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Improved strains for production of xanthan gum by fermentation of *Xanthomonas campestris*

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SUMMARY

Two classes of mutants of *Xanthomonas campestris* B1459 were isolated that accumulate more xanthan gum than the parental wild-type in culture broths of shake flask cultures and both batch and fed-batch fermentations. The first mutant class was resistant to the antibiotic rifampicin and accumulated, on average, about 20% more xanthan gum than wild-type. The second mutant class, a derivative of the first, was resistant to both bacitracin and rifampicin, and accumulated about 10% more xanthan than its parent. On a weight basis, the viscosities of the polysaccharides made by each strain were not distinguishable. Only a subset of the drug-resistant mutants were overproducers of xanthan. The biochemical basis for the overproduction of xanthan by the mutant strains has not been determined. Both new strains served as recipients for recombinant plasmids bearing 'xanthan' genes and further augmented the effects of multiple copies of those genes on xanthan productivity.

INTRODUCTION

Xanthan gum is an exopolysaccharide secreted by the gram-negative bacterium *Xanthomonas campestris* (for a recent comprehensive review see Ref. 6). Due to useful aqueous rheological properties, this biopolymer is widely used as a thickener or viscosifier for both food and industrial applications. The viscosity of a xanthan solution is high even at low concentration, and is not only pseudoplastic, but also insensitive to a wide range of salt concentrations, temperature and pH. These properties, coupled with reported yields exceeding 40 g/l in batch fermentations requiring about 3 days and consistency in quality, translate into the most successful microbial biopolymer to be marketed. Nevertheless, improvements in both productivity and

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properties are possible, and may play a significant role in expanding the industrial use of xanthan in the future.

Strain improvement is an empirical process. Random mutation and selection are the primary classical methods used, but can be supplemented by the application of genetic and biochemical knowledge about the production organism. Thus strain improvement may also be a rational process. We have attempted to use a combination of both standard microbial genetics and recombinant DNA methods to improve productivity and properties of biopolymers made by microorganisms. Our initial work focused on *X. campestris* and xanthan gum. We found that productivity [13] and properties (unpublished) could be improved through recombinant DNA technology. Barrere et al. [1] and Harding et al. [5] have also demonstrated yield increases of 35 and 10% respectively and a 47% increase in the degree of pyruvylation [5] by using gene cloning and transfer techniques. In each study, percentage increases in xanthan accumulation were obtained by increasing the gene copy number by cloning similar DNA fragments of *X. campestris* chromosomal DNA into multi-copy plasmids. One major advantage of using gene cloning methods for strain improvement is the avoidance of an accumulation of deleterious mutations which often occur in classical mutagenesis and screening programs. Not surprisingly, there are few reports in the literature on the results of applying more classical genetic methods to strain improvement for *X. campestris* [6]. This is probably due to decisions by xanthan producers to protect proprietary interests, but may also reflect the already high productivity of bacterial strains that are widely available [2,7,9,12,14]. In this report we describe two mutant strains of *X. campestris* that accumulate elevated levels of xanthan gum. We have cultured these strains in shake flasks and in two fermentation modes: batch and fed-batch, both at the 10-liter scale. The productivity improvements are found with each culture method tested.

MATERIALS AND METHODS

Bacterial strains

We obtained three *X. campestris* 'wild-type' strains from two sources: B1459S-4L-II (our X55) and B1459 (our X56) from the Northern Regional Research Center in Peoria, IL; and ATCC 13951 (our X57) which is also B1459 from the American Type Culture Collection in Rockville, MD. The strains described in this work all derive from X55. We gave the two B1459 strains different designations because they came to us from different collections and because there have been reports of phenotypic instability [6]. However, we have not yet noted any differences between our X56 or X57.

Strain X59 was isolated previously in our laboratory [13] and is a spontaneous rifampicin-resistant derivative of X55. Colonies of strain X59 are fully mucoid on nutrient agar plates with or without added glucose (usually at 2% w/v). This genetically marked strain was originally isolated in order to serve as a recipient in conjugal mating experiments described previously [13]. More recently we have found that X59 and several related rifampicin-resistant mutants accumulate more xanthan gum in culture media than does the X55 antibiotic-sensitive parent strain. All the rifampicin-resistant mutants were spontaneous in origin and selected as able to grow on nutrient agar plates containing rifampicin at 50 or 100 μg/ml. Strain X55 cannot grow under these conditions. In liquid culture X55 does not grow in the presence of rifampicin at 10 μg/ml, whereas X59 and its derivatives grow normally even when rifampicin is as high as 500 μg/ml, or above its solubility limit. The resistant mutants arose at a frequency of about one in $10^8 - 10^9$ cells.

Strain X50 is a spontaneous derivative of X59 and was selected as able to grow on nutrient agar plates containing the cyclic peptide antibiotic, bacitracin, at 0.5 mg/ml. Strain X50 retains resistance to rifampicin and gives rise to colonies that are fully mucoid on plates. While X50 grows in liquid medi-

um containing bacitracin at 250 μg/ml (and slightly even at 500 μg/ml), the X59 and X55 strains grow with bacitracin at 100 μg/ml but not at 250 μg/ml. The predominant class of bacitracin-resistant derivatives of X59 appeared nonmucoid and were not studied further.

Culture conditions for plating and shake flasks

Single colonies were obtained by streaking frozen (−80°C) stored cells onto LB plates containing agar (1.5% w/v) and the appropriate selective antibiotic. When included, rifampicin was 50–100 μg/ml and bacitracin was 250–500 μg/ml, and plates were incubated at 30°C. Inocula for shake flask experiments were grown in the same medium in tubes at 30°C and adjusted by dilution to obtain equivalent cell densities in log phase. Shake flask tests of productivity did not have antibiotics added, nor did the inocula. Both the inocula and shake flask media were either LB broth, YM, XG011, XG004 or PS. LB broth included (per liter): 10 g Bacto tryptone (Difco), 5 g Bacto yeast extract (Difco) and 10 g NaCl. XG011 consisted of (per liter): 1 g $(NH_4)_2HPO_4$, 1 g $NaNO_3$, 1 g Amberex 510 (Amber Laboratories), 10 mg $MgSO_4 \cdot 7H_2O$, 100 mg $CaCl_2 \cdot 2H_2O$, 1 ml of 1000X trace elements and 20 g glucose. The 1000X trace elements comprised (per liter of deionized H_2O): 2.25 g $FeCl_3 \cdot 6H_2O$, 2.2 g $ZnSO_4 \cdot 7H_2O$, 1.41 g $MnSO_4 \cdot H_2O$, 0.4 g $CoCl_2 \cdot 6H_2O$, 0.4 g H_3BO_3, 0.26 g $Na_2MoO_4 \cdot 2H_2O$, 0.25 g $CuSO_4 \cdot 5H_2O$ and 0.06 g KI. XG004 included (per liter): 5 g Bacto tryptone, 2.5 g yeast extract (Difco), 2 g NaCl, 6.8 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 2.2 g L-glutamic acid, 2 g citric acid, 0.1 g $CaCl_2 \cdot 2H_2O$, 1 ml of 1000X trace elements, and 20 g glucose. PS is a modification of a medium described by Cadmus et al [2] and consisted of (per liter): 10 g Bacto peptone (Difco or Scott), 3.5 g K_2HPO_4, 2.6 g KH_2PO_4, 0.26 g $MgSO_4 \cdot 7H_2O$, 6 mg H_3BO_3, 6 mg ZnO, 2.6 mg $FeCl_3 \cdot 6H_2O$, 20 mg $CaCO_3$ and 20 g glucose. YM (Difco) included (per liter): 3 g Bacto yeast extract, 3 g malt extract, 5 g Bacto peptone, and 10 g dextrose. For measurements of xanthan synthesis these media were supplemented with glucose to 20–30 g(final)/l. Flasks were also incubated at 30°C with vigorous shaking.

Fermentation conditions

The fermentor inocula were prepared in two growth steps. Four LB plates containing agar (1.5% w/v) were each spread with a loopful of concentrated cells that were stored frozen at −80°C in 15% (v/v) glycerol. When the plates reached confluency (about 48 h at 30°C), the cells were harvested by scraping and divided between two 2-liter flasks containing 500 ml of LB broth. The flasks were incubated at 30°C with vigorous shaking for about 16 h. Cells from these cultures were used as inocula (10% v/v).

The aerobic fermentations were conducted in a Braun Biostat E fermentor using 10 liters of the 15-liter capacity in either batch of fed-batch mode. The vessel was 43 cm high and 20 cm in diameter and the liquid height was 34 cm. There were four Rushton turbine impellers, each with six flat blades. For batch fermentation, the non-optimized medium XG011 contained 50 g of glucose per liter. The pH was maintained at 7 ± 0.1 by incremental addition of 2.5 N NaOH or 0.5 N HCl and temperature was regulated at 30 ± 0.1°C. Dissolved oxygen was maintained at 60% by air flow variation from 0.5 to 20 l/min and agitation speeds between 300 and 1000 rpm. The probe for dissolved oxygen sampled the vessel 29 cm from the top, or at the midpoint of the culture broth, and about 3 cm from the wall. The fed-batch fermentations were as above, but with these changes. The initial medium was PS with either 15 g peptone or yeast extract per liter and the initial volume was 5 liters. The feeding medium was the same but without the peptone and was six-times-concentrated. The feed was pumped into the vessel at a rate of 60–100 ml/h. The final volume was about 10 liters.

Analytical procedures

The amount of xanthan accumulated in the growth medium was determined by weighing a sample of about 10 ml and precipitating the polysaccharides with two volumes of isopropyl alcohol. The cells were not removed by centrifugation before adding alcohol. The cells were less than 10% of the weight, and the cell weights were uniform for X55, X59 and X50. The precipitate was collected on a

glass filter (Whatman 934-AH), dried in a vacuum at 80°C 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). Glucose was measured enzymatically with glucose oxidase using a Beckman Glucose Analyzer 2.

RESULTS

Synthesis of xanthan gum by antibiotic-resistant mutants in shake flasks

Cultures of the drug-resistant and parental strains were grown in nutrient broth containing glucose at 2% (w/v). The volumes were 10–50 ml in 125–500 ml baffled, capped Erlenmeyer flasks and incubation was at 28–30°C with vigorous shaking (200–300 rpm). For each experiment the inocula were of equivalent cell numbers grown under identical conditions. Samples that were equal by weight were withdrawn at specified times and mixed with two volumes of isopropyl alcohol to precipitate the exopolysaccharide. The precipitates were trapped on filters, dried and weighed. The weights of semi-purified xanthan gum are tabulated for several independent experiments with different culture conditions in Table 1. Within each experiment, the amount of xanthan gum varied less than 5% between replicate flasks. As indicated, there were two classes of rifampicin-resistant mutants, with the minor class accumulating more xanthan gum than either the major class or strain X55. Strains X30 through X49 were a randomly isolated set of rifampicin-resistant siblings, of which only three showed higher production of xanthan gum. Attempts to isolate mutants resistant to higher levels of rifampicin failed to yield strains that were reproducibly more productive than X59. Two high-level xanthan producers and two normal producers from a separate selection with 100 μg/ml rifampicin are included in Table 1 and were designated 'RM101-109'. We have adopted strain X59 as representative of the 'more-productive' class, since no other rifampicin-resistant mutants reproducibly make more xanthan gum.

Properties of xanthan gum synthesized by X. campestris mutants

Samples of xanthan gum made from each strain were tested for shear-dependent viscosity. Strains X55, X59 and X50 were each grown in PS medium plus 2.5% (w/v) glucose in shake flasks at 30°C for 40 h. The bacterial cells were removed by two centrifugation steps and the xanthan collected as before, as a precipitate in isopropyl alcohol. The polysaccharide was dried in a vacuum, ground to a powder, weighed and resuspended in 0.1% (w/v) NaCl at 0.3% (w/v) xanthan. Viscosities were measured at various shear rates using a Brookfield LVT viscometer with a number 18 spindle. Cell-free samples set aside before precipitation with alcohol (in other words, culture broth minus cells) showed shear-thinning viscosity. The viscosities for each strain at a shear rate of 1 s^{-1} were as follows: X50, 730 cps; X59, 500 cps; and X55, 205 cps. However, the alcohol-precipitated xanthan samples that were resuspended at 0.3% (w/v) xanthan showed indistinguishable viscosities at various shear rates. The viscosities were 320 $\pm$ 50 cps at a shear rate of 1 s^{-1}.

Fermentation of mutants of X. campestris at the 10-liter scale

Two modes of fermentation were carried out: batch and fed-batch. The batch mode conditions were compiled from several reports in the literature, were not optimized by us at the time and are now known to be suboptimal. Nevertheless, the results comparing different strains are meaningful. The results of four fermentations are given in Fig. 1. Three control cultures (X55, 56 and 57) were compared to X59, the rifampicin-resistant derivative of X55. The historical relationship between the three control strains is given in Materials and Methods. As shown in the four panels in Fig. 1, the three controls were not distinguishable for cell density, glucose consumed, xanthan produced or culture viscosity.

Table 1

Synthesis of xanthan gum by antibiotic-resistant *X. campestris*

Phenotype	Strain[a]	Dry weight of exopolysaccharide (mg)								
	Expt. No:[b]	1	2[c]	3[c]	4	5[c]	6[c]	7[c]	8	9
Rif^s	X55	37	41	38	32	27	119	159		
Rif^r	X59*	45	46	46	36	36	147	199	177	181
	X30		39							
	X31		41							
	X32		40							
	X33		39	39						
	X34*		45	43			149	180		
	X35		40							
	X36		39							
	X37*		51	44			143	186		
	X38		39							
	X39				29					
	X40				30					
	X41				29					
	X42				30					
	X43				30					
	X44*				33	36	153	196		
	X45				29					
	X46				29					
	X47				31					
	X48				30					
	X49				34	29				
	RM108*								182	
	RM102*								179	
	RM109								158	
	RM101								157	
Rif^{r}Bac^r	X50*					41			195	192

[a] Strains X30 through X49 were consecutively numbered, random, rifampicin-resistant derivatives of X55. RM108, 102, 109 and 101 were not a random set of isolates. They also were spontaneous derivatives of X55, but were retained as representative overproducers and normal strains. Each overproducer is marked by an asterisk.

[b] For experiments 1–5, the sample size was 8 g of culture broth; for experiments 6–9, the sample size was 10 g. The time of harvest was about 48 h. The media were: LB broth plus 2% (w/v) glucose, experiment 1; XG011 plus 2% (w/v) glucose, experiments 2–4; YM plus 2% (w/v) glucose, experiment 5; and XG004 plus 2% (w/v) glucose, experiments 6–9. Only the XG004 medium (experiments 6–9) was buffered with KH_2PO_4.

[c] Average values from two independent flasks. The standard deviation (1σ) for replicates was 5% or less.

The fermentations were continued until the glucose was consumed. The mutant strain, X59, was different in three respects. First, the rate of consumption of glucose was 1.4 times faster for X59 for the time interval beginning at about 20 h and extending until the end of the fermentation. Second, for this interval, xanthan accumulation was about 1.5 times higher for X59. Third, the viscosity of the X59 fermentation broth was about 1.6 times higher than that of the three controls. These three differences are of similar magnitudes. Analogous parallel fermentations of X55 and X59 confirmed these results.

The two strains X59 and X50 were also grown in fed-batch mode in PS medium. This medium allows

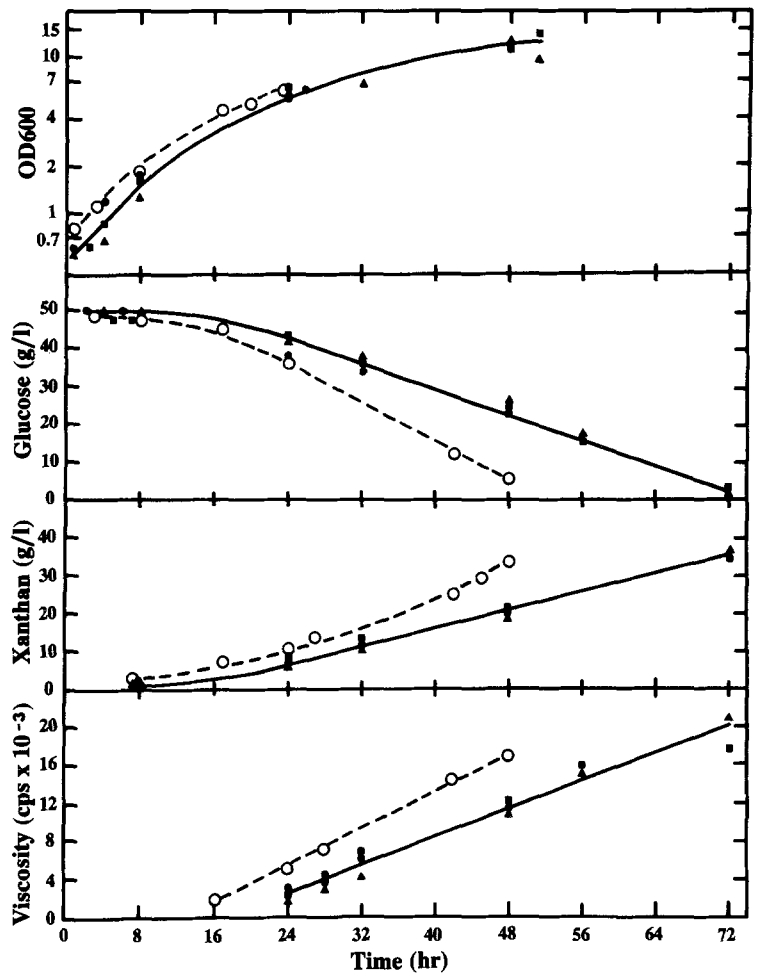

Fig. 1. Batch-mode fermentation of wild-type *X. campestris* and rifampicin-resistant strain X59. Symbols: ●, X55 (wild-type and parent of X59); ▲ X56 (wild-type); ■, X57 (wild-type); O, X59 (rifampicin-resistant mutant).

the accumulation of more cells and xanthan per volume. Modifications of new medium components and conditions were initially tested in shake flasks. The effects on cell growth and xanthan synthesis of initial glucose and nitrogen concentrations, temperature and pH were tested in preliminary experiments in a 10-liter fermentor (data not shown). The quantitative results of the fed-batch fermentations are summarized in Table 2. The fermentation was carried out in two phases. The first phase was a batch culture with initially high nitrogen content and glucose at 20 g/l. The nitrogen source was either yeast extract or peptone from casein at 15 g/l. The high nitrogen content promoted rapid cell growth (doubling time of about 3 h) and dense final cell concentrations (absorbance at 600 nm of 15–17 for the peptone-containing medium in Table 2). The first phase was about 24 h. During the second phase the culture was fed a concentrated solution of glucose and salts so that glucose was maintained at about 10 g/l. After about 24–30 h the feeding was stopped and the culture was allowed to consume the residual glucose.

By this two-phase culture, we could not completely dissociate cell growth from xanthan production. A low amount of xanthan accumulates even in young cultures. Nevertheless, the fed-batch fermentations were more productive than those carried out

Table 2

Fed-batch fermentations

	Strain: X59				X50	
	Medium: yeast extract		peptone		peptone	
	48 h	61 h	48 h	63 h	48 h	71 h
Absorbance (600 nm)	11	12	17	15	15	15
Xanthan[a] (g/l)	47	59	49	58	52	66
Viscosity[b] (cps × 10^{-3})	27	38	37	47	33	45
Yield (g xanthan/g glucose)	–	0.80	–	0.85	–	0.85
Global productivity (g xanthan/l/h)	0.98	0.96	1.04	0.92	1.07	0.92

[a] Xanthan dry weight was measured after precipitation with isopropyl alcohol.

[b] Viscosity was measured for crude culture broths with a Brookfield LVT viscometer with spindle number 4 at 12 rpm.

in batch mode. Final xanthan concentrations exceeded 60 g/l and viscosities were so high that mixing within the vessel was visibly poor. Global productivities exceeded 1 g xanthan per liter per hour for the 48-h period, and as seen earlier in shake flask experiments, X50, the bacitracin-resistant derivative of X59, accumulated more xanthan than its parent. However, we also noted a lower viscosity for the culture broth when expressed as centipoise per g xanthan. As yet, we do not know the basis for this apparent decrease. We should point out that no attempt has yet been made to specifically optimize the culture conditions for strain X50. Finally, the use of peptone as a nitrogen source in the fed-batch fermentations increased cell growth and the viscosity of the culture broth, while the amount of xanthan was unchanged, when compared to yeast extract as the nitrogen source.

Strains X59 and X50 as hosts for a recombinant plasmid bearing 'xanthan' genes

Previously we showed that a recombinant plasmid carrying genes essential for xanthan synthesis enhanced gum accumulation in *X. campestris* strain X59 by about 15–20% compared to the vector plasmid alone [13]. We repeated these measurements with both strains, X59 and X50 as hosts for the recombinant plasmid (c8) and the vector (pRK311). Table 3 shows the extent of xanthan accumulation measured as weight percent and final culture viscosity. As observed previously [13], strain X50 accumulated more xanthan than X59. In Table 3 both strains carry the cloning plasmid pRK311. When

Table 3

Synthesis of xanthan gum by strains X59 and X50 carrying multiple copies of cloned 'xanthan' genes

Plasmid[a]	Host	% (w/v) xanthan[b]	Culture viscosity[c]
pRK311	X59	1.4	700
pRK311	X50	1.6	1300
c8	X59	1.7	1200
c8	X50	1.7	1600

[a] Plasmid c8 comprises vector pRK311 [4] plus a cloned segment of the *X. campestris* chromosome which codes for genes essential for xanthan synthesis [13].

[b] Inocula of 10 ml were grown at 30°C overnight from single colony isolates in LB medium with tetracycline at 7.5 μg/ml and rifampicin at 50 μg/ml. Cultures of 250 ml in PS medium in 1-liter shake flasks were grown at 30°C while shaking for 64 h. The final weight percent of isopropyl alcohol-precipitated material was measured as described in Methods.

[c] Centipoise, at a shear rate of 1 s^{-1} at room temperature.

strain X59 carried multiple copies of the xanthan genes on plasmid c8, the amount of xanthan was further increased. However, because of the relative error of 5% in these measurements, we cannot conclude that c8 in X50 is higher than pRK311 in X50, but the viscosity of the culture broth was highest for plasmid c8 in strain X50. We observed a similar pattern for other recombinant plasmids, including a derivative of c8 (data not shown).

DISCUSSION

Xanthan gum was first described by Rogovin et al. [8] as a commercially promising polysaccharide product from cultures of *X. campestris*. Since then, considerable effort has been devoted to improving the production strain, fermentation conditions and recovery processes used to manufacture xanthan. A useful recent comprehensive review of xanthan production was prepared by Kennedy and Bradshaw [6], but it is likely that many process improvements remain as proprietary information within companies either producing or desiring to produce xanthan gum. Examples of levels of xanthan productivity reported in the literature are described below. The examples can be compared with our results. However, bench-scale fermentors have different internal configurations (baffles, cooling devices, probes and sampling lines) that result in different mixing effects. As xanthan accumulates, the broth viscosity becomes so high that some stagnant regions are observed in most vessels, even with energetic attempts at mixing. These differences in the effectiveness of heat and mass transfer cause the comparisons to be imprecise, at best. Nevertheless, it is interesting to attempt such comparisons.

The results of numerous batch fermentations are reported in the literature. We will mention only one here, as representative of high productivity. Moraine and Rogovin [7] described bench-scale batch fermentations with strain NRRL-B1459 and medium including a complex nitrogen source, distiller's dried solubles. In two 80-h cultures with pH control, 5 and 6% (w/v) glucose was converted to 3 and 3.8% (w/v) xanthan product. Our comparable 'wild-type' strains (X55, 56, 57) behaved similarly. The 5% (w/v) glucose added at the start was consumed in 72 h, and the final xanthan level reached 3.6% (w/v). However, as seen previously in shake flask tests, the rifampicin-resistant strain X59 completed the same conversion of carbohydrate to xanthan in only 48 h. We have not characterized the mutation in X59 to determine whether, in fact, an alteration in the cellular RNA polymerase has arisen, as is usually the case for a rifampicin resistance phenotype. We think a single mutation gives rise to the xanthan-overproduction phenotype, since the two classes of rifampicin-resistant mutants, normal and overproducers, were obtained at similar frequencies. Yet how such an alteration might shift the cell's macromolecular syntheses toward exopolysaccharide production for the overproducing class remains conjecture at this point.

For fed-batch fermentation, Wernau [14] reported about 70% conversion of glucose to a final 5.1% (w/v) xanthan gum. The 7-liter culture was begun with a 10% (v/v) inoculum and lasted for 136 h. The final culture viscosity was about 42 750 centipoise measured at 12 rpm with a Brookfield viscometer. The *X. campestris* strain was NRRL-B1459, and comparable if not identical to our strain X55. Although we have not fermented the parent strain X55 in fed-batch mode, the two mutated strains, X59 and X50, accumulated about 5% (w/v) xanthan in 48 h starting with a 10% (v/v) inoculum. Under our conditions the culture viscosity reached about 35 000 centipoise (Brookfield viscometer at 12 rpm) by 48 h and higher values later. We do not know why substitution of peptone for yeast extract resulted in higher culture viscosity per gram of xanthan accumulated. Our xanthan yield as a fraction of the amount of glucose added to the culture was about 80% (g xanthan/g glucose). Although admittedly not a precise comparison, the nearly threefold time difference in achieving comparable levels of xanthan gum justifies a continued effort to identify optimal fermentation conditions for these strains. An obvious requirement is to now demonstrate the productivity improvement at the pilot scale, or above 100 liters.

The reason why a bacitracin-resistant mutant

might accumulate more exopolysaccharide has been discussed by Sutherland [11] with particular emphasis on *Salmonella* and *Klebsiella* as examples. The idea is that exopolysaccharide synthesis is a lower cellular priority than synthesis of cell wall peptidoglycan and lipopolysaccharide, and that all three macromolecular syntheses may depend on a limited supply of the recyclable C_{55}-isoprenyl phosphate carrier molecule required for polymerization. Bacitracin was shown to form a complex with isoprenyl pyrophosphate and divalent cations [10] and indirect evidence [11] suggested that resistance to bacitracin for *Klebsiella* might be due to an abundance of isoprenoid lipids. On the contrary, we reasoned at the outset of our work that the total amount of lipid carrier in *X. campestris* was probably not a limitation to xanthan synthesis, since actually more xanthan is accumulated per cell per hour in exponentially dividing cells than after cell growth and active peptidoglycan synthesis has ceased. Therefore, we were surprised to find that the predominant class of bacitracin-resistant mutants of *X. campestris* were non-producers of xanthan, as if by not making xanthan, more carrier is available for essential peptidoglycan and lipopolysaccharide synthesis. A minor class, represented by the strain X50, produced more xanthan than its parent X59 and cell growth appeared to be depressed slightly. In order to resolve the apparent contradiction of these two mutant classes and to determine whether Sutherland's [11] notion of availability of carrier is true for *X. campestris,* we would need to measure the amount of carrier at different growth phases.

In evaluating the economic benefit from an apparent improvement in productivity from an altered bacterial strain, a key consideration must be the quality of the product made under the new conditions. We determined whether or not the xanthan gum accumulated by these new strains was qualitatively the same. Our measurements of specific viscosity based on grams of material at various shear rates revealed no differences. To rigorously prove identity we would need to chemically analyze the products and carefully measure molecular size, but that is beyond the scope of the present work. The essential determinant of specific viscosity in dilute solutions appears to be molecular chain length and not the amount of acetyl or pyruvate groups attached to the xanthan side chains [3]. The chemical makeup of xanthan may play a more significant role in more concentrated polysaccharide solutions where intermolecular associations are possible. Furthermore, culture conditions can influence the degrees of pyruvylation and acetylation of xanthan [8,12].

Recently, we have used the X59 and X50 mutant strains as hosts for multi-copy plasmids that carry genes previously cloned from a wild-type *X. campestris* strain and that code for products essential for xanthan synthesis [13]. Certain of the plasmid-carried multiple copies of cloned DNA previously were shown to increase xanthan gum accumulation in strain X59 [13]. The two mutant strains retained their relative productivities while carrying different multi-copy recombinant plasmids: in each case the X50 constructions produced more xanthan than the X59 constructions. Therefore, we believe that the rifampicin- and bacitracin-resistant strains described here can be used in concert with recombinant DNA technology to improve xanthan production.

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