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Note

DNA-DNA homology between plasmids from *Streptococcus thermophilus*

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Summary

Fifty strains of *Streptococcus thermophilus* isolated from raw milk, industrial starters or issued from collections were studied for their plasmid complement. Most of them did not contain plasmid DNA. Six strains only harboured one plasmid and three strains two plasmids. All these plasmids were small sized : 2.9 to 7.6 kilobases. Restriction analysis and DNA-DNA hybridization were used to demonstrate similarity between plasmids of these strains and to distinguish several homology groups among them.

Key words : Streptococcus thermophilus - Plasmids - DNA-DNA hybridization - Restriction analysis.

Résumé

Homologie de l'ADN des plasmides de Streptococcus thermophilus

Nous avons étudié le contenu plasmidique de cinquante souches de *Streptococcus thermophilus* isolées de laits crus, de ferments industriels ou issues de collection. La plupart d'entre elles ne contiennent pas de plasmides. Six souches possèdent un seul plasmide et trois souches possèdent deux plasmides. Tous ces plasmides sont de petite taille : de 2,9 à 7,6 kilobases. L'analyse de restriction et l'hybridation ADN-ADN ont permis de montrer des similitudes entre les plasmides de ces souches et de distinguer plusieurs groupes d'homologie parmi eux.

Mots clés : Streptococcus thermophilus - Plasmides - Hybridation ADN-ADN - Analyse de restriction.

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Introduction

Streptococcus thermophilus is used in making dairy products when a high fermentation temperature is necessary (ACCOLAS *et al.*, 1980). This bacterium has only been isolated from heat treated milk (OTTOGALLI *et al.*, 1978) and milk is considered as its main habitat. In spite of its industrial importance, little is known about this species : few reports have appeared concerning the presence of extrachromosomal elements in this species. PECHMANN *et al.* (1982) claimed the existence of plasmid DNA only in 1 out of 8 strains. SOMKUTI and STEINBERG (1981) described up to 5 plasmids in 26 out of 36 thermophilic strains but they did not differentiate between *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. In the report of DAVIES and GASSON (1983), DAVIES and UNDERWOOD (unpublished data) mentioned that 18 of 53 strains carried between 2 and 5 plasmids.

Recently, HERMAN and Mc KAY (1985) analysed 23 strains from commercial starters and collections and found only 5 strains possessing a single plasmid which ranged in size from 2.2 to 3.5 kb. SOMKUTI and STEINBERG (1986) analysed 35 strains and 13 out of them were found to contain plasmid DNA ranging in size from 2.2 to 14.75 kb.

In our study the plasmid complement of 21 strains of *S. thermophilus* that have been isolated from raw milk was examined and compared with 20 strains used as industrial starters and with 9 collection strains. An attempt has been made to establish the extent of homology existing among these extrachromosomal DNA molecules using restriction analysis and DNA-DNA hybridization.

I. Materials and methods

A. *Strains and cultivation*

Strains were isolated from different samples of raw milk, industrial starters and from the Centre National de Recherche Zootechnique Collection (CNRZ-INRA, Jouy-en-Josas, France) and were characterized by physiological, biochemical and serological tests. Bacteria were grown in M17 medium (TERZAGHI and SANDINE, 1975).

B. *Preparation and restriction analysis of plasmid DNA*

Plasmid DNA was isolated according to ANDERSON and MCKAY (1983) and eventually purified in cesium chloride - ethidium bromide density gradient ultracentrifugation (240 000 × *g*, 22 h, 20 °C). Covalently closed circular (CCC) configuration was determined according to HINTERMANN *et al.* (1981). Restriction endonucleases were purchased from Bethesda Research Laboratory and used as recommended by the manufacturer.

C. *Electrophoresis of plasmid DNA*

Plasmid profiles and plasmid digests were performed on an horizontal slab gel electrophoresis apparatus with 0.6 to 1 % agarose (Sigma, type II) gels in 40 mM Tris-acetate, pH 7.9 and 2 mM di-sodium EDTA running buffer.

Plasmid DNA of ML3 *S. lactis* (DAVIES *et al.*, 1981) and *Hind* III fragments of lambda phage DNA (SANGER *et al.*, 1982) were used as size markers. The average size of plasmids was obtained with at least five measurements. DNA was transferred from gels to nitrocellulose filters (BA85 from Schleicher & Schuell, GmbH) by the SOUTHERN blot technique (SOUTHERN *et al.*, 1975).

D. *Preparation of ^{32}P-labelled DNA*

Whole plasmid DNA was extracted, purified and nick translated by incubation at 14 °C for 90 min with 10 microcuries of ^{32}P-labelled dCTP in the presence of DNase and DNA polymerase (Amersham). The reaction was stopped by the addition of 10 mM di-sodium EDTA and unbound labelled nucleotides separated using Sephadex G50 (Pharmacia) ; before use labelled DNA was boiled for 5 min and rapidly cooled on ice.

E. *DNA-DNA hybridization on filters*

Prehybridization was performed on nitrocellulose filters in Denhardt's solution (MANIATIS *et al.*, 1982) for 2 h at 60 °C in the presence of calf thymus DNA (Sigma). Hybridization was carried out for 18 h at 60 °C ; filters were washed 5 times in a solution containing 450 mM NaCl, 45 mM tri-sodium citrate, 0.1 % (w/v) sodium dodecyl sulphate (SDS) and 1 mM di-sodium EDTA, then exposed on X-ray film (Kodak X OMAT AR) for 2 to 3 days.

II. Results

A. *Number of plasmids in strains of* S. thermophilus

50 strains were checked for their agreement with the species *S. thermophilus* and examined for their plasmid complement. No plasmid DNA was detected in 41 strains which included the following strains : CNRZ 160, CNRZ 308, CNRZ 310, CNRZ 368, CNRZ 411, CNRZ 456, CNRZ 560 (NCDO 1611), HO_S from D^r J. STADHOUDERS (NIZO, Department of Microbiology, Ede, Netherlands). Single plasmids were found in 6 strains and 3 contained 2 plasmids. All were checked for CCC configuration. Plasmids were present in 1 out 9 CNRZ collection strains, 2 out of 20 industrial strains, and 6 out of 21 isolated strains (table 1).

TABLE 1

Restriction digests from plasmids of S. thermophilus

Analyse de restriction des plasmides de S. thermophilus

Strain	Plasmids	Restriction Endonucleases	Size (kb) of fragment	Total size (kb) of plasmid
4 [c]	pUCT4a	*Bst* EII	2.8	2.8
		Eco RI	2.8	2.8
	pUCT4b	*Mbo* I	2.4, 2.1	4.5
43 [b]	pUCT43	*Bgl* II	5.5, 2.0	7.5
		Mbo I	5.5, 1.0, 0.9	7.4
		Taq I	2.5, 1.7, 1.0, 0.9, 0.8, 0.7	7,6
44 [b]	pUCT44	*Bgl* II	5.9, 2.0	7.9
		Mbo I	5.4, 1.2, 1.1	7.7
		Taq I	2.3, 1.4, 1.1, 1.0, 0.9, 0.8	7.5
53 [c]	pUCT53a	*Bst* EII	3.0	3.0
		Eco RI	3.0	3.0
	pUCT53b	*Eco* RI	1.8, 1.7	3.5
127 [c]	pUCT127	*Eco* RI	3.8	3.8
		Mbo I	3.8	3.8
130 [c]	pUCT130	*Bst* EII	2.9, 1.0	3.9
		Eco RI	3.8	3.8
		Mbo I	3.8	3.8
132 [c]	pUCT132	*Bst* EII	2.9, 1.0	3.9
		Eco RI	3.8	3.8
		Mbo I	3.8	3.8
240 [c]	pUCT240	*Bst* EII	2.8	2.8
		Eco RI	3.0	3.0
*T129 [a]	pUCT129b	*Bgl* II	5.0	5.0

[a] CNRZ312.
[b] origin : industrial starter.
[c] origin : raw milk.
* pUCT129a was cut with neither endonucleases used.

B. *Size of the plasmids*

Several plasmid types were found in size from 2.9 ± 0.1 to 7.6 ± 0.2 kb (table 1). The unique plasmid of strains 43 and 44 is the same size : 7.6 kb ; they are respectively named pUCT43 and pUCT44. Strains 127, 130, and 132 each harboured a plasmid of identical size : 3.8 kb, respectively named : pUCT127, pUCT130, and pUCT132.

C. *Restriction analysis of plasmid DNA*

Each plasmid DNA was treated with a variety of restriction endonucleases. No activity was obtained with *Ava* I, *Bam* HI, *Hae* III, *Hha* I, *Hind* III, *Kpn* I, *Pst* I, *Pvu* II, *Sal* I, *Xba* I or *Xho* I.

Results of restriction analysis with *Bgl* II, *Bst* EII, *Eco* RI, *Mbo* I, and *Taq* I are reported in table 1. Restriction pattern of pUCT43 and pUCT44 with *Mbo* I, *Bgl* II and *Taq* I was very similar (table 1). The same can be said of plasmids pUCT4a, pUCT53a and pUCT240 hydrolysed with *Bst* EII and *Eco* RI. Plasmids pUCT127, pUCT130, and pUCT132 were similarly restricted with *Mbo* I and *Eco* RI (table 1) ; pUCT127 was not cleaved by *Bst* EII. The pUCT129b was only linearized by *Bgl* II and pUCT129a was cut with neither endonucleases used.

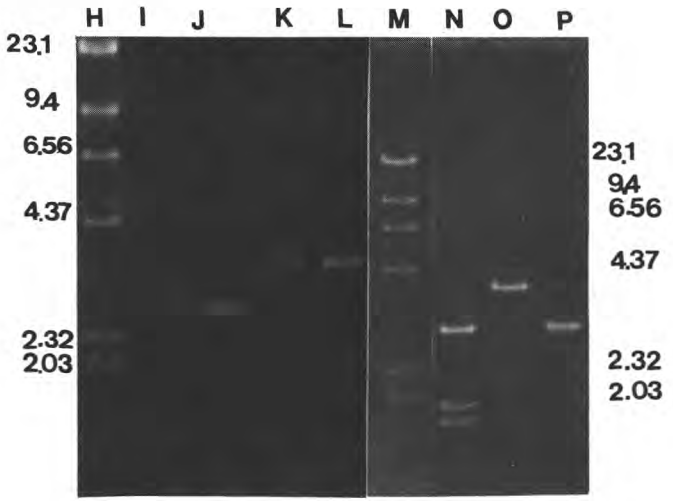

Fig. 1A

Agarose gel electrophoresis of plasmids from Streptococcus thermophilus *hydrolysed by various restriction endonucleases. Tracks H to L : Agarose concentration : 0.8 % ; (H) : lambda phage DNA* Hind *III fragments (control) ; (I) : pUCT 130 digested by* Bst *EII ; (J) : pUCT132 digested with* Bst *EII ; (K) : pUCT130 digested with* Eco *RI ; (L) : pUCT132 digested with* Eco *RI ; Tracks M to P : Agarose concentration : 1 % ; (M) : lambda phage DNA* Hind *III fragments (control) ; (N) : pUCT53a and pUCT53b digested with* Eco *RI ; (O) : pUCT127 digested with* Eco *RI ; (P) : pUCT240 digested with* Eco *RI.*

Electrophorèse en gel d'agarose de plamides de Streptococcus thermophilus *hydrolysés par diverses endonucléases de restriction.*

Bandes H à L : concentration d'agarose de 0,8 % ; (H) : fragments Hind *III du phage lambda (contrôle) ; (I) : pUCT130 coupé par* Bst *EII ; (J) : pUCT132 coupé par* Eco*RI ; (L) : pUCT132 coupé par* Eco *RI. Bandes M à P : concentration d'agarose de 1,0 % ; (M) : fragments* Hind *III du phage lambda (contrôle) ; (N) : pUCT53a et pUCT53b coupés par* Eco *RI ; (O) : pUCT127 coupé par* Eco *RI ; (P) : pUCT240 coupé par* Eco *RI.*

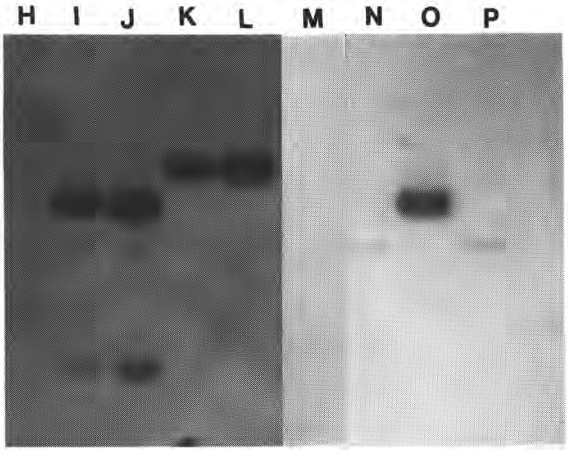

Fig. 1B

Autoradiogram of the Southern blot of both gels shown in figure 1A with ^{32}P-labelled DNA pUCT130 used as a probe.

Autoradiogramme du buvardage selon Southern des deux gels représentés sur la figure 1A après hybridation avec l'ADN du pUCT130 marqué au ^{32}P et utilisé comme sonde.

D. *DNA-DNA hybridization with ^{32}P-labelled DNA probes from different plasmids*

Labelled pUCT43 probe hybridized with pUCT43 and pUCT44 plasmids and all their restriction fragments. No hybridization was observed with any other plasmids using this probe.

The pUCT130 probe only hybridized with pUCT130, pUCT127, and pUCT132 (fig. 1A and 1B).

Whole labelled plasmid DNA from the strain 53 hybridized with pUCT240, pUCT4a, and both plasmids of the strain 53 : pUCT53a and pUCT53b. No hybridization was observed with any other plasmids using this probe.

Neither plasmid from strain T129 nor pUCT4b ever hybridized with any probe assayed.

III. Discussion-Conclusion

By analysing 50 strains of different origins, we confirmed the paucity of plasmids in *S. thermophilus* (Herman and McKay, 1985 ; Somkuti and Steinberg, 1986) : 9/50 of the strains (18 %) harboured 1 or 2 plasmids and their small size : 2.9 to 7.6 kb. Some of them were identical in size and in restriction analysis (table 1).

Most of the plasmid types bore a single restriction site for one or two endonucleases and could be a potential source of plasmid vectors.

Among 23 strains isolated from commercial starters or obtained from collections, HERMAN and MCKAY (1985) found only 5 strains (from commercial sources) containing a single plasmid. Using DNA-DNA hybridization, they showed that all these plasmids belong to a single group of homology. In our study, we distinguished 8 plasmids by their size and their restriction fragments ; we could easily classify them in 3 completely distinct groups of homology :

— pUCT43 and pUCT44 ;

— pUCT130, pUCT132 and pUCT127 ;

— pUCT240, pUCT4a, pUCT54b and/or pUCT53b.

These results indicated that the plasmids studied did not originate from a common ancestor as suggested by HERMAN and MCKAY (1985).

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