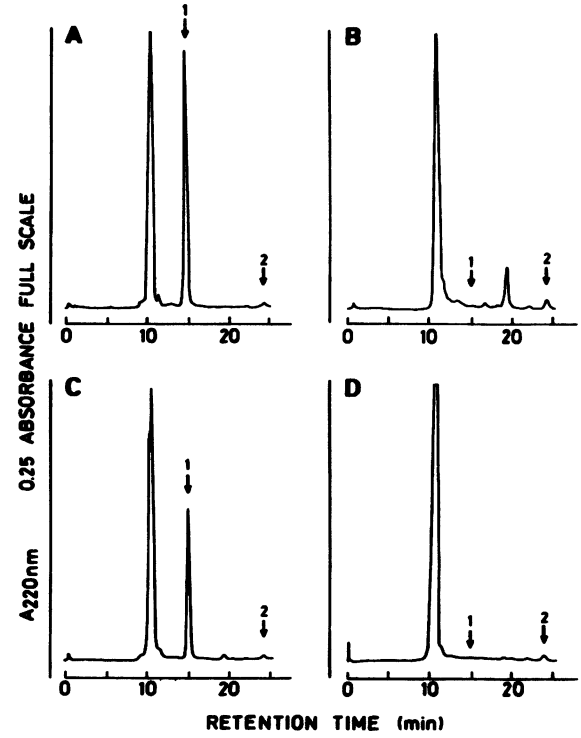

FIG. 4. High-pressure liquid chromatograms of xanthan hydrolysates on an Aminex HPX-87H ion-exclusion column. Polysaccharides, hydrolyzed in 0.25 M H_2SO_4 at 96°C for 4 h, were obtained from supernatants of the following strains: wild type (A), 3972 (B), 31313(pBXP3) (C), and 31313(pBXP4) (D). The retention times of pyruvic and acetic acid are indicated by arrows (1 and 2, respectively). See the legend to Fig. 3 for a description of mutant strain 3972.

have not tested the expression of the *kpt* gene of pBXP3 in *E. coli* JZ279 or pCHC3 in *E. coli* TB1. The low expression of the *kpt* gene on pCHC3 in JZ279 is probably due to the absence of an *E. coli*-like promoter region in the *X. campestris xps* gene cluster.

Site-directed mutagenesis of the *X. campestris kpt* gene. Mini-Mu(Tet^r) mutagenesis of pCHC3 resulted in a mini-Mu(Tet^r) insertion that mapped inside the 1.4-kb *Bam*HI fragment (16). A *kpt* mutant was constructed by crossing the transposon from pCHC3::mini-Mu(Tet^r) into the chromosome of strain B-1459 (Fig. 3). The mutant strain, designated 3972, was tested for content of pyruvyl residues in the polysaccharide and production of pyr-ps-P-P-lipid by the in vitro assay. The amount of pyruvic acid recovered from the polysaccharide was severely reduced as compared with that of the wild-type strain (Fig. 4B). In addition, activity of the enzyme KPT was absent, and in vitro synthesis of ps-P-P-lipid was not affected by the mini-Mu(Tet^r) insertion (data not shown).

DISCUSSION

The application of recombinant DNA technology to study the biosynthesis and genetic regulation of biopolymers is being investigated by using *X. campestris*. In this work, by biochemical characterization, complementation analysis, and site-directed mutagenesis, we have identified the structural gene for the KPT enzyme on a 1.4-kb *Bam*HI fragment of the *X. campestris xps* chromosomal region.

There are several potential explanations for the depyruvylated xanthan gum production of the mutant strain 31313. For example, the mutation could be located in the structural gene for KPT or in some other gene which affected the activity of the enzyme, the ability of the ps-P-P-lipid to function as an acceptor of pyruvyl residues, or the ability of the polymerase enzyme to recognize the pyr-ps-P-P-lipid as a substrate. In vitro analysis indicated that strain 31313, although producing ps-P-P-lipid in amounts similar to that from the wild-type strain, is unable to synthesize the pyruvylated intermediate. In addition, we found that the ps-P-P-lipid synthesized by the mutant is functionally equivalent to the ps-P-P-lipid synthesized by the wild-type strain, since it could be used as an in vitro acceptor of pyruvyl substituents. Heterologous expression in *A. xylinum* of pCHC3, a subclone of the *xps* region, provided direct proof that the *kpt* structural gene is located in this region. By using complementation data and subcloning, we have cloned the *kpt* gene as a 1.4-kb *Bam*HI fragment. A functional KPT enzyme was detected in transformed *X. campestris* and *E. coli*. Our results suggest that the expression of the KPT enzyme was dependent on the *lac* promoter from the vector.

In vitro analysis showed that the defect in the KPT enzyme did not affect the activity of the glycosyl transferases involved in ps-P-P-lipid synthesis. In addition, introduction of the pCHC3 into the wild-type strain resulted in a significant increase in the pyruvate content of xanthan gum (16). Accordingly, a higher ratio of pyruvylated to nonpyruvylated ps-P-P-lipid was observed (Fig. 2).

Similar mutants producing pyruvate-free xanthan have been described by others (18). The lack of pyruvate was presumed to be due to the lack of ketal transferase; however, no evidence for the defects being located in the structural gene was presented.

Recently, Müller et al. (31) reported the isolation of two Tn*5* *Rhizobium meliloti* mutants, which produce two to three times more acidic exopolysaccharide than the wild-type strain, with no pyruvyl substituents. No biological function of the corresponding gene product was reported.

Many of the genes involved in exopolysaccharide synthesis are often clustered. In *X. campestris*, as reported for *Agrobacterium tumefaciens* (5, 26), *Erwinia stewartii* (7), *R. meliloti* (27), *Rhizobium leguminosarum* biovar phaseoli (11), *Rhizobium* sp. strain NGR234 (6), *Pseudomonas aeruginosa* (10), *Zoogloea ramigera* (14), a cluster of genes essential for xanthan polysaccharide synthesis has been isolated (2, 16, 34). Experiments designed to determine the function of the remaining *xps* gene products are in progress.

It will be of interest to study the effect of the pyruvyl residue in the polymerization process as well as in the assembly of the ps-P-P-lipid. Earlier investigations have revealed that pyr-ps-P-P-lipid is not required for in vitro synthesis of xanthan gum (21, 22). Isolation and characterization of mutants deficient in KPT activity will help to delineate the mechanism regulating synthesis, elongation, and assembly of the xanthan gum. Although the lesion in the *kpt* gene has no apparent effect on the synthesis of ps-P-P-lipid and pyr-ps-P-P-lipid is not essential for the polymerization process, whether or not pyruvylation influences the rate of synthesis remains to be determined.

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