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Note

Determination of genome size of four *Propionibacterium* species by pulsed-field gel electrophoresis

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Summary — The 2 restriction enzymes *Xba*I and *Ssp*I were found to produce DNA fragment distributions convenient for genome analysis of dairy propionibacteria species (*P acidipropionici*, *P freudenreichii*, *P jensenii* and *P thoenii*) by pulsed-field gel electrophoresis. These restriction enzymes produced 10–19 fragments with sizes ranging from 5–1 000 kilobases (kb) depending on the strains. The entire genome size was estimated to be approximately 2 300–3 200 kb depending on the species.

***Propionibacterium* spp / genome size / pulsed-field gel electrophoresis**

Résumé — Détermination, par électrophorèse en champs pulsés, de la taille du génome de 4 espèces de *Propionibacterium*. *Parmi plusieurs endonucléases de restriction testées, deux enzymes (*Xba*I et* Ssp*I) ont été utilisées de manière à générer une distribution de fragments bien adaptée à une analyse en champs pulsés du génome des bactéries propioniques laitières (*P acidipropionici, P freudenreichii, P jensenii *et* P thoenii*). Ces enzymes de restriction produisent suivant les souches 10 à 19 bandes dont les tailles varient de 5 à 1 000 kilobases (kb). La taille du génome varie suivant les espèces et s'échelonne de 2 300 à 3 200 kb.*

Propionibacterium *spp / taille du chromosome / électrophorèse en champs pulsés*

INTRODUCTION

Dairy propionibacteria are involved in Swiss cheese-making as essential ripening starters (Langsrud and Reinbold, 1973a, b) and in several industrial fermentations (production of propionic acid and vitamin B_{12}). Existing knowledge on the *Propionibacterium* genome is quite limited (Hofherr and Glatz, 1983; Perez Chaia *et al,* 1988; Rehberger and Glatz, 1990) and needs to be extended in order to improve existing industrial strains used in dairy starters.

The genomes of dairy propionibacteria have a G+C content that ranges from 64–68% (Johnson and Cummins, 1972) but its size is still unknown. This high G+C content means that restriction enzymes with recognition sequences rich in A and

T nucleotides should digest the *Propionibacterium* chromosome only in a few sites, yielding large fragments that may be separated by pulsed-field gel electrophoresis techniques (PFGE) (Cantor *et al*, 1988; Lai *et al*, 1989). This technique has been used to separate whole chromosomes up to 9 000 kb in size (Chu *et al*, 1986; Cantor *et al*, 1988; Smith *et al*, 1988). The analysis of restriction fragments has been applied to estimate the size of bacterial chromosomes (Chen *et al*, 1986; Su and Baseman, 1990).

In this paper we describe the use of PFGE to determine the genome size of *Propionibacterium* using chromosomal digestion by restriction endonucleases *Xba*I (T/CTAGA) and *Ssp*I (AAT/ATT), which as expected, produce large fragments compared to other endonucleases.

MATERIALS AND METHODS

Bacterial strains

The *Propionibacterium* strains (table I) were obtained from the ATCC (American Type Culture Collection), CIP (Institute Pasteur Collection), CNRZ (Centre National de Recherches Zootechniques, France), DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) and the TL (INRA Dairy Technology Laboratory Collection, Rennes, France). The lytic enzymes lysozyme or mutanolysin were not efficient enough to digest the cell wall of most strains of dairy *Propionibacterium*; the cells were therefore made fragile by growth for 2 days on YEL medium (Hettingha *et al*, 1968), which contained 0.5 mol/l sucrose supplemented with various concentrations of glycine (w/v) depending on the strains: 0.5% for CIP 6434 and CNRZ 729, 1% for TL 2500 and CNRZ 732, 1.5% for CIP 6435 and CNRZ 85, 2% for CNRZ 79, ATCC 4875 and CIP 103026, 3% for CNRZ 87. All the cultures were harvested at an optical density (OD) of 0.3 at 650 nm. The *Escherichia coli* K12 strain was grown at 37 °C on Luria–Bertani medium (Maniatis *et al*, 1982) to an OD of 0.4. In order to synchronize replication forks, chloramphenicol (180 μg/ml) was added (Ohki and Smith, 1989), and shaking was continued for 1 h.

Genomic DNA preparation

DNA was embedded in low melting point agarose to prepare DNA insert as described by McClelland *et al* (1987). Ten ml of culture, grown as described previously, were harvested and suspended in 10 ml of TES buffer (Tris–HCl 0.05 mol/l, Na_2 EDTA 0.010 mol/l, pH 8.0, 0.5 mol/l sucrose). After centrifugation (5 000 g, 10 min) the cells were suspended in 0.5 ml of TE buffer (Tris–HCl 0.05 mol/l, Na_2 EDTA 0.01 mol/l, pH 8.0). Low melting point agarose (1%) (NuSieve) in TE buffer was added to each cell suspension. A 100-μl volume of the cell–agarose mixture was poured into 150-μl molds. Each agarose block was washed in TE and incubated at 37 °C for 4 h in a buffer: TE with lysozyme (Aflact France, 20 mg/ml), and 0.05% lauroyl sarcosine. The blocks were washed with TE and suspended in a lysis solution (0.1 mol/l EDTA, Tris 0.01 mol/l pH 8.0 1% SDS, 1 mg/ml proteinase K (Boehringer Mannheim). This was followed by incubation at 50 °C for 24 h. The blocks were washed with TE, suspended in a phenylmethylsulfonylfluoride (PMSF) solution (175 μg/ml in TE), and incubated at room temperature for 1 h. The blocks were finally washed 3 times with TE buffer and stored in the buffer at 4 °C.

Genomic DNA digestion and pulsed-field gel electrophoresis

Restriction enzymes were obtained from Boehringer. The agarose blocks were washed extensively with TE. Each plug was incubated at room temperature for 12 h in 500 μl of restriction enzyme buffer. For digestion, each plug was incubated overnight in 200 μl of restriction enzyme buffer with 50 U of restriction enzyme. The restricted block could be stored at 4 °C in 0.5 mol/l EDTA for a few days.

The pulsed field gel electrophoresis was performed on a Bio-Rad CHEF DRII electrophoresis cell (Lai *et al*, 1989). The samples were migrated through 1% (w/v) agarose gels in a

Table I. Size (kb) of restriction fragments obtained after digestion with *Xba*I or *Ssp*I of the chromosome of various *Propionibacterium* strains.
Taille des bandes (kb) obtenues après digestion du chromosome de différentes souches de Propionibacterium *avec* Xba*I et* Ssp*I.*

P acidipropionici			P freudenreichii				P jensenii				P thoenii			
ATCC 4875		*DSM20273*	*CIP 103208*		*TL 2500*	*CNRZ 729*	*CIP 6435*		*CNRZ 87*	*CNRZ 79*	*CIP 6434*		*CNRZ 732*	*CNRZ 85*
Ssp*I*	Xba*I*	Xba*I*	Ssp*I*	Xba*I*	Xba*I*	Xba*I*	Ssp*I*	Xba*I*	Xba*I*	Xba*I*	Ssp*I*	Xba*I*	Xba*I*	Xba*I*
360	270	300	450	650	360	360	1000	270	270	260	360	270	250	360
270	245	270	270	275	270	270	280	260	260	245	250	230	245	260
250	240	240	245	245	250	270	270	245	260	215	240	230	240	250
230	240	235	245	245	250	245	245	240	248	215	225	215	210	245
230	220	230	210	210	230	245	208	210	245	205	210	215	205	245
218	220	220	203	203	225	210	130	200	245	205	210	205	203	205
210	210	205	195	200	200	208	80	200	210	195	200	205	195	205
205	205	205	130	190	200	204	40	180	205	195	175	200	195	190
205	205	180	110	110	191	180	25	180	190	103	150	105	190	105
200	200	120	110	100	100	106	10	105	110	102	120	105	160	105
165	190	120	80	95	80	100		100	105	100	100	100	103	100
165	190	110	80	50	60	50		60	100	95	90	70	103	95
130	150	105	40	20	60	25		60	45	90	70	70	45	45
125	125	100	20	10		10		35	40	45	60	45	45	40
110	125	98	15					35	30	40		40		20
100		95	20					25	20	20		20		20
		70	10					10		20		20		20
		70						5				20		
3 173	3 035	2 433	2 433	2 603	2 476	2 483	2 288	2 420	2 583	2 350	2 460	2 365	2 389	2 510

solution of TBE gel buffer (0.089 mol/l Tris–borate, 0.089 mol/l boric acid, 0.002 mol/l EDTA, pH 8.3) 0.5x. The electrophoretic conditions have been indicated above the figures.

RESULTS AND DISCUSSION

A first selection of restriction endonucleases was made using the results obtained from bacteria showing a high G+C DNA. We chose the enzymes *Not*I and *Sfi*I, which recognize 8 base-pair high G+C sequences and which have been successfully used to determine the size of the *E coli* K12 chromosome (Smith *et al,* 1987). Some enzymes with recognition sequences rich in A+T (*Dra*I, *Hpa*I, *Nde*I, *Ssp*I, *Xba*I and *Asn*I) used to determine the size of the *Pseudomonas aeruginosa* genome which has a high G+C content were also employed (Hector and Johnson, 1990). Moreover, the restriction site of *Xba*I and *Ssp*I containing the stop codon TAG appears very infrequently on the *E coli* chromosome (Condemine and Smith, 1990). All these restriction enzymes have been tested on 4 representative strains of *Propionibacterium* (*P acidipropionici* ATCC 4875, *P freudenreichii* CIP 103026, *P jensenii* CIP 6435 and *P thoenii* CIP 6434). The results obtained with strain ATCC

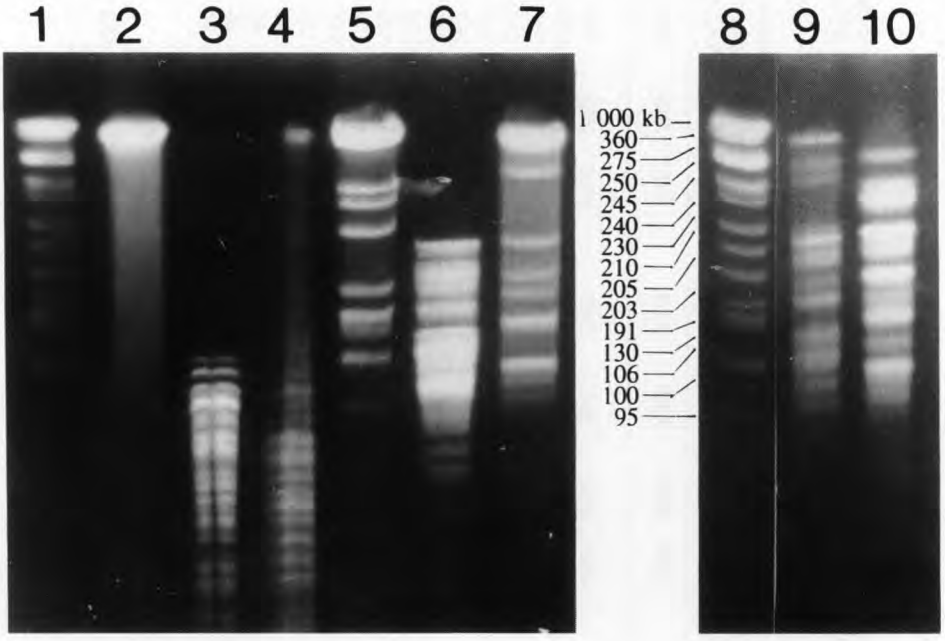

Fig 1. PFGE of DNA from *P acidipropionici* strain ATCC4875 digested with various restriction enzymes. Lane 2: *Dra*I. Lane 3: *Not*I. Lane 4: *Sfi*I. Lane 5: *Asn*I. Lane 6: *Nde*I. Lane 7: *Hpa*I. Lane 9: *Ssp*I. Lane 10: *Xba*I. Lane 1 and Lane 8, *E coli* K12 chromosome digested with *Not*I. Running conditions were 200 V for 16 h with a 20 s pulse time at 14 °C.
PFGE de l'ADN de la souche de P acidipropionici *ATCC4875 digéré avec différentes endonucléases de restriction. Piste 2 :* Dra*I. Piste 3 :* Not*I. Piste 4 :* Sfi*I. Piste 5:* Asn*I. Piste 6 :* Nde*I. Piste 7 :* Hpa*I. Piste 9 :* Ssp*I. Piste 10 :* Xba*I. Pistes 1 et 8 : chromosome de* E coli *K12 digéré avec* Not*I. Les paramètres de migration étaient de 200 V à 14 °C, 16 h avec une fréquence d'impulsion de 20 s.*

4875 are presented in figure 1; they were similar for the 3 other strains (data not shown). Some enzymes used to determine the size of the *Pseudomonas* genome (*Dra*I and *Asn*I) presented none or only a few cut sites in the Propionibacteria genomes. Other enzymes, such as *Spe*I, had more complex patterns which were difficult to analyse. The restriction endonucleases *Not*I and *Sfi*I produced too many restriction fragments. Digestion of the chromosomal DNA with *Xba*I and *Ssp*I (fig 1, lanes 9 and 10) seemed to be most suitable for genome size determination, since the resulting pattern was more simple to analyse, the fragments produced were large and the genome was cut into fewer than 20 bands.

Figure 2 shows the restriction profiles obtained with *Xba*I digestion of the chromosome of a representative strain of each species. The patterns obtained with *Ssp*I (data not shown) were similar and constituted 10–16 bands, ranging in size from kb approximately 10–1 000. Lambda concatemers were used as markers and *E coli* K12 was digested with *Not*I (Smith *et al*, 1987) in order to compare the restriction profiles. Band size has been indicated in table I.

The chromosome molecular size of each *Propionibacterium* species was determined by averaging the size obtained by *Xba*I or *Ssp*I digestion of 2 (for *P acidipropionici*) or 3 strains of each species. For *P acidipropionici Xba*I and *Ssp*I consistently gave a genome size of approximately 3 060 kb. For *P freudenreichii*, these enzymes gave a size of about 2 500 kb. The genome size of *P jensenii* was estimated to be 2 410 kb and that of *P thoenii* to be 2 440 kb. Moreover, the restriction patterns of these different species of *Propionibacterium* did not resemble one another. In comparison, the genome size of *E coli* obtained by PFGE analysis was 4 550 kb and that of *L lactis* ranged from 2 300–2 600 kb (Le Bourgeois *et al*, 1989).

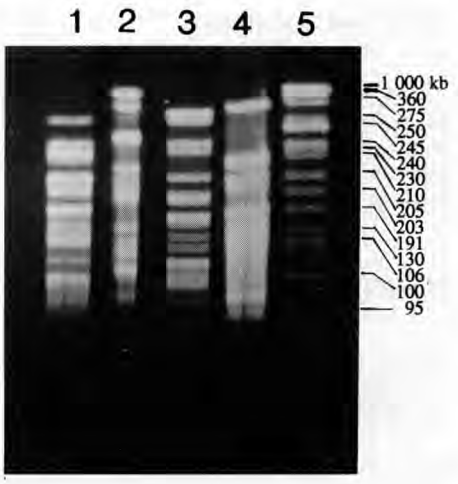

Fig 2. PFGE separation of *Propionibacterium* chromosome digested with *Xba*I. Lane 1: *P acidipropionici* ATCC4875. Lane 2: *P freudenreichii* CIP103026. Lane 3: *P jensenii* CIP6435. Lane 4: *P thoenii* CIP6434. Lane 5: *E coli* chromosome digested with *Not*I. Running conditions were 200 V for 16 h with a 20 s pulse time at 14 °C.
PFGE du génome de différentes espèces de Propionibacterium *digéré avec* Xba*I. Piste 1 :* P acidipropionici *ATCC4875. Piste 2 : P* freudenreichii *CIP103026. Piste 3 :* P jensenii *CIP6435. Piste 4 :* P thoenii *CIP6434. Piste 5 : chromosome de* E coli *K12 digéré avec* Not*I. Les conditions de migration étaient 200 V à 14 °C pendant 16 h avec une fréquence d'impulsion de 20 s.*

The PFGE method provides a good means of determining the genome size of *Propionibacterium*, a study which has not been yet published. Unlike the chemical method (Herdman, 1985) PFGE provides a direct measurement of the physical length of the DNA. Using PFGE, the fragments produced by *Xba*I and *Ssp*I were separated and genome size determination was facilitated. This technique will be used to compare strains in our collection as has recently been proposed for lactococci (Tankskanen *et al*, 1990) in order to develop a simple method of identification.

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