Possible Plasmid Nature of the Determinant for Production of the Antibiotic Nisin in Some Strains of *Streptococcus lactis*

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INTRODUCTION

During recent investigations into the genetics of lactic streptococci performed in our laboratory, some observations were made which indicated that gene(s) determining the production of the polypeptide antibiotic nisin (Nis) in some strains of *Streptococcus lactis* may be located on a plasmid (Kozak, Rajchert-Trzpił & Dobrzański, 1973*a*, 1974). The evidence included (i) the frequent and spontaneous occurrence of stable nisin-negative (Nis⁻) clones in populations of some nisin-producing (Nis⁺) strains at frequencies of 0.03 to 0.82% and (ii) an increase in the frequency of stable Nis⁻ clones as a result of growth of some Nis⁺ strains in the presence of proflavin (Pro), ethidium bromide (EB) or at the elevated temperature of 40 °C. A 5.4- to 95-fold increase occurred following growth with Pro or EB and a 1.4- to 20-fold increase as a result of growth at 40 °C.

We tested for the presence of plasmid DNA in certain of these *S. lactis* nisin-producing strains and in Nis⁻ clones isolated from them after treatment with Pro and EB (Kozak *et al.* 1973*a*, 1974).

METHODS

Organisms. All *Streptococcus lactis* strains used in the study originated in this laboratory (Kozak *et al.* 1973*a*, 1974). The strains and their relevant characters are listed in Table 1.

Media. Bacteria were stored at 4 °C in a 10% (w/v) aqueous suspension of powdered skim milk, enriched with 0.5% yeast extract (Difco). Radioactive labelling of DNA was performed in a semi-synthetic medium (SSM; Parsons *et al.* 1973). Lactose-fermenting ability was tested using a medium composed of (% w/v): dehydrated meat broth (Biomed, Poland), 1; yeast extract (Difco), 1; NaCl, 1.5; Na₂HPO₄, 0.5; MgSO₄·7H₂O, 0.5; lactose, 1; bromocresol purple, 0.004; agar, 1.5; pH 7.2.

Nisin was determined by the method of Tramer & Fowler (1964).

Lysogeny was tested using the method of Kozak *et al.* (1973*b*).

DNA labelling and preparation of cleared lysates. Samples (24 ml) of SSM supplemented with 2-deoxyadenosine (250 µg/ml) and [³H]thymidine (5 µCi/ml; specific activity 5 Ci/mmol) were inoculated with 3% (v/v) of an overnight culture of *S. lactis* in SSM (3 × 10⁸ colony-forming units/ml) and grown for 5.5 h at 30 °C. Bacteria were collected by centrifugation, washed once with TES buffer (0.03 M-tris, 0.005 M-Na₂-EDTA, 0.05 M-NaCl, pH 8.0) and suspended in 2 ml of TES. A portion (0.4 ml) of lysozyme solution (12 mg/ml) was added and the samples were incubated for 1.5 h at 37 °C. Lysis was then performed with Brij-58 by the method of Courvalin, Carlier & Chabbert (1972). After lysis was completed, samples were centrifuged at 20000 g for 30 min at 4 °C to remove the bulk of the chromosomal DNA.

Table 1. *Strains of S. lactis*

<i>S. lactis</i> strain no.	Ability to:		Lysogeny
	Produce nisin	Ferment lactose	
2	+	+	ND
27	+	+	ND
29	+	+	ND
40	+	+	+
45	+	+	+
49	+	+	ND
49/v	+	+	ND
51/v	+	+	ND
49/v/Pro*	—	—	ND
49/v/EB*	—	—	ND
51/v/Pro*	—	—	ND
51/v/EB*	—	—	ND

ND, Not detected.

* Clones isolated after treatment of strains 49/v and 51/v with proflavin or ethidium bromide.

Preparation of the gradients and ultracentrifugation. Cleared lysates were mixed with solid caesium chloride and the refractive indices were adjusted either to 1.4024 (CsCl gradients) or to 1.3929 (CsCl-EB gradients). The mixtures were then transferred to centrifuge tubes and 0.05 ml ethidium bromide solution (10 mg/ml) was added to appropriate gradients immediately before centrifugation. Samples were overlaid with liquid paraffin and centrifugation was performed for 40 h at 150000 g in the 65 Ti rotor of a Beckman L2-65B ultracentrifuge at 20 °C. Fractions were collected by puncturing the bottom of the tubes. A 10 µl portion of each fraction was then put on to a filter-paper disc (Whatman 3 MM), washed twice with cold 5% (w/v) TCA solution and once with acetone, and dried. Radioactivity was counted with a Packard model 2425 liquid scintillation spectrometer using a Liquifluor scintillation mixture.

Chemicals. [Methyl-³H]thymidine was purchased from the Radiochemical Centre, Amersham, Buckinghamshire. Ethidium bromide, Brij-58 and CsCl (optical grade) were obtained from Serva Feinbiochemica, West Germany, 2-deoxyadenosine from Sigma, proflavin from Loba-Chemie, Austria, and Liquifluor from NEN Chemicals, West Germany.

RESULTS AND DISCUSSION

As shown in Table 1, in four clones derived from strains 49/v and 51/v loss of the ability to produce nisin as a result of treatment with Pro or EB was accompanied by loss of the ability to ferment lactose.

Cleared lysates of strains were investigated by ultracentrifugation in CsCl-EB gradients (Radloff, Bauer & Vinograd, 1967) for the occurrence of a more dense fraction of DNA, indicating the presence of covalently closed circular (c.c.c.) DNA characteristic of plasmids.

Among eight *S. lactis* strains tested, six (nos. 27, 40, 45, 49, 49/v and 51/v) were found to have c.c.c. DNA, but in two strains (nos. 2 and 29) it was not observed. When centrifuging the cleared lysates in a gradient of CsCl alone, the denser DNA fraction was absent.

Though c.c.c. DNA was not observed in cleared lysates prepared from clones 49/v/Pro and 49/v/EB, it was detectable in lysates of clones 51/v/Pro and 51/v/EB (Fig. 1). In clone

Fig. 1. CsCl and CsCl-EB gradient analysis of cleared lysates from *S. lactis* strains 49/v, 51/v and Nis⁻ derivatives isolated after proflavin or ethidium bromide treatment.

51/v/Pro the c.c.c. fraction was very small in comparison with the same fraction in the parental Nis⁺ strain.

The lack of c.c.c. DNA in two of the strains studied (nos. 2 and 29) may indicate the absence of plasmids in these strains or the presence of plasmid material integrated into the bacterial chromosome. Since the majority of the strains tested were non-lysogenic, it is unlikely that that phage genomes contribute to c.c.c. DNA.

The loss of Nis⁺Lac⁺ characters in strain 49/v, accompanied by the disappearance of

c.c.c. DNA, may indicate that genes responsible for these characters are located on a plasmid(s). However, clones 51/v/Pro and 51/v/EB, although Nis⁻Lac⁻, still contain c.c.c. DNA. The occurrence of a minute c.c.c. DNA peak in clone 51/v/Pro may be characteristic, since it was observed in each of three independent experiments.

The results of Kozak *et al.* (1974) suggest that (i) ethidium bromide and proflavin are less effective curing agents for strain 51/v than for 49/v, and (ii) in strain 51/v proflavin may be more effective in eliminating the nisin gene(s) than ethidium bromide. Plasmids differ in their susceptibility to various curing agents (Clowes, 1972) and it is possible that in strain 51/v the genes for nisin production and lactose fermentation are located on a plasmid(s) which has been removed by the curing agents, while certain other plasmid DNA remains in the bacterial population.

Since the completion of these experiments, Cords, McKay & Guerry (1974) have reported the presence of various forms of c.c.c. DNA in different strains of lactic streptococci. They found that *S. lactis* C2 and a lactose-negative clone isolated from this strain after acriflavine treatment both contained c.c.c. DNA.

Further experiments are necessary to determine the properties of *S. lactis* plasmids. Such experiments are in progress in our laboratory.

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