GENETIC CONSTRUCTION OF XANTHOMONAS CAMPESTRIS AND XANTHAN GUM PRODUCTION FROM WHEY

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SUMMARY

Plasmids pUR291 and pNZ521 containing lacZ gene, maturation protein and proteinase P genes, were transferred into X. campestris either by conjugation or by transformation. Plasmid pNZ521 was also conjugally transferred into X. campestris m1 a transformant carrying plasmid pUR291. All the constructed strains were evaluated for xanthan gum production in either a medium of 50% whey or the same medium supplemented with 1.5% lactose or 1.5% glucose. Mixed cultures either with transconjugants or with transformants were tested for xanthan gum production as well.

INTRODUCTION

Xanthomonas campestris, a Gram- bacterium, produces xanthan gum, a water soluble, extracellular polysaccharide. Xanthan is composed of pentasaccharide repeating units, containing D-glucose, D-mannose, D-glucoronic acid, acetal-linked pyruvic acid and D-acetyl groups (Moorhouse et al. 1977). Xanthan gum has unique properties which contributed to the establishment of its use in numerous applications, mainly in the techniques of oil drilling, in textile and food industry, where it is used as thickener, emulsifier and stabiliser (Andrew 1977, Jeans 1974, Moorhouse et al. 1977).

The construction of lactose-utilizing X. campestris was recently reported (Fu and Tseng 1990, Fu et al. 1992, Konicek et al. 1992, Thorne et al. 1984, Schwartz and Bodie 1985, Walsh et al. 1984). All these constructed strains were capable of utilizing lactose from whey, but do not producing xanthan gum in large quantities. X. campestris conjugated with Lactococcus lactis containing the pNZ521 plasmid with phospho-8-
galactosidase, maturation protein and proteinase P genes, was capable to produce xanthan gum in high quantities from whey media (Ekateriniadou et al. 1994).

In this paper we describe the construction of *X. campestris* strains either by conjugation or by transformation with *Escherichia coli*, *Lactococcus lactis*, or *X. campestris* XLM1521. Most of the constructed strains were capable of producing xanthan gum from whey in large quantities. Results from fermentations with mixed cultures were presented as well.

**MATERIAL AND METHODS**

**Bacterial strains and plasmids.** The bacterial strains and plasmids used in this study are listed in Table 1.

**Media and cultivation.** LB medium was used as a general-purpose medium with 0.2% glucose (LBG), (Maniatis et al. 1989). For *X. campestris* XLM1, XMT1 and XLM1521, LBG medium was supplemented with 20μg/ml Streptomycin (St), 50μg/ml Ampicillin (Am) and 8μg/ml Chloramphenicol (Ch), respectively. *E. coli* XL1 was cultured in LBG medium with 50μg/ml Am. *L. lactis* MG1820 was maintained in M17 medium containing 0.5% lactose (LM17) or glucose (GM17) and supplemented with 10μg/ml Ch (Terzaghi, B.E. and Sandine, W.E., 1975). The whey was supplied from the dairy industry "D. Kolios Inc." Thessaloniki, Greece. All media were sterilised by autoclaving. Lactose and glucose solutions were sterilised by filtration. Strains of *X. campesristis*, *L. lactis* and *E. coli* were incubated at 28°C, 28°C-30°C and 37°C, respectively. For liquid cultures, bacteria were grown in 250-ml flasks containing 20ml of liquid with vigorous shaking.

**Conjugation.** Plasmid pUR291 was transferred by conjugation from *E. coli* into *X. campestris* XLM1 by the filter mating procedure (Walsh et al.1984). The lac phenotype of the recipient and transconjugant was tested on LBG agar containing X-gal and isopropyl-β-D-thiogalactoside (IPTG) at a concentration of 1mM. Blue colonies were selected for further analysis. Plasmid pNZ521 was transferred by conjugation from *L. lactis* MG1820 into *X. campestris* XLM1 as it was previously described. (Ekateriniadou et al. 1994). Plasmid pNZ521 was also transferred by conjugation from *X. campestris* XLM1521 into *X. campestris* XMT1 as it was described above. In all cases transfer frequencies are expressed as the number of transconjugant per donor.

**Transformation.** A single colony of *X. campestris* w.t. was transferred into 5ml LB broth and incubated overnight at 28°C. 1% inoculum of that culture was transferred into 20ml LB broth and incubated at 28°C until it reached an optical density (600nm) of ca. 0.9 (10^8 cells per ml). Cells were pelleted by centrifugation at 1130xg for 15min. Pellet was suspended in 1/6 of the culture volume of 0.1M MgCl₂ and incubated on ice for 30 min. After centrifugation at 6000xg for 10min the pellet was suspended in 1/6 of the culture volume of 0.1M CaCl₂ and incubated on ice for 20min. After a new centrifugation at 6000xg for 10min the pellet was resuspended in 1/20 of the culture volume of 0.1M CaCl₂. 100μl of the competent cells were mixed with plasmid DNA (final concentration 2μg/ml), incubated on ice for 60min and heated at 42°C for 1min. Cells then were inoculated into 900μl LBG broth and incubated at 28°C for 4h with mild shaking. Transformants were selected on LB agar containing X-gal, IPTG and Am (50μg/ml) for pUR291 or Ch (10μg/ml) for pNZ521. As control were used competent