HIGH PRODUCTION of XANTHAN GUM by a STRAIN of *Xanthomonas campestris* CONJUGATED with *Lactococcus lactis*

by

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SUMMARY

Plasmid pNZ52L, containing phospho-β-galactosidase, maturation protein and proteinase P genes, was conjugally transferred for the first time from *Lactis* into *X.campestris* XLM1. After 20 generations 67% of the tested colonies were resistant to chloramphenicol. In the transconjugant proteinase activity appeared in the growth medium, whereas in *Lactis* MG1820 it was extracted from the cell wall. Proteinase activity and production of xanthan gum were studied in different concentrations of whey. Xanthan gum production was found much higher in all cultures with the transconjugant strain XLM152L.

INTRODUCTION

Whey, a byproduct of dairy industries, is a fluid containing very low quantities of milk solids and it possesses high biological value, due to its consistency in lactose and proteins.

*Xanthomonas campestris*, a Gram- mobile bacillus, produces xanthan gum, a water soluble extracellular polysaccharide. 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). *X.campestris* possesses β-galactosidase activity, though does not produce large amounts of xanthan gum from whey.

The construction of lactose-utilizing *X.campestris* strains was recently reported (Fu and Tseng 1990, Fu et al 1992, Konicek et al 1992, Walsh et al 1984). All these constructed strains were capable of utilizing lactose from cheese whey, but do not producing xanthan gum in large quantities.

In this paper we describe the construction of a strain of *X.campestris* XLM1 by conjugation with *Lactococcus lactis* MG1820, capable of producing xanthan gum from whey in large quantities.
Bacterial strain. *Xanthomonas campestris* XLM1 (Str+) was derived in our laboratory from *X. campestris* ATCC 13951 (obtained from the lab. of Technological Institute of Thessaloniki, Greece) by serial plating in LB agar with 20 μg/ml streptomycin. *Lactococcus lactis* MG1820 (Maeda and Gasson 1986, De Vos et al 1989), carrying pNZ521 (De Vos et al 1989) and MG1363 (Gasson 1983) were obtained from National Institute of Research, Athens, Greece.

Media. *X. campestris* ATCC 13951 and XLM1 were maintained in Luria-Bertani medium (Maniatis et al 1989) plus 0.2% glucose (LBG). For *X. campestris* XLM1 LBG medium was supplemented with 20 μg/ml streptomycin (Str) (Sigma Chemical Co.). *Lactis MG1820* and MG1363 were maintained in M17 medium contained 0.5% lactose (LM17) and glucose (GM17) respectively (Terzaghi and Sandine 1975). For *Lactis MG1820* M17 medium was supplemented with 10 μg/ml chloramphenicol (Ch) (Sigma Chemical Co.). The sweet whey was supplied from the dairy industry "D.Kolios Inc", Thessaloniki, Greece. All media were sterilised by autoclaving.

Conjugation. The filter mating procedure described by Gasson and Davies (1980) was used with the following modifications. From an overnight culture of *Lactis MG1820*, 5% transfer was made into 15 ml LM17. The culture was incubated at 28-30°C until it reached an optical density (600 nm) of 0.4. Mating mixtures were prepared by using the donor and recipient cultures at a ratio 1:10. Filter mating experiment involved collection of 5 ml of the mating mixture on a 0.45 μm (pore diameter) filter (type HA, Millipore Corp.) and incubation on GM17 agar for 1 or 2 hours at 28-30°C. Cells were suspended in 2 ml Ringer solution (Oxoid). Serial dilutions of the wash were plated onto McConkey or LBG agar containing Str (20 μg/ml) and Ch (8 μg/ml) and incubated at 28-30°C for 72 hours. Transfer frequencies are expressed as the number of transconjugant colonies per recipient colonies.

Isolation of cell wall proteinase. Proteinase P (prtP) from *Lactis MG1820* was extracted in the absence of Ca++, as described by Exterkate and de Veer (1984).

Assay of proteinase. Proteinase activity was determined by using N-CBZ-Glycine-p-nitrophenyl ester as substrate, diluted in acetonitrile (Korrr and Teh-Yung Lin 1973). The assay mixture contained 50 mM CH3COONH4-8% w/v CH3CN pH 5.0; 10 mM CaCl2; 0.05 mM dithiothreitol; 0.5 mM substrate and enzyme preparation in a final volume of 10 ml. The reactions were terminated by the addition of 0.5 ml 0.3 N NaOH. Absorbance was measured at 410 nm and the amount of p-nitrophenol released was determined from a standard curve. One unit of proteinase was defined as the amount of 1 amol p-nitrophenol released at 25°C under the above specified conditions.

Plasmid isolation. Single colonies of transconjugant and donor were transferred into 10 ml LB broth containing the appropriate antibiotics. The cultures were incubated at 28°C until they reached an optical density of 0.6-0.8 at 600 nm. A small scale isolation procedure of plasmid DNA (Maniatis et al 1989) was done in transconjugants. Plasmid DNA from *Lactis MG1820* was isolated by CaCl2 (Gasson 1983). The samples were analysed in horizontal gel containing 1% agarose in TAE buffer (40 mM Tris- acetate, 1 mM EDTA pH 8.0) and visualised under UV by ethidium bromide (0.5 μg/ml). As markers were used λDNA/BstEII (New England Biolabs).

Plasmid stability. Plasmid stability was checked as it was described by Walsh et al. (1984).

Lactose determination. Lactose/D-galactose kit from Boehringer Mannheim was used for this purpose.

Xanthan gum determination. Xanthan gum was determined by measuring the dry weight of xanthan gum, as described by Chin -Hang Shu et al. (1990) with some modifications. Cultures of 10 ml were diluted with water by a factor of 2-5 and then were centrifuged at 10000xg for 20 min at 4°C to precipitate the suspended cells. Two volumes of alcohol and KCl, at a final concentration of 5% w/v, were added to the supernatant to precipitate xanthan gum. Polysaccharide solution was then filtered off and dried at 40°C for 24 h.

Growth curves. Single colonies of donor, recipient and transconjugant were transferred into LB+Str, LM17+Ch and LB+Str+Ch, respectively. The cultures were incubated at 28°C until they reached an optical density (600 nm) of 0.6-0.8. One ml of this was transferred into 100 ml of each medium. The cultures were incubated at 28°C in 1-liter flasks with shaking. Growth of the cultures was estimated by measuring the optical density at 600 nm.