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Direct utilization of lactose in clarified cheese whey for xanthan gum synthesis by *Xanthomonas campestris*

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SUMMARY

A derivative of *Xanthomonas campestris* B1459 was constructed that utilizes lactose in clarified cheese whey for xanthan gum synthesis. Genes conferring lactose utilization carried by transposon Tn951 were inserted into the bacterial chromosome. The ability to use lactose for xanthan gum synthesis was stably inherited and the amount of xanthan produced suggested carbohydrate conversion efficiencies similar to wild-type *X. campestris* growing in the presence of glucose. Bench-scale fermentation of this organism and identification of the optimal whey sources and pretreatments can now proceed.

INTRODUCTION

Xanthan gum is usually produced by fermentation of *Xanthomonas campestris* with glucose or corn syrup as the major carbon source [9]. However, it is also possible to convert the glucose and galactose in hydrolyzed whey to xanthan gum [2,13]. Whey is a high BOD waste from the manufacture of cheese. The composition of whey varies according to the type of cheese produced, but can be up to 5% (w/v) as lactose or 70% lactose when dried. The remainder is made up of protein, organic acids, minerals and vitamins. The amount of waste whey generated from just a few typical cheese plants could supply all the worldwide fermentation substrate needs for the production of xanthan gum.

Because wild-type strains of *X. campestris* utilize lactose poorly, the whey must first be hydrolyzed enzymatically with lactase or β-galactosidase. The efficiency of conversion of hydrolyzed whey carbohydrate to xanthan gum ranges from over 75% to near the theoretical maximum [2]. One possible reason for the poor utilization of unhydrolyzed lactose may be that the β-galactosidase of *X. campestris* has a low affinity for lactose [8]. In order to overcome this defect and to generate a strain of *X. campestris* that could utilize lactose more efficiently, Walsh et al. [15] transferred exogenous *lac* (lactose) genes into *X. campestris*. The *lac* genes were carried by transposon Tn951 which was in turn inserted within the mobilizable broad host range plasmid RP1 [4]. In the absence of a plasmid-selective antibiotic the plasmid, and therefore the *lac* genes,

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were not stable. A different approach was taken by Schwartz and Bodie [11] when they isolated a spontaneous derivative of *X. campestris* B1459 that could convert unhydrolyzed lactose in whey to xanthan gum. They demonstrated substrate conversion efficiencies with this strain of less than 40% for long fermentation times (about 70 h). The nature of the mutation was not known and the strain proved to be unstable for xanthan production, losing considerable productivity within 40 generations under non-selective conditions.

In this report we describe the construction of a plasmid vector that is useful for integrating foreign DNA into the chromosome of *X. campestris*. Using this vector we inserted the *lac* genes from pGC9114 (RP1::Tn951) into a rifampicin-resistant derivative of *X. campestris* B1459. The genetic stability of lactose utilization and conversion of lactose or lactose in clarified whey to xanthan gum was determined. In addition, a preliminary characterization of the quality of the xanthan gum made by this strain from clarified cheese whey is described.

MATERIALS AND METHODS

Bacterial strains, plasmids and growth conditions. *X. campestris* B1459S-4L-II (our strain X55) was obtained from the Northern Regional Research Center in Peoria, Illinois and is the parent of all *X. campestris* strains used in this work. Strain X59 is a spontaneous rifampicin-resistant derivative of X55 [14]. Both are fully positive for xanthan gum synthesis (Xgs^+). *Escherichia coli* strain MC1009 (*Δlac ipozy*-X74, *galK, galU, Δara-leu*-7697, *strA, recA*) was obtained from J. Hoch and strain JC3272 (*his, trp, lys, Δlac ipozy*-X74, *strA*) containing plasmid pGC9114 (RP1::Tn951) from G. Somkuti. Plasmids pRK290, pRK311 and pRK2013 were obtained from D. Helinski and pUC13 from Bethesda Research Laboratories. *Xanthomonas* strains were cultured at 30°C in four related liquid or solid (with agar) media: YT (10 g/l Difco yeast extract, 16 g/l Difco tryptone, 5 g/l NaCl); YTS (5 g/l Difco yeast extract, 5 g/l Difco tryptone, 3.5 g/l K_2HPO_4, 2.6 g/l KH_2PO_4, 0.26 g/l $MgSO_4 \cdot 7H_2O$, 6 mg/l H_3BO_3, 6 mg/l ZnO, 2.6 mg/l $FeCl_3 \cdot 6H_2O$, 20 mg/l $CaCO_3$); YPS (with an equal weight of peptone substituted for tryptone in YTS); PS (10 g/l peptone substituted for yeast extract and tryptone in YTS); S (2–4 g/l $(NH_4)_2SO_4$ substituted for yeast extract and tryptone in YTS). The volume of culture was always one-tenth to one-fifth the flask capacity. *E. coli* strains were grown in LB broth or YT. Antibiotics and carbohydrate were added as needed. Whey was 'sweet whey' from Sigma. It was 65% lactose by dry weight, 13% protein, 8% ash and 2% lactic acid. A 30% (w/v) solution was autoclaved at 121°C for 20 min and centrifuged to clarify. The pH before autoclaving and after clarification was about 6. The phenol-H_2SO_4 assay was used to measure final lactose concentration [7].

DNA preparation and analysis. DNA was prepared by the boiling method [10] or the Birnboim and Doly procedure [1] and when necessary purified by equilibrium sedimentation in density gradients of CsCl containing ethidium bromide [10]. Restriction enzymes and DNA ligase were used according to the instructions of the manufacturer. Chromosomal DNA digested with *Hin*dIII was separated by gel electrophoresis and transferred to nitrocellulose filters by the method of Southern [10,12]. Plasmid DNA for use as hybridization probes was radioactively labeled with ^{32}P by nick translation [10]. Transformation of *E. coli* cells with plasmids or ligation mixtures was standard [10] and conjugal transfer of plasmids into *X. campestris* followed the triparental mating scheme [6].

Xanthan gum isolation and analysis. In order to measure amounts of xanthan gum, culture samples (without prior removal of cells) were added to 2 volumes of isopropyl alcohol. The precipitated material was collected by filtration onto Whatman 934-AH filters, then dried at 80°C in a vacuum oven and weighed. For viscosity measurements the dried precipitate was ground in a mortar and sieved through a 250 micron mesh before resuspending in 0.1% (w/v) NaCl. Viscosity measurements over a range of shear rates at room temperature were made with a Brookfield LVT viscometer. Protein concentrations were determined with the Bio-Rad Protein Assay and standards of bovine serum albumin (Sigma).

RESULTS

Construction of lactose-positive X. campestris

Plasmid pGC9114 is a derivative of plasmid RP1 and carries Tn951, a transposon that confers lactose utilization [4]. We verified that a subfragment of pGC9114 of about 10.5 kbp and flanked by *Bam*HI restriction sites carried the *lac* genes [3]. We subcloned that fragment to pUC13 and transformed Lac$^-$ *E. coli* MC1009 to Lac$^+$ (blue colonies on nutrient plates containing 5-bromo-4-chloro-3-indoxyl-β-D-galactoside (Xgal) and isopropyl thio-galactopyranoside (IPTG)). The same 10.5 kbp fragment was subcloned into a plasmid 'integration' vector (pSY1181) that could be conjugally transferred from *E. coli* to *X. campestris* but could not replicate in the latter. We call this an 'integration' vector since the only way that the *lac* genes can be stably maintained in the recipient is if they recombine with and become integrated into the bacterial chromosome. The four steps in the construction of plasmids pSY1181 and pSY1232 are illustrated in Fig. 1 and explained below.

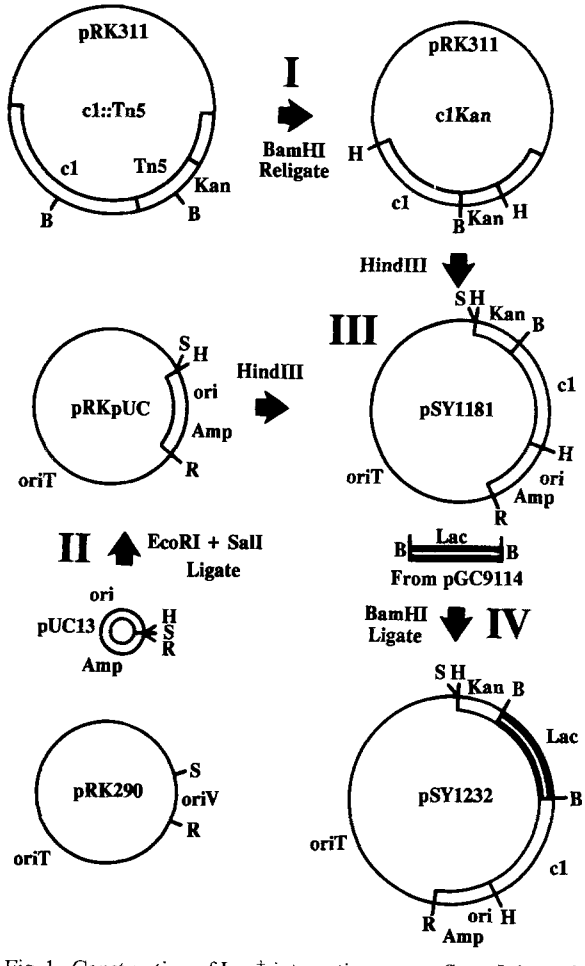

Fig. 1. Construction of Lac$^+$ integration vector. Steps I through IV are described in the text. The circular genetic maps are drawn roughly to scale. Plasmid pSY1181 is a general purpose integration vector and carries a DNA segment (c1) that is identical to an *X. campestris* chromosomal sequence. Plasmid pSY1232 carries in addition the *lac* genes from Tn951.

To promote integration into the chromosome we included a fragment of chromosomal DNA in pSY1232. Previously we isolated fragments of the *X. campestris* wild-type chromosome that complemented or restored xanthan gum synthesis to mutants unable to make the polysaccharide [14]. Colonies of both the wild-type and mutants carrying the 'xanthan' genes cloned on cosmid vectors were mucoid, while the mutants alone were non-mucoid. One such clone was c1, a recombinant between the cosmid vector pRK311 [5] and an approximately 22 kbp chromosomal fragment. A derivative of c1, that carried transposon Tn5 (kanamycin-resistance) in a site within c1 but which did not inactivate complementation by c1 for the corresponding mutant m1, was our starting material. As shown by step I of Fig. 1, this plasmid was restricted with *Bam*HI enzyme and recircularized to create a single *Bam*HI cloning site flanked by the c1 complementing DNA and the kanamycin-resistance gene of Tn5. The c1-Kan region is bounded by *Hin*dIII sites.

The second step was to convert the matable broad host range plasmid pRK290 [6] to narrow host range by substituting the origin of replication from pUC13 for the *oriV* of pRK290. The third step was to fuse c1-Kan with pRKpUC via the *Hin*dIII sites to create the 'integration' vector pSY1181. The last step was to insert the *lac* genes at the *Bam*HI site of pSY1181 to generate pSY1232. *E. coli* MC1009 transformed with pSY1232 are resistant to ampicillin and kanamycin and give blue colonies on plates containing Xgal and IPTG.

In order to allow *X. campestris* to utilize lactose we transferred pSY1232 into strain X59 (a rifam-

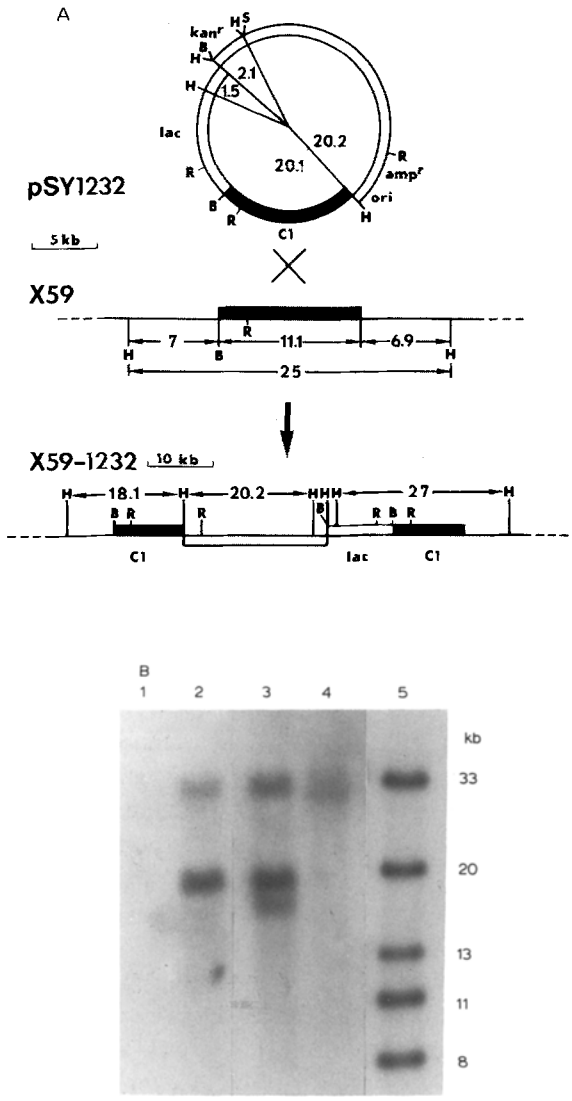

Fig. 2. Integration of plasmid pSY1232 into the X59 chromosome. Panel A: restriction maps and *Hin*dIII fragment sizes for plasmid pSY1232, the chromosomal c1 region of strain X59, and the expected recombinant chromosome of strain X59-1232. The filled-in boxes show the homologous c1 sequences. Panel B: hybridization of labelled DNA probes to *Hin*dIII digests of chromosomal DNA from strains X59 and X59-1232. Lanes 1 and 4, X59 DNA; lanes 2 and 3, X59-1232 DNA. Lanes 1 and 2, hybridization to radioactively labelled plasmid pGC9114; lanes 3 and 4, hybridization to plasmid pSY1232. Plasmid pGC9114 [4] carries the *lac* genes and sequences homologous to the 20.2 kb fragment of pSY1232, but does not include the *X. campestris* c1 DNA. Lane 5, molecular size markers. Plasmid pSY1232 was separately digested with *Bam*HI alone and *Bam*HI plus *Eco*RI, and then the two digests were mixed before electrophoresis. Sizes for molecular markers are given in kb at right.

picin-resistant derivative of *X. campestris* B1459S-4L-II) using a triparental conjugation scheme with pRK2013 as the helper plasmid [6]. Exconjugants were initially selected on kanamycin since *X. campestris* is naturally resistant to ampicillin, the other resistance gene carried on pSY1232. All the Kan^r exconjugants were then shown to grow on minimal plates with lactose, unlike X59. The Kan^r Lac$^+$ exconjugants were indistinguishable, and one, named X59-1232, was chosen as representative for further work. Similar results were obtained by mobilizing the Lac$^+$ plasmid pGC9114 into X59, as earlier demonstrated by Walsh et al. [15]. In liquid cultures we found that the plasmid-bearing X59-pGC9114 grew more slowly than either X59 or X59-1232, which grew at similar rates. We tentatively attributed this slower growth to the 'cost' of maintaining the multi-copy plasmid.

Lactose genes are integrated in the chromosome of strain X59-1232

We immediately noticed that in the absence of tetracycline selection the plasmid pGC9114 was lost from a culture of X59, whereas in the absence of kanamycin selection for strain X59-1232 the cryptic marker was retained. More importantly the ability to utilize lactose behaved in the same way. This was consistent with at least part of the pSY1232 DNA being stably integrated in the bacterial chromosome. By DNA hybridization analysis [12], we confirmed that the narrow host range plasmid had integrated into the chromosome. Furthermore, the restriction fragment sizes were consistent with insertion into the homologous c1 chromosomal region. The expected results are illustrated in Fig. 2, panel A, and the observed hybridization pattern is shown in panel B. The c1 region of the chromosome was characterized for diagnostic *Hin*dIII restriction sites after cloning the region on two overlapping fragments of DNA. The portion of the c1 region in common with pSY1232 is indicated by the filled-in boxes. Insertion of plasmid pSY1232 into the c1 region of the chromosome by homologous recombination (one double-stranded crossover) should replace the 25 kb *Hin*dIII fragment with fragments of 18.1, 20.2 and 27 kb. Chromosomal DNA was

Table 1

Genetic stability of utilization of lactose for xanthan gum synthesis

Passage number[a]	Generation number[b]	Xanthan gum (% w/w)					
		X59pGC9114			X59-1232		
		Lac	Glc	Lac/Glc	Lac	Glc	Lac/Glc
0	7	1.6	2.0	0.8	1.6	1.9	0.8
1	14	1.7	2.0	0.9	1.7	2.0	0.9
3	28	0.6	1.5	0.4	1.5	1.7	0.9
4	35	0.1	1.8	0.1	1.5	2.0	0.8
5	42	0.2	1.7	0.1	1.7	2.2	0.8

[a] Initial inocula were grown in YT plus rifampicin (50 μg/ml) with tetracycline (7.5 μg/ml) for X59pGC9114 or kanamycin (50 μg/ml) for X59-1232. Each passage was in YT plus rifampicin with an inoculum of 10^7 cells/ml and was ended at about 10^9 cells/ml (A_{600} = 1). After each passage, shake flasks containing YPS medium with either lactose (Lac) or glucose (Glc) at 2% (w/v) were inoculated with 10^7 cells/ml. After 48 h the amount of xanthan gum in each flask was measured by precipitation with 2 volumes of isopropyl alcohol and then dried and weighed.

[b] Includes about seven generations per passage and about seven generations during carbohydrate conversion assay.

prepared from strains X59 and X59-1232, digested with *Hin*dIII and the fragments separated by gel electrophoresis. After transfer of the DNA to filters [12], the DNA was hybridized to either of two radioactively labelled plasmid probes: pGC9114 or pSY1232. The plasmid pGC9114 carries the *lac* genes and is also homologous to the 20.2 kb *Hin*dIII fragment of pSY1232, but does not hybridize to the X59 chromosomal DNA (panel B, lane 1). Lane 2 shows that the pGC9114 probe hybridizes to chromosomal *Hin*dIII fragments of about 20 and 27 kb for strain X59-1232. When pSY1232, carrying a portion of the c1 region, was used as the hybridization probe, a chromosomal fragment of 25 kb was detected in the X59 DNA. This band was absent from strain X59-1232 and in its place were bands of about 18, 20 and 27 kb. This is consistent with integration of the entire plasmid pSY1232 and the *lac* genes into the c1 chromosomal locus.

Stability of integrated lactose genes

Since the overall objective was to generate a stable strain for converting lactose to xanthan gum, we measured stability for this trait after serially subculturing X59-pGC9114 and X59-1232 for many generations without tetracycline for selection of plasmid pGC9114 or kanamycin in the case of X59-1232. In either case, the X59 host is resistant to rifampicin. This allows a selection against rifampicin-sensitive accidental contamination during repeated serial transfer. Each strain was grown both in glucose and lactose and the ratio of the amount of xanthan produced from lactose to glucose was calculated. The results are given in Table 1. The ability to convert lactose to xanthan gum by the plasmid-bearing strain, X59pGC9114, decreased to half its original level at the third passage. In contrast, X59-1232 carrying the *lac* genes integrated into the chromosome showed stable conversion of lactose to xanthan gum through the end of the parallel experiment, a total of 42 generations.

Utilization of carbohydrate substrate for xanthan gum synthesis

Parallel shake flask cultures of strains X59 (Lac^-) and X59-1232 (Lac^+) were tested for utilization of carbohydrate for the synthesis of xanthan gum. Exopolysaccharide accumulation was measured with glucose, lactose and clarified cheese whey at equivalent weight percents of glucose or lactose. The results are given in Table 2. The Lac^- parental strain X59 did not convert appreciable lac-

Table 2

Utilization of carbohydrate substrate for xanthan gum synthesis

Strain	Carbohydrate substrate	Xanthan gum (% w/w)[a]	
		12 h	24h
X59 (Lac$^-$)	glucose	1.2	2.1
	lactose	0.2	0.2
	whey lactose	0.0	0.4
X59-1232 (Lac$^+$)	glucose	1.1	2.0
	lactose	1.6	2.0
	whey lactose	1.7	1.8

[a] Inocula were grown in YT medium with rifampicin (50 μg/ml), centrifuged, washed with LB broth and resuspended at 2 × 10^9 cells/ml in YTS medium plus carbohydrate substrate at 2% (w/v). Samples were withdrawn and xanthan was precipitated with 2 volumes of isopropyl alcohol, and then dried and weighed.

tose or lactose in clarified whey to xanthan gum, compared to the stable Lac$^+$ strain X59-1232. Since the residual amounts of substrates from the carbohydrate, yeast extract, tryptone and whey were not determined, we could not calculate the absolute conversion efficiencies. However, the amounts of xanthan gum shown in Table 2 are similar to those of our most productive strains of *X. campestris*, which can convert over 70% of substrate to xanthan during controlled fermentations.

Quality of xanthan gum produced from glucose, lactose and clarified cheese whey

The following cultures were grown in shake flasks containing 200 ml of PS medium supplemented with the indicated carbohydrate at 2% (w/v): strain X59, glucose; X59-1232, lactose; X59-1232, clarified cheese whey (lactose). After 48 h growth the culture contents were precipitated with 2 volumes of isopropyl alcohol, dried and ground to uniform particle size (about 100–200 microns). Samples of each were resuspended in 0.1% (w/v) NaCl at specific weight percentages, with the weights determined to the exclusion of water, protein and ash. Viscosities were measured over a range of shear rates and the results are given in Fig. 3. The solution viscosities for the xanthan-containing material made by X59 from glucose or X59-1232 from lactose were not distinguishable. However, the material made in the presence of clarified cheese whey appeared to be less viscous, requiring almost twice as much by weight to give equal viscosity. A subsequent mixing experiment indicated that an unknown clarified whey component lowers the viscosity of xanthan gum. We prepared a separate culture of X59-1232 grown on PS medium plus lactose. The xanthan-containing material was precipitated either in the presence or absence of added clarified whey. Enough clarified whey was added to make the final lactose concentration 2% (w/v). The resulting viscosities from this mixing experiment are superimposed on Fig. 3. Most of the apparent qualitative difference is accounted for by the whey effect on viscosity. Whether the remaining difference repesents an alteration in the chemical composition or molecular size of the xanthan gum is not yet known.

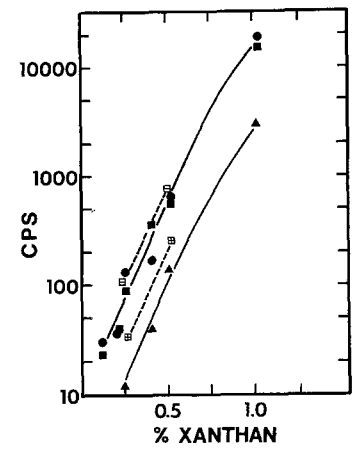

Fig. 3. Viscosity of xanthan-containing material made from glucose, lactose or clarified whey. Solutions of xanthan gum were prepared at defined concentrations and to the exclusion of water, ash and protein. Viscosities at different shear rates were measured and the values from a shear rate of 1.32 s^{-1} (1 rpm for spindle number 18, Brookfield), were plotted. Symbols, strains and growth or processing conditions: ●, X59 (Lac$^-$), glucose; ■, X59-1232 (Lac$^+$), lactose; ▲, X59-1232 (Lac$^+$), clarified whey; ⊟, X59-1232 (Lac$^+$), lactose, without clarified whey added at harvest; ⊞, X59-1232 (Lac$^+$), lactose, with clarified whey added at time of harvest.

DISCUSSION

This study extends previous investigations of the use of waste cheese whey for production of xanthan gum by *X. campestris* [2,8,11,13,15]. We have constructed a stable recombinant strain, X59-1232, which produces quality xanthan gum from lactose by inserting the *lac* genes from transposon Tn951 into the chromosome of *X. campestris* strain X59. The apparent conversion efficiency of lactose to xanthan gum by this strain was equivalent to the conversion of glucose to xanthan gum by strain X59, a strain which efficiently carries out the latter conversion. The viscosities of the xanthan gums from these two sources were equivalent. After more than 14 generations of growth without positive selection for genetically linked traits, the apparent conversion efficiency from lactose by strain X59-1232 was superior to that of strain X59-pGC9114, a strain which carried the Tn951 *lac* genes on a multicopy plasmid (pGC9114), which, in turn, was superior to strain X59, which lacks the Tn951 *lac* genes. The superiority of X59-1232 could be attributed to the stable integration of the *lac* genes into the chromosome of X59-1232.

The apparent efficiency of conversion for clarified cheese whey to xanthan gum by X59-1232 was approximately 90% that of lactose to xanthan gum. This is probably within the experimental error of the measurements employed. Precise conversion efficiencies must be determined by including measurements of residual substrates from the added carbohydrate, yeast extract, tryptone, and clarified whey in future studies. The high utilization of clarified whey for xanthan gum synthesis by our strain X59-1232 has not been achieved before and represents a significant advance in investigating the use of waste cheese whey for production of xanthan gum. The high utilization must now be confirmed using conditions which more closely mimic manufacturing conditions, such as bench-scale batch and fed-batch fermentations in stirred reactors with forced aeration.

A significant phenomenon which was identified by our study could prevent the use of clarified cheese whey for xanthan gum production by currently accepted manufacturing procedures. Isopropyl alcohol, used to precipitate xanthan gum from *X. campestris* broths in FDA accepted procedures, appears to precipitate a component of whey which lowers the viscosity of isopropyl alcohol-precipitated xanthan gum. The final quality of the xanthan gum is as important to the overall production economics of this specialty chemical as cellular productivity. Hence, this study indicates that the identification and elimination of the interfering component in whey is a key issue for investigators interested in using waste cheese whey for xanthan production by *X. campestris*.

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REFERENCES

1 Birnboim, H.C. and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7: 1513–1523.

2 Charles, M. and M.K. Radjai. 1977. Xanthan gum from acid whey. In: Extracellular Microbial Polysaccharides (Sandford, P.A. and A. Laskin, eds.), pp. 27–39, American Chemical Society, Washington, DC.

3 Cornelius, G. 1981. Sequence relationships between plasmids carrying genes for lactose utilization. J. Gen. Microbiol. 124: 91–97.

4 Cornelius, G., D. Ghosal and H. Saedler. 1978. Tn951: a new transposon carrying a lactose operon. Mol. Gen. Genet. 160: 215–224.

5 Ditta, G., T. Schmidhauser, E. Yakobsen, P. Lu, X.-W. Liang, D.R. Finlay, D. Guiney and D.R. Helinski. 1985. Plasmids related to the broad host range vector pRK290, useful for gene cloning and for monitoring gene expression. Plasmid 13: 149–153.

6 Ditta, G., S. Stanfield, D. Corbin and D.R. Helinski. 1980. Broad host range DNA cloning system for Gram-negative bacteria: construction of a gene bank of *Rhizobium melitoti*. Proc. Natl. Acad. Sci. USA 77: 7347–7351.

7 Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350–356.

8 Frank, J.F. and G.A. Somkuti. 1979. General properties of β-galactosidase of *Xanthomonas campestris*. Appl. Environ. Microbiol. 38: 554–556.

9 Kennedy, J.F. and I.J. Bradshaw. 1984. Production, properties and applications of xanthan. In: Progress in Industrial Microbiology (Bushell, M.E., ed.), pp. 319–371, Elsevier/North-Holland, Amsterdam.

10 Maniatis, T., E.F. Fritsch and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

11 Schwartz, R.D. and E.A. Bodie. 1985. Production of high-viscosity whey broths by a lactose-utilizing *Xanthomonas campestris* strain. Appl. Environ. Microbiol. 50: 1483–1485.

12 Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98: 503–517.

13 Stauffer, K.R. and J.G. Leeder. 1978. Extracellular microbial polysaccharide production by fermentation on whey or hydrolyzed whey. J. Food Sci. 43: 756–758.

14 Thorne, L., L. Tansey and T.J. Pollock. 1987. Clustering of mutations blocking synthesis of xanthan gum by *Xanthomonas campestris*. J. Bacteriol. 169: 3593–3600.

15 Walsh, P.M., M.J. Haas and G.A. Somkuti. 1984. Genetic construction of lactose-utilizing *Xanthomonas campestris*. Appl. Environ. Microbiol. 47: 253–257.