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(54) **Title:** GENETIC CLUSTER OF STRAINS OF *STREPTOCOCCUS THERMOPHILUS* HAVING APPROPRIATE ACIDIFYING AND TEXTURIZING PROPERTIES FOR DAIRY FERMENTATIONS

(57) **Abstract:** The invention relates to a genetic cluster of strains of *Streptococcus thermophilus,* which have a lysotype distinct from that of the strains currently used. Within this cluster novel acidifying and lexturizing strains have been identified.

WO 2008/040734 A1

Genetic cluster of strains of *Streptococcus thermophilus* having appropriate acidifying and texturizing properties for dairy fermentations

The invention relates to a genetic cluster of strains of *Streptococcus thermophilus* (*S. thermophilus*) having appropriate acidifying and texturizing properties for dairy fermentations.

Bacteriophages are viruses capable of attacking bacteria. During these viral attacks, the bacteriophages infect the bacterial culture, and multiply in order to finally destroy this culture. The technological impact on the milk processing industry (production of cheese, yogurt, fermented milk) of these bacteriophages is significant since they can cause a complete cessation of fermentation and therefore prevent the production of these milk-derived products.

One way of combating the problems linked to the infection of the fermentations by bacteriophages is the use of strains having appropriate sensitivity spectra to the phages. In particular, producers of dairy ferments have developed strategies to combat the bacteriophages by constructing ferments constituted by several strains having distinct lysotypes, and using several of these ferments in rotation. It is clear that in order to be able to adopt this strategy it is important to possess a diversity of strains having the same functionalities (such as for example acidification, thickening power, flavouring etc.) but distinct lysotypes.

S. thermophilus are used extensively alone or in combination with other bacteria for the production of fermented food products. They are included in particular in the formulation of the ferments used for producing yogurts. Strains of *S. thermophilus* are expected to participate in the formation of lactic curd by acidification of milk and in the development of the texture of the fermented product. A distinction is generally drawn between 4 groups of *S. thermophilus* based on these functional properties: 1) the non-texturizing and non-acidifying strains, 2) the non-texturizing and acidifying strains, 3) the texturizing and non-acidifying strains, and 4) the texturizing and acidifying strains. A texturizing

strain is a strain making it possible to obtain fermented milks the gels of which can be described by their rheological properties.

Hitherto only four strains of *S. thermophilus* corresponding to the criteria of acidifying and texturizing strains have been described in the literature: Sfi39, CNCM I-2423, CNCM I-2426 and the strain CNCM I-2980 described in the Application WO2004/085607.

The rarity of such strains (rapid acidification and texturizing) makes it difficult to combat the bacteriophages during the fermentation of the milk. In fact, ideally, the fight against the bacteriophages would involve the combination in the same ferment of strains having similar technological properties but distinct lysotypes, then the use of ferments of this type in rotation. The ferments used in rotation should also have distinct lysotypes but similar technological properties. In the case of the texturizing and rapidly acidifying ferments, this approach is difficult in view of the small number of strains having these functional qualities.

Thus one of the problems which the invention proposes to resolve is to provide novel strains of *S. thermophilus* which have a lysotype distinct from the strains currently used, in particular strains which are acidifying and texturizing.

For this purpose, the invention relates to the strains of *S. thermophilus* in a genetic cluster which have a lysotype distinct from that of the acidifying and texturizing strains of *S. thermophilus* currently used. Within this cluster novel acidifying and texturizing strains have been identified.

The invention also describes a method making it possible to predict a strain's membership of a family of strains having identical or related lysotypes. This method analyzes the restriction polymorphism of the *epsA-B-C-D* region of the genome of *S. thermophilus*.

By the *epsA-B-C-D* region is meant the region of the chromosome of *S. thermophilus* overlapping the *epsA* to *epsD* genes of the *eps* locus. The DNA fragment corresponding to this region, called the epsAD fragment, can be obtained by PCR reaction on the chromosomic DNA of *S. thermophilus* using the oligonucleotides of SEQ ID N°1 and SEQ ID N°2 as primers.

A subject of the present invention is a strain of *Streptococcus thermophilus* the epsAD fragment of which, after digestion by the restriction enzymes *Mnl*I, *Fok*I and *Hin*dIII, has a restriction profile characterized by DNA fragments of 344±2 base pairs (bp), 341±2 bp, 305±2 bp, 299±2 bp, 277±2 bp, 210±2 bp, 160±2 bp, 142±2 bp, 100±2 bp, 79±2 bp, 75±2 bp, 66±2 bp, 42±2 bp, 23±2 bp and 9±2 bp.

The restriction profile of the epsAD fragment is determined by standard sequencing of the epsAD fragment followed by *in silico* determination of the restriction profile.

Typically the sequencing can be carried out with the CEQ8000 equipment (Beckman) and the *in silico* determination of the restriction profile can be carried out starting from the sequence of the epsAD fragment using the NEBcutter V2.0 tool accessible on the internet via the website http://tools.neb.com/.

Typically a strain according to the invention comprises a nucleotide sequence having at least 80%, preferentially at least 90%, at least 95% or at least 97% and still more preferentially 100% identity with the nucleotide sequence of SEQ ID N°4.

Typically a strain according to the invention comprises a nucleotide sequence having at least 80%, preferentially at least 90%, at least 95% or at least 97% and still more preferentially 100% identity with the nucleotide sequence of SEQ ID N°5.

In order to calculate the percentage identity, a person skilled in the art will for example use the "BLAST 2 Sequences" tool (Tatusova & Madden, 1999; http://www.ncbi.nlm.nih.gov/BLAST/) with the default parameters of the "blastn" program (for alignment of nucleotide sequences) or of the "blastp" program (for alignment of protein sequences). The percentage similarity between two protein sequences is calculated using the BLOSUM62 matrix.

Preferentially the strain according to the invention is texturizing. Preferentially the strain according to the invention acidifies rapidly. Still more preferentially the strain according to the invention is texturizing and acidifies rapidly.

By texturizing strain of *Streptococcus thermophilus* is meant a strain which produces fermented milks having, under the conditions described in the example, a viscosity greater than approximately 35 Pa.s, a thixotropic area of less than approximately 2000 Pa/s and/or a yield point of less than approximately 14 Pa.

By rapidly acidifying strain is meant a strain which under the conditions described in the example, has a Vm of less than -0.0100 upH/min.

Preferentially, a strain according to the invention is the strain of *Streptococcus thermophilus* deposited on 14th June 2006 at the Collection Nationale de Cultures de Microorganismes under no. CNCM I-3617 or a mutant strain which can be obtained from the latter.

Typically in order to obtain such mutant strains, a person skilled in the art can use the usual mutagenesis techniques such as UV irradiation or exposure to mutagenic chemical products (ethyl-methane-sulphonate, nitrosoguanidine, nitrous acid etc.).

Preferentially, a subject of the invention is the strain of *Streptococcus thermophilus* deposited on 14th June 2006 in the name of Danisco France SAS, 20 rue de Brunel, 75017 Paris at the Collection Nationale de Cultures de Microorganismes under no. CNCM I-3617.

A person skilled in the art, starting from the restriction profiles described previously and/or from the sequences of SEQ ID N°4 and/or N°5, can identify the strains which belong to the same genetic cluster as the strain CNCM I-3617. Typically in order to do this, he can use PCR and/or hybridization and/or DNA sequencing techniques.

A subject of the invention is also a bacterial composition comprising at least one strain according to the invention. By bacterial composition is meant a mixture of different strains, in particular a ferment, or a leaven.

The mixtures of preferred strains according to the invention are mixtures of *Streptococcus thermophilus* with other *Streptococcus thermophilus*, or mixtures of *Streptococcus thermophilus* with *Lactobacillus delbrueckii* subsp. *bulgaricus*, or mixtures of *Streptococcus thermophilus* with other *Lactobacillus* and/or with *Bifidobacterium*, or mixtures of *Streptococcus thermophilus* with *Lactococcus*, or mixtures of *Streptococcus thermophilus* with other strains of lactic bacteria and/or yeasts.

A subject of the invention is also a manufacturing process for a food product, a food complement, a dietary supplement or a product with probiotic properties, comprising a stage in which a strain according to the invention is used.

Typically the food product, the food complement, the dietary supplement or the product with probiotic properties is a dairy product, a meat product, a cereal product, a drink, a foam or a powder.

Preferentially the food product, the food complement, the dietary supplement or the product with probiotic properties is a dairy product. It is for example a fermented milk, a yogurt, a matured cream, a cheese, a fromage frais, a milk drink, a dairy product retentate, a processed cheese, a cream dessert, a cottage cheese or an infant milk. Typically the dairy product comprises milk of animal and/or plant origin.

A subject of the invention is also a food product, a food complement, a dietary supplement or a product with probiotic properties comprising at least one strain according to the invention or the bacterial composition described previously.

The invention also describes a method for predicting the lysotype of a strain of *S. thermophilus* starting from analysis of the restriction polymorphism of the *epsA-B-C-D* region of its genome, comprising the following stages:

a) amplification of the epsAD fragment by PCR reaction on the chromosomic DNA of *S. thermophilus* using the oligonucleotides of SEQ ID N°1 and SEQ ID N°2 as primers;

b) sequencing of the epsAD fragment;

c) *in silico* determination of the restriction profile of the epsAD fragment after digestion by the restriction enzymes *Mnl*I, *Fok*I and *Hin*dIII; and

d) comparison of the restriction profile obtained in Stage c) with the restriction profiles of the epsAD region of strains of *S. thermophilus* the lysotype of which is known.

Examples of strains of *S. thermophilus* the lysotype of which is known are listed in Table 3.

The present invention is better illustrated below using the examples which follow. These examples are given only by way of illustration of the subject-matter of the invention, of which they in no way constitute a limitation.

Brief descriptions of the figures:

Figure 1: Diagrammatic representation of the *eps* locus of various *S. thermophilus* having similarities in organization and sequence of the 5' region including the *epsA, epsB, epsC* and *epsD* genes. The GENBANK access numbers of the nucleotide sequences are given in parentheses.

Figure 2: Alignment of partial sequences of the *epsA* (A) and *epsD* (B) genes at the level of the regions targeted by the primers of SEQ ID N°1 (in the *epsA* gene) and SEQ ID N°2 (in the *epsD* gene).

Figure 3: *In silico* determination of the profiles obtained according to the epsAD method of the various strains of *S. thermophilus* the sequence of the *eps* locus of which is described in the literature. The images of the restriction profiles were generated using the NEBcutter tool (http://tools.neb.com/) with the following parameters : Gel Type = 2% agarose; Marker = 100 bp DNA Ladder; DNA Type = Unmethylated; L = 102 mm. The GENBANK access numbers of these strains are: AF448502 (MTC310), AF373595 (Sfi39), AF410175 (Type I), AJ289861 (IP6757), AF454496 (Type V), Y17900 (NCFB 2393), AJ272341 (FI9186), AF454497 (Type VI), NZ_AAGS01000017 (LMD-9), AF454501 (Type XI), AF454498 (Type VII), AF454495 (Type IV), AF454500 (Type X), AF454499 (Type IX), AY057915 (Type III), CP000023 (LMG 18311), CP000024 (CNRZ 1066), AF434993 (MTC360), AF430847 (MTC330), AY061649 (MR-2C), U40830 (Sfi6), Z98171 (CNRZ 368), AF448249 (MR-1C). The *eps* sequence of the strain CNCM I-2980 is described in the Application WO2004/085607.

Figure 4: Diagrammatic representation of the organization of the *eps* locus of the strain CNCM I-3617, corresponding to the sequence SEQ ID N°3. The open reading frames (ORF) represented by vertical hatching (region 1) have significant similarities with the *epsA-epsB-epsC-epsD* genes situated at the start of the *eps* locus in the great majority of the strains of *S. thermophilus*. The ORFs represented by horizontal hatching (region 2) have significant similarities with the *eps11E*, *eps11F*, *eps11G* and *eps11H* genes of the type XI strain. The ORFs represented by a chequered pattern (region 4) have significant similarities with the *eps4F* gene of the type IV strain. The grey rectangle between regions 2 and 3 represents a non-coding region having sequence homologies with a region of the *eps* locus of the type IV strain. The ORFs represented by diagonal hatching (region 6) have significant similarities with the *epsP*, *epsQ*, *epsS*, *epsR* and *epsT* genes of the CNRZ368 strain. The unshaded ORFs (regions 3 and 5) have no significant similarities with the *eps* genes described for other *S. thermophilus*. The two black bands at the bottom of the figure represent the position of the sequences SEQ ID N°4 and SEQ ID N°5.

Examples

Biological material

Table 1 and Table 3 show some of the strains used for the study. Some of these strains are obtained from the Danisco collection of strains and phages (DGCC: Danisco Global Culture Collection). The preparation of cultures of these strains was carried out according to the standard methods of microbiology.

Table 1: Description of some of the strains used for the study

Strain	Other name	Texturizing property [a]	Acidifying property [a]	Related strains [b]	Bibliographical references [c]
CNCM I-3617		Yes	Yes	Unknown	None
CNCM I-2980		Yes	Yes	DGCC2056, DGCC8013, DGCC8015	WO2004/085607
CNCM I-2423	MTC310	Yes	Yes	Sfi39, SY102 FI9186, Type I, DGCC945	Lemoine *et al.* (1997); Germond *et al.* (2001); Marshall *et al.* (2001)
CNCM I-2978	MTC360	Yes	No	Sfi6, CNCM I-733, CNCM I-734, CNCM I-735, IMDO1, 2, 3, NCFB859, EU21, MR-2C, DGCC7773	Lemoine *et al.* (1997); Doco *et al.* (1990); Marshall *et al.* (2001); Stingele *et al.* (1996)
CNCM I-2432	Type IV	Yes	No	Unknown	Rallu *et al.* (2002)
CNCM I-2429	Type VII	Yes	No	Unknown	Rallu *et al.* (2002)
CNCM I-2979		Yes	No	CNRZ368, MR-1C	Bourgoin *et al.* (1999); Low *et al.* (1998)
DGCC7966		Yes	No	Unknown	None
DGCC7919		No	Yes	Unknown	None
DGCC7766		No	No	Unknown	None

a: on the basis of industrial use and the present study.

b: on the basis of published sequence results of the *eps* locus and/or the structure of the polysaccharide and data internal to Danisco.

c: on the strain studied and/or on related strains.

The strain CNCM I-3617 belongs to a novel genetic cluster

The recent determination of the complete sequence of the chromosome of two strains of *S. thermophilus* CNRZ1066 and LMG18311 shows a high level of conservation of the genetic content and the organization of the genes in this species) Bolotin *et al.*, 2004). One of the rare regions exhibiting major genetic

differences between these two strains corresponds to the *eps* locus which codes for the genes involved in the biosynthesis of exopolysaccharides. Moreover, a great diversity has already been described at the level of this genetic locus (cf. Figure 1) since several *eps* sequences had been determined for various strains of *S. thermophilus* (for a journal article, see Broadbent *et al.*, 2003). Flanked by the *deoD* (coding for a purine-nucleoside phosphorylase) and *pgm* (coding for a phospho-glucomutase) genes, all the clusters of *eps* genes in *S. thermophilus* are composed of a conserved proximal region (from the *epsA* gene to the *epsD* gene) and of a highly variable distal region. In spite of a conserved organization of the *epsA-B-C-D* genes, a significant sequence polymorphism exists in this region. The polymorphism of this region has been used to develop a tool for genetic typing of the strains of *S. thermophilus:* the epsAD method.

epsAD method: The tool developed is based on the specific amplification (PCR) of the *epsA-B-C-D* region followed by the analysis of its restriction polymorphism (RFLP). For this purpose primers have been determined which allow the PCR amplification of this region for the great majority of the strains of *S. thermophilus*. They have been determined by the alignment of the sequences of the epsAD region (cf. Figure 2) and allow the amplification of a DNA fragment of approximately 2480 base pairs (bp). The genomic DNA of *S. thermophilus* is purified using the "DNeasy Tissue Kit" (Qiagen), then the epsAD region is amplified by PCR according to the following parameters:

Composition of the reaction mixture (50 µL): buffer for the DNA polymerase ×1, $MgCl_2$ 2 mM, dNTP 200 µM each, genomic DNA 100 to 500 ng, primer EPSA632 (5'-AAATgAATTCAgAgCAAgCACTTg-3' (SEQ ID N°1)) 200 nM, primer EPSD1064 (5'-gTCATgTCAACTTTATTAAggACg-3' (SEQ ID N°2)) 200 nM, DNA polymerase 1.25 units, H_2O qsf 50 µL.

Amplification parameters :

- predenaturation at 94°C for 1 min
- 35 cycles alternating denaturation at 94°C for 30 s, hybridization at 56°C for 30 s, elongation at 72°C for 3 min

- post-elongation at 72°C for 6 min.

After amplification, the PCR product is checked by electrophoresis on 1.5% agarose gel. The size of the amplified product is approximately 2480 bp.

The sequence of the PCR fragment is determined (according to a method derived from Sanger *et al.*, 1977) with CEQ8000 equipment (Beckman). The sequence is processed by the NEBcutter tool (for example, http://tools.neb.com/) by selecting the restriction enzymes *Mnl*I, *Fok*I and *Hin*dIII in order to establish its restriction profile *in silico*, and in particular in order to define the size of the restriction fragments.

For the strains of *S. thermophilus* the sequence of the *eps* locus of which is partially or completely available in the public databases (GENBANK for example), the *in silico* analysis of the theoretical restriction products of the PCR fragment of approximately 2480 bp with the restriction enzymes *Mnl*I, *Fok*I and *Hin*dIII produces the restriction profiles shown in Figure 3. These restriction profiles were established *in silico* on the basis of the sizes of the restriction fragments provided by the NEBcutter tool (cf. Table 2).

Table 2: Size of the DNA fragments determined by *in silico* digestion of the epsAD fragment by the restriction enzymes *Mnl*I, *Fok*I and *Hin*dIII, for the strains of *S. thermophilus* the sequence of the *epsA-B-C-D* genes of which is available.

Strain of *S. thermophilus*	GENBANK access number of the *eps* locus, or other reference	Size of the *Mnl*I, *Fok*I and *Hin*dIII digestion fragments of the epsAD region (size in base pairs determined by NEBcutter, http://tools.neb.com/)
MTC310	AF448502	778; 371; 344; 299; 175; 142; 100; 79; 75; 42; 35; 23; 9
Sfi39	AF373595	778; 371; 344; 299; 175; 142; 100; 79; 75; 42; 35; 23; 9
Type I	AF410175	778; 371; 344; 299; 175; 142; 100; 79; 75; 42; 35; 23; 9
IP6757	AJ289861	778; 371; 299; 247; 239; 175; 100; 76; 75; 42; 35; 23;

		9; 3
Type V	AF454496	1264; 374; 305; 210; 123; 76; 66; 42; 9; 3
NCFB2393	Y17900	1264; 374; 305; 210; 123; 76; 66; 42; 9; 3
FI9186	AJ272341	1264; 374; 305; 210; 123; 76; 66; 42; 9; 3
Type VI	AF454497	1286; 305; 277; 210; 100; 76; 75; 66; 42; 23; 9; 3
LMD-9	NZ_AAGS01000017	848; 655; 371; 175; 111; 100; 75; 60; 42; 23; 9; 3
Type XI	AF454501	851; 356; 305; 299; 175; 111; 100; 75; 60; 46; 42; 23; 20; 9; 3
CNCM I-3617	SEQ ID N°3	344; 341; 305; 299; 277; 210; 160; 142; 100; 79; 75; 66; 42; 23; 9
Type VII	AF454498	486; 341; 305; 299; 277; 210; 160; 100; 76; 75; 66; 42; 23; 9; 3
Type IV	AF454495	618; 551; 305; 299; 210; 100; 95; 79; 75; 46; 42; 23; 20; 9
Type X	AF454500	571; 356; 305; 299; 277; 210; 100; 79; 75; 66; 60; 42; 23; 9
Type IX	AF454499	987; 305; 299; 277; 210; 100; 79; 75; 66; 42; 23; 9
Type III	AY057915	341; 305; 299; 277; 247; 239; 210; 160; 100; 76; 75; 66; 42; 23; 9; 3
CNCM I-2980	WO2004/085607	778; 374; 305; 247; 239; 210; 123; 76; 66; 42; 9; 3
LMG18311	CP000023	778; 371; 299; 247; 239; 210; 100; 76; 75; 42; 23; 9; 3
CNRZ1066	CP000024	778; 371; 299; 247; 239; 175; 100; 76; 75; 42; 35; 23; 9; 3
MTC360	AF434993	778; 371; 299; 247; 239; 175; 100; 76; 75; 42; 35; 23; 9; 3
MTC330	AF430847	778; 371; 299; 247; 239; 175; 100; 76; 75; 42; 35; 23; 9; 3
MR-2C	AY061649	778; 371; 299; 247; 239; 175; 100; 76; 75; 42; 35; 23; 9; 3
Sfi6	U40830	778; 371; 299; 247; 239; 175; 100; 76; 75; 42; 35; 23; 9; 3
CNRZ368	Z98171	691; 371; 312; 305; 239; 112; 100; 76; 75; 63; 42; 35; 23; 22; 9; 3
MR-1C	AF448249	691; 371; 312; 305; 239; 112; 100; 76; 75; 63; 42; 35; 23; 22; 9; 3

Alternatively, the PCR product can be digested by the restriction enzymes *Mnl*I, *Fok*I and *Hin*dIII under the following conditions: PCR product 15 to 30 µL, buffer 2 (New England Biolabs) ×1, bovine serum albumin (New England Biolabs) ×1, enzyme *Mnl*I (New England Biolabs) 1 unit, enzyme *Fok*I (New England Biolabs) 1 unit, enzyme *Hin*dIII (New England Biolabs) 1 unit, H_2O qsf 50 µL. Incubation at 37°C for 1 hour.

The restriction fragments are then analyzed by electrophoresis. Electrophoresis on agarose gel can be used. However, in order to remedy the low resolution power of this type of electrophoresis (precision +/- 10%) and the difficulty of visualizing fragments smaller than 100 bp, methods with a higher resolution (+/- 0.1 to 1%) such as micro-fluidic electrophoresis (Agilent) or capillary electrophoresis may be preferred.

These methods (*in silico* analysis of the restriction profile or electrophoresis analysis of the restriction fragments) were applied to several hundreds of strains from the Danisco collection of *S. thermophilus* strains and the reference strains described in the literature.

The strains which have the same restriction profile have been grouped together in genetic clusters (or genetic groups) denoted CL-1 to CL-12. Comparison of the restriction profiles was carried out by means of Bionumerics software version 3.5. Table 3 summarizes some of the results obtained.

Tableau 3: Summary of the results of genotyping and lysotyping

Strain	Genotype	Lysotype	Owner	Texturizing
CNCM I-3617	CL-1	Resistant	Danisco	YES
CNCM I-2423	CL-2	LT-2	Danisco	YES
CNCM I-2426			Danisco	YES
CNCM I-2424			Danisco	YES
Sfi39			Private collection	YES
DGCC945			Danisco	YES
CNCM I-2432	CL-3	LT-4	Danisco	YES
CNCM I-2978	CL-4		Danisco	YES
DGCC7773			Danisco	YES

CNRZ1066			Public collection	YES
DGCC7785			Danisco	YES
DGCC7788			Danisco	YES
DGCC7966			Danisco	YES
DGCC47			Danisco	YES
CNCM I-2980	CL-5	LT-5	Danisco	YES
DGCC2056			Danisco	YES
DGCC8013			Danisco	YES
DGCC8015			Danisco	YES
DGCC7790	CL-6	LT-6	Danisco	YES
DGCC7813			Danisco	YES
CNCM I-2979			Danisco	YES
CNRZ368			Public collection	YES
MR-1C			University	YES
CNCM I-2429	CL-7	LT-7	Danisco	YES
ATCC BAA-491	CL-8	LT-8	Public collection	NO
DGCC7689			Danisco	NO
DGCC1086			Danisco	NO
SMQ-301	CL-9		University	NO
DGCC7853			Danisco	NO
DGCC7919	CL-10	LT-10	Danisco	NO
DGCC7766	CL-11	LT-11	Danisco	NO
DGCC7809	CL-12	LT-12	Danisco	NO

Method for determining the sensitivity of a strain to a bacteriophage: The sensitivity of a strain to a bacteriophage is established by the lysis plaque method. 100 µl of a culture of the strain to be tested and 100 µl of an appropriate dilution of a serum containing the bacteriophage to be studied are used in order to seed 5 ml of an agar medium under surfusion (0.6 % agar weight/volume) M17+glucose supplemented at 10 mM with $CaCl_2$. The mixture is poured onto the surface of a solidified agar medium (1.5 % agar weight/volume) M17+glucose supplemented at 10 mM with $CaCl_2$. After incubation overnight at 42°C, the strain's sensitivity to the bacteriophage is evaluated by the presence of lysis plaques. The absence of lysis plaque signifies this strain's resistance to the bacteriophages tested. The

spectrum of a strain's sensitivity to the bacteriophages, also called lysotype, is constituted by all of the sensitivities and resistances to the bacteriophages studied. A reference system with approximately sixty phages has been implemented in order to establish the lysotype of the strains of *S. thermophilus* in the Danisco collection. The strains which have the same lysotype have been grouped together in different groups denoted LT-n.

Some of the results are given in Table 3. It demonstrates in particular that the strains of the same genetic cluster virtually always have the same lysotype. The strain CNCM I-3617 belongs to a novel genetic group called CL-1 which has the very particular lysotype of being resistant to all the bacteriophages tested.

Sequence of the *eps* locus

The genetic knowledge acquired with regard to *S. thermophilus* has shown that the *eps* locus is one of the major sites of heterogeneity between strains. This characteristic has already been exploited in part in order to develop the abovementioned genotyping method which uses the diversity in the *epsA-B-C-D* region which is the proximal region of the *eps* locus. An even greater diversity appears in the distal region of the *eps* locus (region encoding the glycosyl-transferases, see Figure 1). This region gives the specificity of the exopolysaccharide and therefore induces at least in part the specificity of the strain's thickening power. This is therefore a region of the chromosome particularly indicated for unambiguously describing the strain CNCM I-3617 and the strains which are related to it. The nucleotide sequence of the *eps* locus of the strain CNCM I-3617 (starting from the *epsA* gene) was obtained from a synthetic DNA fragment. This fragment was synthesized by PCR on a purified genomic DNA matrix of the strain CNCM I-3617 using two specific primers of conserved genes (*deoD* encoding a purine-nucleotide phosphorylase, and *orf14.9* of unknown function) generally framing the *eps* locus in the *S. thermophilus* described in the literature. The sequence of 16037 bp originating from the strain CNCM I-3617 corresponds to the SEQ ID N°3.

The sequences SEQ ID N°4 and SEQ ID N°5 (cf. Figure 4) correspond respectively to the positions 6592 to 9391 and 10331 to 11373 of SEQ ID N°3.

Genetic organization of the *eps* locus

Figure 4 shows diagrammatically the genetic structure of the *eps* locus of the strain CNCM I-3617 established by analysis of its nucleotide sequence. The part upstream of the *epsA* open reading frame (ORF), the sequence of the start of the *epsA* ORF, and the part downstream of the *epsT* ORF are not known. Analysis of the sequence identifies 20 ORFs which are all oriented in the same direction. Due to their similarity of sequence with other known genes, and/or by the presence of specific protein units within products deduced from these ORFs, it is possible to attribute a putative function to them.

The structural analysis of the *eps* locus of the strain CNCM I-3617 shows that it possesses an overall organization similar to that of the *eps* loci already known (cf. Figure 1).

The results of sequence comparison between the potential proteins deduced from the ORFs of the *eps* locus of CNCM I-3617 and those available in the public of databases (GENBANK) are summarized in Table 4. The *eps* locus of the strain CNCM I-3617 codes for proteins potentially involved in the synthesis of polysaccharide such as for example glycosyltransferases.

On the basis of these data, 6 regions can be distinguished (region 1 to region 6, from the 5' end to the 3' end of the *eps* locus; see Figure 4):

- Region 1: this region is formed from 4 ORFs (*epsA, epsB, epsC, epsD*) for which the deduced proteins exhibit very great similarities (between 95.7 and 99.6%) with the proteins deduced from ORFs situated in the *eps* locus of strains of *S. thermophilus*.
- Region 2: this region is formed from 5 ORFs (*epsE, epsF, epsG, epsG'* and *epsH*) for which the deduced proteins exhibit very great similarities (between 96.6 and 99.3%) with the proteins deduced from ORFs situated in the *eps* locus of the type XI strain (GENBANK access no. AF454501).

- Region 3: this region is formed from 3 ORFs (*epsJ, epsK* and *epsL*) for which the deduced proteins exhibit sufficient similarities (less than 81%) with proteins described in the literature to assign a probable function to them. However these ORFs are clearly distinct from ORFs already described in the literature.
- Region 4: this region is formed from one ORF (*epsM*) for which the deduced protein exhibits very great similarities (95.4%) with the protein deduced from the *eps4F* ORF situated in the *eps* locus of the type IV strain.
- Region 5: this region is formed from 2 ORFs (*epsN and epsO*) for which the deduced proteins exhibit sufficient similarities (less than 91%) with proteins of lactobacillae described in the literature to assign a probable function to them. However these ORFs are clearly distinct from ORFs already described in the literature in *S. thermophilus*.
- Region 6: this region is formed from 5 ORFs (*epsP, epsQ, epsS, epsR* and *epsT*) for which the deduced proteins exhibit very great similarities (between 98.9 and 100%) with the proteins deduced from ORFs situated in the *eps* locus of the strain CNRZ368 (GENBANK access no. Z98171).

Overall, the distal part of the *eps* locus resembles a hybrid assembly of genes certain of which had never previously been described.

Table 4: Analysis of the ORFs of the *eps* locus of the strain CNCM I-3617. The position corresponds to the nucleotides at the start and end of the ORF in the sequence SEQ ID N°3. The probable function is that of the proteins deduced from the ORF sequence. The best similarity is the protein or the deduced protein (the GENBANK access number of the protein sequence is indicated in parentheses) having the greatest similarity with the protein deduced from the ORF. The score is the percentage similarity obtained for the best similarity using the "blastp" tool (Protein-protein BLAST, http://www.ncbi.nlm.nih.gov/BLAST/).

ORF	Position	Probable function	Best Similarity	Score
epsA	1 to 491	Transcriptional Regulator	Eps10A (AAN63761), *S. thermophilus* Type X	99.4
epsB	492 to 1223	Polymerization and/or export of polysaccharides	Eps5B (AAN63698), *S. thermophilus* Type V	99.6
epsC	1232 to 1924	Polymerization and/or export of polysaccharides	Eps1C (AAN63508), *S. thermophilus* Type I	97.0
epsD	1934 to 2674	Polymerization and/or export of polysaccharides	Eps5D (AAN63700), *S. thermophilus* Type V	99.2
epsE	2731 to 4098	Undecaprenyl-phosphate glycosyl-1-phosphate transferase	Eps11E (AAN63787), *S. thermophilus* Type XI	98.9
epsF	4131 to 4574	Rhamnosyltransferase	Eps11F (AAN63788), *S. thermophilus* Type XI	99.3
epsG	4546 to 5073	Epimerase	Eps11G (AAN63789), *S. thermophilus* Type XI	96.6
epsG'	5089 to 5553	Epimerase	Eps11G (AAN63789), *S. thermophilus* Type XI	96.8
epsH	5644 to 5940	UDP-glucose 6-dehydrogenase	Eps11H (AAN63790), *S. thermophilus* Type XI	98.9
epsJ	6661 to 7812	UDP-galactopyranose mutase	Glf (ZP_00045853), *Lactobacillus gasseri* ATCC 33323	81.1
epsK	7814 to 8896	Glycosyltransferase	EpsF (AAG44710), *Lactobacillus delbrueckii* subsp. *bulgaricus* Lfi5	64.9
epsL	8896 to 9441	Glycosyltransferase	CpsI (CAC81257), *S. thermophilus* FI9186	65.7
epsM	8896 to 9441	Glycosyltransferase	Eps4F (AAN63682), *S. thermophilus* Type IV	95.4
epsN	9540 to 10370	dTDP-4-dehydrorhamnose reductase	RfbD (YP_619620), *Lactobacillus delbrueckii* subsp. *bulgaricus* ATCC 11842	81.4
epsO	10955 to 11563	dTDP-4-dehydrorhamnose 3,5-epimerase	RfbC (NP_964906), *Lactobacillus johnsonii* NCC 533	90.6
epsP	11580 to 12422	Glycosyltransferase	EpsP (CAB52238), *S. thermophilus* CNRZ368	99.3

epsQ	12519 to 13358	Glycosyltransferase	EpsQ (CAB52237), *S. thermophilus* CNRZ368	98.9
epsS	13355 to 14641	unknown	EpsS (CAB52236), *S. thermophilus* CNRZ368	99.5
epsR	14648 to 15790	Glycosyltransferase	EpsR (CAB52235), *S. thermophilus* CNRZ368	100
epsT	15790 to 16037	unknown	EpsT (CAB52234), *S. thermophilus* CNRZ368	100

Acidifying property

The fermentation support is obtained by supplementing 100 ml of semi-skimmed UHT milk (Le Petit Vendéen®) with 3% (weight/volume) of skimmed milk powder (SUP'R TOP®, Eurial Poitouraine). The sterility of the solution is obtained by pasteurization for 10 min at 90°C (at the core). The fermentation support thus obtained is inoculated with the strain to be tested at a rate of 10^6 cfu/ml, then incubated at 43°C (in a water bath). The pH is continuously monitored using a CINAC apparatus (Ysebaert).

The acidifying properties of the strains of *S. thermophilus* can be described by the maximum rate of acidification, Vm (pH unit/min (pHu/min)), calculated by the maximum value of the first derivative of the pH curve as a function of time. Under these operating conditions, it is estimated that this variable is characteristic of the strain. Its value is constant whatever the physiological state of the micro-organism upon inoculation of the milk and the level of seeding. Two groups of strains are distinguished, the strains with so-called slow acidification, the Vm of which is greater than – 0.0100 pHu/min, and the strains with so-called rapid acidification the Vm of which is les than -0.0100 pHu/min. The strain CNCM I-3617 clearly belongs to the group of the so-called rapid acidification strains (Table 5). This property is very often linked to the presence in the genome of the strains of a gene encoding the wall protease PrtS which could be detected in the genome of CNCM I-3617.

Table 5: Maximum rate of acidification of different strains of *Streptococcus thermophilus* evaluated under the operating conditions described.

Strain	Maximum rate (× -1.E5 upH/min)		
	Average	Standard deviation	*prtS* gene
CNCM I-2429	66	24	absent
DGCC7966	68	12	absent
CNCM I-2432	80	13	absent
DGCC7766	82	13	absent
CNCM I-2978	88	12	absent
DGCC7773	92	17	absent
CNCM I-2979	102	33	absent
CNCM I-2423	129	24	present
CNCM I-3617	144	7	present
CNCM I-2980	167	27	present
DGCC7919	190	22	present

Texturizing property

The fermentation support is obtained by supplementing 100 ml of semi-skimmed UHT milk (Le Petit Vendéen®) with 3% (weight/volume) of skimmed milk powder (SUP'R TOP®, Eurial Poitouraine). The sterility of the solution is obtained by pasteurization for 10 min at 90°C (at the core). The fermentation support thus obtained is inoculated with the strain to be tested at a rate of 10^6 cfu/ml, then incubated at 43°C (in a water bath) until a pH of 4.6 is obtained. The pH is continuously monitored using a CINAC apparatus (Ysebaert). The fermented milks thus obtained are placed in a ventilated oven at 6°C, until they are analyzed. Two types of rheological measurements are carried out: viscosity and flow. The viscosity measurements are carried out at a temperature of 8°C on fermented milks after storage for 1, 7, 14 and 28 days at 6°C. The equipment used is a RVF-type Brookfield® viscosimeter (Brookfield Engineering Laboratories, Inc.) mounted on a Helipath stand (Brookfield Engineering Laboratories, Inc.). The viscosimeter is equipped with a type C needle and the oscillation speed

applied to the needle is 10 rpm. The flow measurements are carried out at a temperature of 8°C on previously-stirred fermented milks, after storage for 14 days at 6°C. The equipment used is an AR1000-N rheometer (TA Instrument) equipped with co-axial cylinders (Radius 1 = 15 mm, Radius 2 = 13.83 mm, Height = 32 mm, Air gap = 2 mm). For the ascending segment, the stress applied in a continuous sweep varies from 0 to 60 Pa for a duration of 1 min according to a linear mode. For the descending segment, the stress applied in a continuous sweep varies from 60 to 0 Pa for a duration of 1 min according to a linear mode. The values taken into account are the thixotropic area and the yield point; the latter is calculated according to the Casson model.

The texturizing ability of a strain can be evaluated in a first phase by a viscosity measurement of the curd obtained under the operating conditions described above. The recognized non-texturizing strains provide viscosity values close to 30 Pa.s while the texturizing strains exceed 40 Pa.s. This texturizing ability can be more or less pronounced (Table 6). For example the strain CNCM I-2979 produces a curd the viscosity of which reaches 42 Pa.s, and the strain DGCC7966 makes it possible to obtain a clearly higher viscosity, of 70 Pa.s. The strain CNCM I-3617 provides curds the viscosity of which amounts to 54 Pa.s (Table 6). This value places this strain among the group of strains with a texturizing ability fully comparable to the industrial strains currently used to devise lactic ferments for the production of yogurts and fermented milks.

Table 6: Viscosity of the fermented milks obtained with the different strains tested, after storage at 6°C for 14 days.

Strain	Viscosity in Pa.s	
	Average	Standard deviation
DGCC7966	70.0	Nd
DGCC7773	55.0	3.3
CNCM I-3617	54.0	2.5
CNCM I-2980	53.0	2.9
CNCM I-2429	51.0	3.1
CNCM I-2978	49.6	4.2
CNCM I-2432	43.0	4.0
CNCM I-2979	42.2	3.0
CNCM I-2423	42.0	4.6
DGCC7919	28.0	Nd
DGCC7766	30.0	Nd

Nd: not determined

The rheological analyses using the AR1000-N rheometer made it possible to measure two rheological descriptors relevant for qualifying fermented milks: the yield point of the product (Pa) and the thixotropic area (Pa/s). These measurements are reported in Table 7 for each of the strains. For the fermented milk with the strain CNCM I-3617, the average values are 11.25 Pa and 352 Pa/s respectively. These values are significantly different from those measured on curds obtained with strains deemed non-texturizing (DGCC7766 or DGCC7919).

Tableau 7: Values of yield point and thixotropic area, Casson model, measurements by the AR1000-N on fermented dairy products with different strains after storage for 14 days at 6°C.

Strain	Yield point (Pa)		Thixotropic area (Pa/s)	
	Average	Standard deviation	Average	Standard deviation
CNCM I-2980	5.89	0.9	488	107
CNCM I-2423	8.86	0.9	1344	574
CNCM I-2978	10.51	0.4	728	153
CNCM I-3617	11.25	0.4	352	53
CNCM I-2432	12.27	1.3	1245	181
CNCM I-2429	13.32	1.2	1215	255
CNCM I-2979	13.56	Nd	1786	250
DGCC7773	14.00	Nd	60	Nd
DGCC7966	15.00	Nd	43	Nd
DGCC7919	15.91	0.2	33100	1415
DGCC7766	17.01	0.1	17083	1520

Nd: not determined

Conclusion

The strain CNCM I-3617 has several characteristics of interest for the construction of ferments and in particular for ferments used during the production of yogurts or fermented milks. It exhibits a rare combination of functional properties (acidifying and thickening strain) and its lysotype is distinct from that of the other strains used in a standard manner for these applications.

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Claims

1. Strain of *Streptococcus thermophilus* the epsAD fragment of which, after digestion by the restriction enzymes *Mnl*I, *Fok*I and *Hin*dIII, has a restriction profile characterized by DNA fragments of 344±2 bp, 341±2 bp, 305±2 bp, 299±2 bp, 277±2 bp, 210±2 bp, 160±2 bp, 142±2 bp, 100±2 bp, 79±2 bp, 75±2 bp, 66±2 bp, 42±2 bp, 23±2 bp and 9±2 bp.

2. Strain according to claim 1 comprising a nucleotide sequence having at least 80% identity with the nucleotide sequence of SEQ ID N°4.

3. Strain according to one of the claims 1 to 2 comprising a nucleotide sequence having at least 80% identity with the nucleotide sequence of SEQ ID N°5.

4. Strain according to one of the preceding claims characterized in that it is texturizing.

5. Strain according to one of the preceding claims characterized in that it acidifies rapidly.

6. Strain according to one of the preceding claims characterized in that the strain is the strain of *Streptococcus thermophilus* deposited on 14th June 2006 at the Collection Nationale de Cultures de Microorganismes under no. CNCM I-3617 or a mutant strain which can be obtained from the latter.

7. Strain of *Streptococcus thermophilus* deposited on 14th June 2006 at the Collection Nationale of Culture of Microorganismes under no. CNCM I-3617.

8. Bacterial composition comprising at least one strain according to one of the preceding claims.

9. Production process for a food product, a food complement, a dietary supplement or a product with probiotic properties comprising at least one stage in which the strain according to any one of the claims 1 to 7 is used.

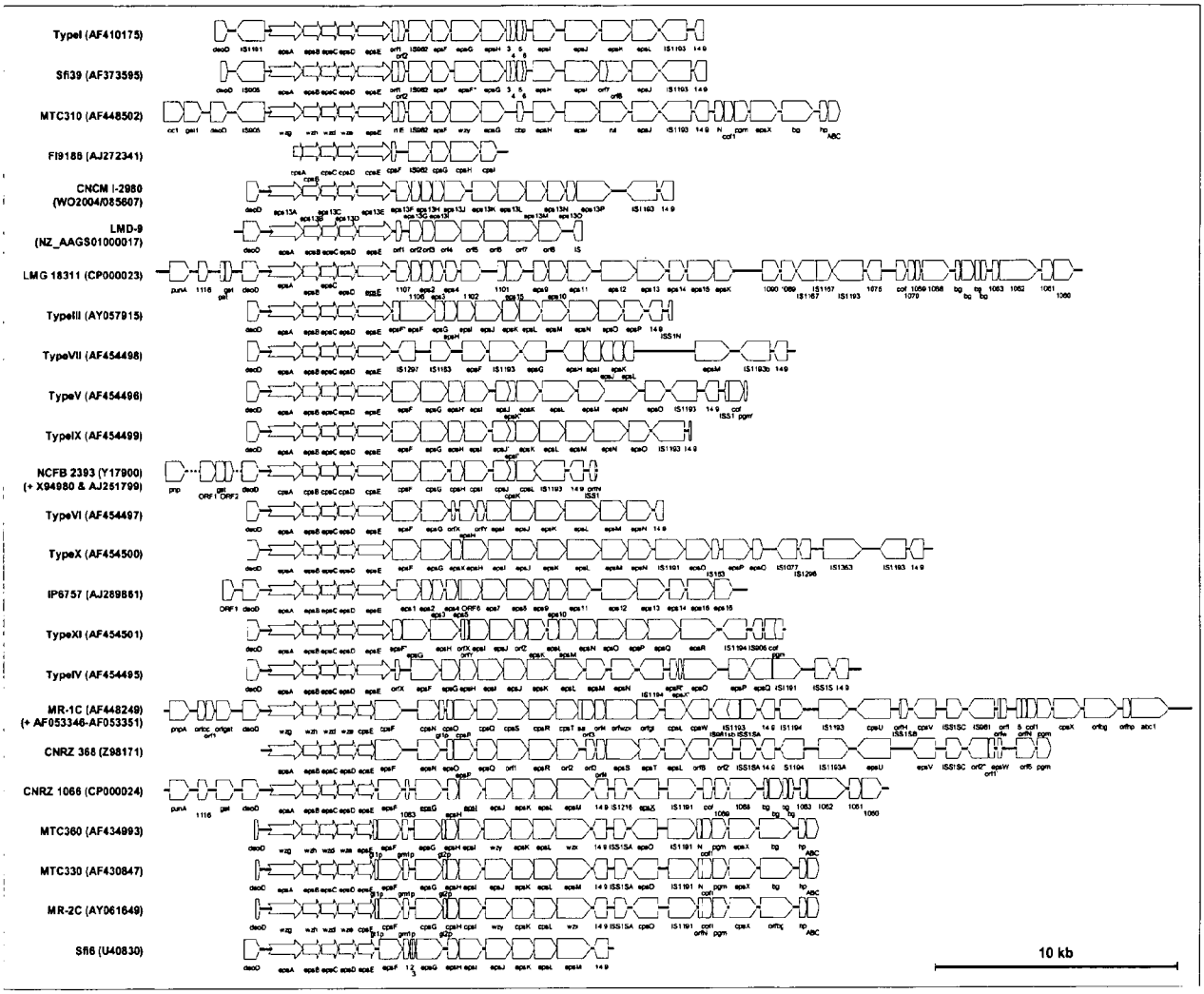
10. Process according to claim 9 in which the food product, the food complement, the dietary supplement or the product with probiotic properties is a dairy product, a meat product, a cereal product, a drink, a foam or a powder.

11. Food product, food complement, dietary supplement or product with probiotic properties comprising at least one strain according to any one of claims 1 to 7 or the bacterial composition according to claim 8.

12. Dairy product comprising at least the strain according to claims 1 to 7 or the bacterial composition according to claim 8.

13. Dairy product according to claim 12 characterized in that it is a fermented milk, a yogurt, a matured cream, a cheese, a fromage frais, a milk drink, a dairy product retentate, a processed cheese, a cream dessert, a cottage cheese or an infant milk.

14. Dairy product according to claim 12 or 13 characterized in that it comprises milk of animal and/or plant origin.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TypeIV (AF454495)
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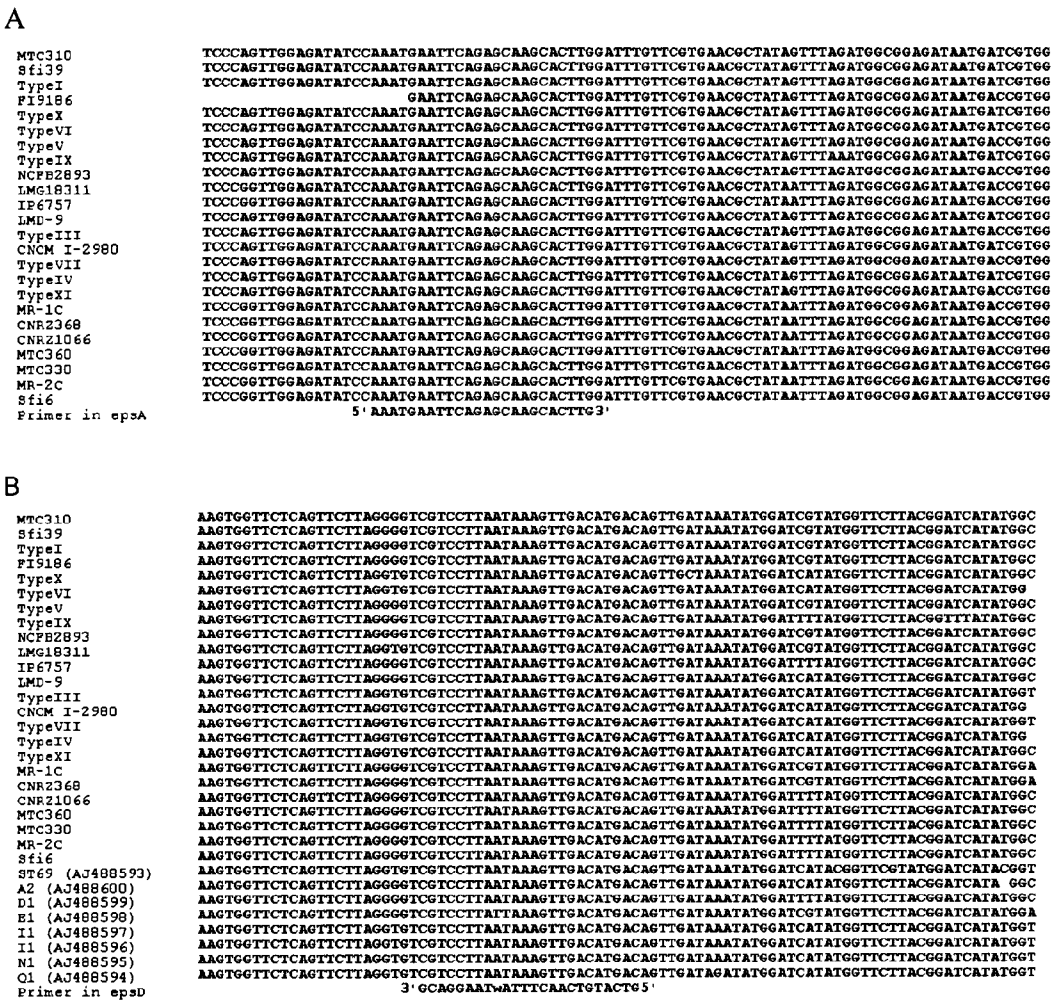
Figure 1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Sfi6
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MTC310
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Figure 2

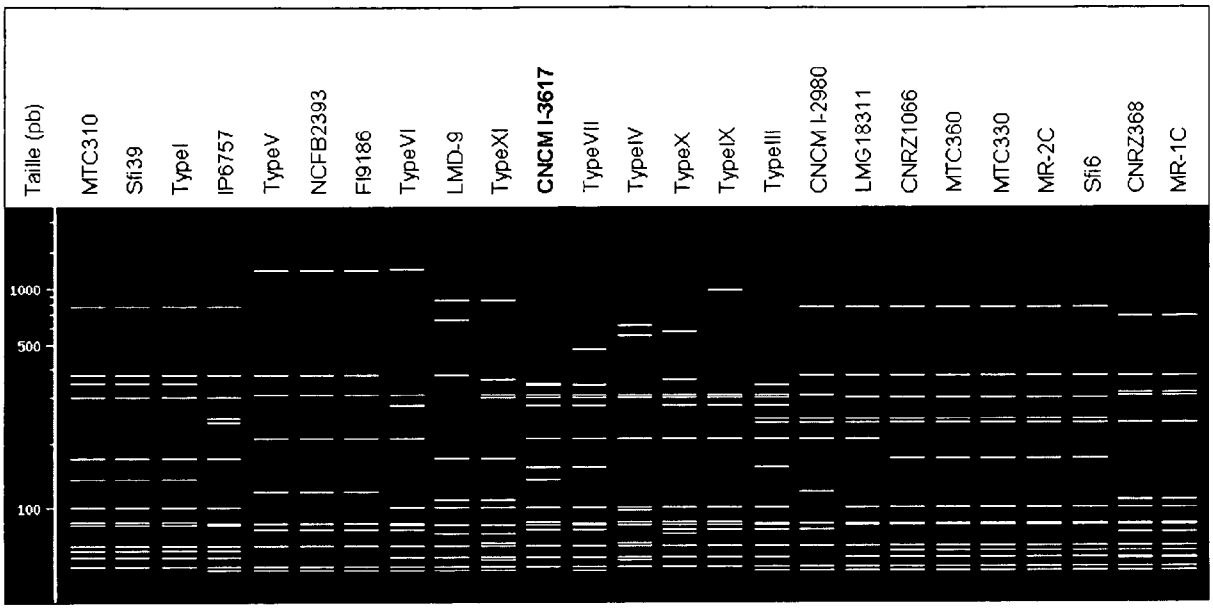

Figure 3

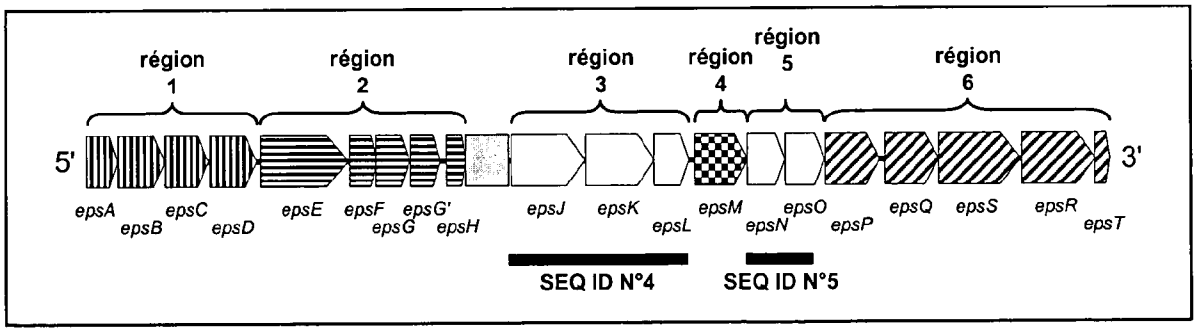

Figure 4

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO :

MAUD GODIGNON
Patent Manager

DANISCO FRANCE SAS
20 RUE BRUNEL - CS 70080
75617 PARIS CEDEX 17

NAME AND ADDRESS OF THE PARTY TO WHOM THE VIABILITY STATEMENT IS ISSUED

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name : **DANISCO FRANCE SAS**	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY : **CNCM I-3617**
Address : **20, RUE DE BRUNEL 75017 PARIS**	Date of the deposit or of the transfer[1] : **14 June 2006**

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on **14 June 2006**[2]. On that date, the said microorganism was

[X][3] viable

[ ][3] no longer viable

1 Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2 In the cases referred to in Rule 10.2 (a)(ii) and (iii), refer to the most recent viability test.

3 Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED [4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name :

COLLECTION NATIONALE DE CULTURES DE MICROORGANISMES (CNCM)

Address :

Institut Pasteur
25, rue du Docteur Roux
F-75724 Paris Cedex 15 (France)

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) :

Georges WAGENER
CNCM, Executive Head

Date : Paris, 7 August 2007

C. N. C. M. COLLECTION NATIONALE DE CULTURES DE MICROORGANISMES Institut Pasteur

4 Fill in if the information has been requested and if the results of the test were negative.

DECLARATION

I, Didier Carcano, an authorised signatory of Danisco France SAS, 20 rue Brunel 75017 Paris, France (Headquarter), FRANCE MAKE OATH and say as follows :

I confirm that Danisco France SAS has authorised Danisco A/S of Langebrogade 1, P.O.. Box 17, DK-1001, Copenhagen K, Denmark to refer to the deposited biological material deposited as *Streptococcus thermophilus* under the Budapest Treaty by Danisco France SAS at the Collection Nationale de Culture de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 16, France under the deposit number CNCM I-3617 on the 14th of June 2006, in patent application filed as French patent application number 06 08657 and any patent application(s) deriving therefrom or claiming priority therefrom. Danisco France SAS has given its unreserved and irrevocable consent to the deposited material being made available to the public.

SWORN and SUBSCRIBED in the presence of a witness.

Signature : 

Date : 5 juillet 2007

Witness signature :

Witness name and address :

Pierrette KRAWCZYNSKI
DANISCO FRANCE SAS
20 rue Brunel
75017 Paris

TRAITE DE BUDAPEST SUR LA RECONNAISSANCE
INTERNATIONALE DU DEPOT DES MICRO-ORGANISMES
AUX FINS DE LA PROCEDURE EN MATIERE DE BREVETS

CERTIFIED COPY OF THE ORIGINAL RECEIPT
for:
MAUD GODIGNON
Patent Manager
DANISCO FRANCE SAS
20 RUE BRUNEL - CS 70080
75617 PARIS CEDEX 17

FORMULE INTERNATIONALE

DESTINATAIRE :

DANISCO FRANCE SAS
20, RUE DE BRUNEL
75017 PARIS

RECEPISSE EN CAS DE DEPOT INITIAL, délivré en vertu de la règle 7.1 par l'AUTORITE DE DEPOT INTERNATIONALE identifiée au bas de cette page

Paris, 14 September 2007

Dr Georges Wagener
CNCM, Executive Head

NOM ET ADRESSE DU DEPOSANT

I. IDENTIFICATION DU MICRO-ORGANISME

Référence d'identification donnée par le DEPOSANT :	Numéro d'ordre attribué par l'AUTORITE DE DEPOT INTERNATIONALE :
DGCC 8014	**CNCM I-3617**

II. DESCRIPTION SCIENTIFIQUE ET/OU DESIGNATION TAXONOMIQUE PROPOSEE

Le micro-organisme identifié sous chiffre I était accompagné :

☐ d'une description scientifique

☒ d'une désignation taxonomique proposée

(Cocher ce qui convient)

III. RECEPTION ET ACCEPTATION

La présente autorité de dépôt internationale accepte le micro-organisme identifié sous chiffre I, qu'elle a reçu le **14 juin 2006** (date du dépôt initial) [1]

IV. RECEPTION D'UNE REQUETE EN CONVERSION

La présente autorité de dépôt internationale a reçu le micro-organisme identifié sous chiffre I le (date du dépôt initial)
et a reçu une requête en conversion du dépôt initial en dépôt conforme au Traité de Budapest le (date de réception de la requête en conversion)

V. AUTORITE DE DEPOT INTERNATIONALE

Nom : **COLLECTION NATIONALE DE CULTURES DE MICROORGANISMES (CNCM)**

Adresse : **Institut Pasteur**
25, rue du Docteur Roux
F-75724 Paris Cedex 15 (France)

Signature(s) de la (des) personne(s) compétente(s) pour représenter l'autorité de dépôt internationale ou de l'(des) organisme(s) autorisé(s) : Georges Wagener

Date : Paris, le 12 septembre 2006

1 En cas d'application de la règle 6.4 d), cette date est la date à laquelle le statut d'autorité de dépôt internationale a été acquis.

Formule BP/4 (page unique)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/060463

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N1/20 A23C9/123
ADD. C12R1/46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2004/085607 A (RHONE POULENC CHIMIE [FR]; HORVATH PHILIPPE [FR]; MANOURY ELISE [FR];) 7 October 2004 (2004-10-07) claims; examples	1-14
A	BROADBENT J R ET AL: "Biochemistry, genetics, and applications of exopolysaccharide production in Streptococcus thermophilus: a review" JOURNAL OF DAIRY SCIENCE, AMERICAN DAIRY SCIENCE ASSOCIATION, SAVOY, IL, US, vol. 86, no. 2, February 2003 (2003-02), pages 407-423, XP009018436 ISSN: 0022-0302 the whole document	1-14

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 January 2008	21/01/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Didelon, Frédéric

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/060463

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOLLY LAURE ET AL: "Molecular organization and functionality of exopolysaccharide gene clusters in lactic acid bacteria" INTERNATIONAL DAIRY JOURNAL, vol. 11, no. 9, 2001, pages 733-745, XP002423540 ISSN: 0958-6946 the whole document	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2007/060463

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004085607 A	07-10-2004	AU 2004223691 A1	07-10-2004
		CN 1761752 A	19-04-2006
		EP 1604025 A2	14-12-2005
		FR 2852604 A1	24-09-2004
		JP 2006520590 T	14-09-2006
		US 2006240539 A1	26-10-2006