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A large scale *in vitro* screening of *Streptococcus thermophilus* strains revealed strains with a high anti-inflammatory potential

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ABSTRACT

In spite of its contribution to health benefits of yogurt, probiotic properties of *Streptococcus thermophilus* remain less explored. Hence, we evaluated the capacities of 30 strains of different origins, to resist the stresses prevailing in digestive tracts, of adhering to the mucus producing HT29-MTX cells, as well as their anti-inflammatory properties. First, on the basis of results obtained by multilocus sequence typing, two very closely related groups were distinguished phylogenetically. However, it appeared that in spite of this phylogenetic proximity, resistance to low pH, bile salts and H_2O_2 and their capacities of adhesion highly varied from one strain to another. Furthermore, most of the strains reduced the production of the pro-inflammatory interleukin IL-8 after co-incubation with HT-29 cells, while they induced production of the anti-inflammatory interleukin IL-10, when incubated with Peripheral Blood Mononuclear Cells. On the basis of ratio of synthesis of IL-10 and of IL-12, currently used to evaluate the anti-inflammatory potential of a probiotic bacterium, three strains appeared to display a strong and promising *in vitro* anti-inflammatory potential, suggesting that they could be appropriate for elaborating anti-inflammatory functional fermented foods. Finally, the Principal Component Analysis method enabled us to cluster strains into 6 classes displaying distinct phenotypic properties.

1. Introduction

The increasing costs of health as well as the desire of consumers to improve their quality of life stimulates the researches and developments of functional foods which are claimed to display health specific properties. Such products can be developed by adding health promoting component(s), reducing/removing harmful component(s) or modifying nature or bioavailability of specific functional component(s) (Gupta & Abu-Ghannam, 2012). Among the health promoting component(s), the probiotics, defined by the Food and Agricultural Organization/World Health Organization as live microorganisms which confer a health benefit to the host, when they are administered in adequate amount, are intensively studied and new probiotics have been searched for. The main criteria used to select probiotics include resistance to stresses encountered in the gastrointestinal tract (GIT), beneficial impacts on the host health and resistance to technological processing stresses when incorporated into a desired food. Hence, many works aim to screen various strains of lactic acid bacteria (LAB) currently used in food industry for probiotic properties.

Among LAB used in food industry, *Streptococcus thermophilus* is traditionally used as starter for cheeses and yogurts. In yogurt,

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S. thermophilus is associated with *Lactobacillus delbrueckii* ssp. *bulgaricus* and contributes to the health benefits of this product, as it is already recognized for improving lactose digestion in individuals suffering from lactose intolerance (EFSA, 2010). Although it has long been believed that *S. thermophilus* does not survive in the GIT, the capacity of certain strains to survive in this given environment and to adapt their metabolism were established (Brigidi, Swennen, Vitali, Rossi, & Matteuzzi, 2003; Elli et al., 2006; Mater et al., 2005; Rul et al., 2011; Thomas et al., 2011). Besides its contribution to the health properties of yogurt, particularly through its capacity to reduce amount of lactose in the small intestine by producing an active β-galactosidase (Mater et al., 2006), *S. thermophilus* is capable of producing folate which is required for certain biological reactions (Iyer, Tomar, Kapila, Mani, & Singh, 2010) or to modulate immunological response (Kekkhonen et al., 2008; Ogita, Tanii, Morita, Suzuki, & Tanabe, 2011).

Knowing that probiotic properties are strain-dependent, we screened 30 *S. thermophilus* strains of different origins for their capacity to resist stresses prevailing in the digestive tract, to adhere to enterocytes and to modulate inflammatory responses.

2. Materials and methods

2.1. Bacterial strains, media and growth conditions

S. thermophilus strains used in this work are listed in Table 1. They come either from the CNRZ and LMG collections or have been isolated in our laboratory (Table 1). Finally, the strain LMD-9, originally isolated from yogurt, was also used in this study (Makarova et al., 2006). They were stored at −80 °C or grown overnight at 42 °C in reconstituted skim milk (100 g/L) before growth in M17 medium (Terzaghi & Sandine, 1975) supplemented with lactose (20 g/L). *Lactobacillus acidophilus* NCFM was grown under limited aeration at 37 °C in MRS medium (Difco).

2.2. Genomic DNA extraction and multi locus sequence typing (MLST) analysis

Genomic DNA was extracted from 24 h *S. thermophilus* cultures and the MLST analysis performed as described elsewhere (Delorme, Bartholini, Bolotine, Ehrlich, & Renault, 2010; Delorme, Poyart, Ehrlich, & Renault, 2007; El-Sharoud, Delorme, Darwish, & Renault, 2012; Galia, Perrin, Genay, & Dary, 2009). An internal part of the nine genes *ddlA*, *pepO*, *glcK*, *ilvC*, *thrS*, *tkt*, *dnaE*, *pyrE* and *serB* was amplified using specific primers (Table 2), and sequenced on both strands (Beckman Coulter Genomics). After creation of a concatenate sequence, the phylogenetic tree was built using the neighbor joining method (bootstrap: 1000 replicates) with the MEGA 5.05 software, and rooted using the corresponding genes of *Streptococcus salivarius* JIM8777, CCHSS3 and 57.I.

2.3. Cell lines, culture configurations, adhesion and immunomodulatory property determination

The three cell lines HT29 (European Collection of Cell Cultures), PBMC (Peripheral Blood Mononuclear Cells; StemCell™ Technologies SARL) and HT29-MTX (INSERM UMR S 938, Paris, France) were respectively employed to determine the capacity of *S. thermophilus*

Table 1
S. thermophilus strains used in this work and allelic composition established by Multi Locus Sequencing Typing.

Strain	Origin	Allele no.								
		glcK	*ddlA*	*pepO*	*ilvC*	*thrS*	*tkt*	*pyrE*	*dnaE*	*serB*
ATE11PB6MJ[a]	Yoghurt	1	1	2	1	2	7	5	1	3
EBL1066[a]	Yoghurt	1	2	3	2	2	3	8	2	7
CNRZ160	Yoghurt	2	2	4 or 1	1	1	3	1	1	2
CNRZ21	Yoghurt	5	2	4 or 1	1	1	1	3	1	4
CNRZ302	Cheese	1	2	3	2	2	3	6	1	3
CNRZ391	Yoghurt	1	1	2	1	2	7	5	1	3
CNRZ455	Unknown	1	1	2	1	2	7	5	1	3
EBLHAD17[a]	Yoghurt	1	2	4 or 1	1	1	1	3	1	4
EBLHAD8α[a]	Yoghurt	1	2	4 or 1	2	1	1	3	1	18
EBL307[a]	Cheese	1	1	2	1	2	7	5	1	3
EBL308[a]	Cheese	2	N	N	1	1	4	6	2	3
EBL385[a]		1	2	3	2	2	3	6	1	3
EBL404[a]	Yoghurt	1	1	2	1	2	7	5	1	3
EBL407[a]	Yoghurt	1	1	2	1	2	7	5	1	3
EBL445[a]	Cheese	1	1	2	1	2	7	5	1	3
EBLST19[a]	Yoghurt	1	1	4 or 1	1	2	2	6	1	25
EBLST20[a]	Yoghurt	1	1	2	1	2	7	5	1	3
EBLY4[a]	Yoghurt	1	1	2	1	2	7	5	1	3
4F44[a]	Cheese	1	2	4 or 1	1	2	3	6	1	3
LMD9	Yoghurt	1	1	2	1	2	7	5	1	3
LMG18311	Yoghurt	1	2	4 or 1	1	2	1	3	1	1
PB18[a]	Cheese	1	1	2	1	2	7	5	1	3
PB18O[a]	Cheese	1	1	2	1	2	7	5	1	3
PB2 (B17[a])	Unknown	1	1	2	1	2	7	5	1	3
PB302[a]	Yoghurt	1	1	2	1	2	7	5	1	3
PB385[a]	Yoghurt	1	1	2	1	2	7	5	1	3
PB5MJ[a]	Yoghurt	1	1	2	1	2	7	5	1	3
ST14[a]	Yoghurt	3	2	4 or 1	1	1	8	13	1	9
ST88[a]	Cheese	1	1	2	1	2	7	5	1	3
WG19258[a]	Milk	1	2	3	2	2	3	8	2	7

The gene *glcK* encodes a glucose kinase, *ddlA* an D-alanine D-alanine ligase, *pepO* an endopeptidase, *ilvC* a ketol acid-reductoisomerase, *thrS* a threonyl-tRNA synthetase, *tkt* a transketolase, *pyrE* an orotate phosphoribosyltransferase, *dnaE* the DNA polymerase III, and *serB* a phosphoserine phosphatase.
For each gene, the attributed allele number corresponded to that deposited in the Genebank (Delorme et al., 2007; El-Sharoud et al., 2012) and in the MLST databases (http://www.pasteur.fr/mlst). The primers used to amplify the gene *pepO* did not permit to distinguish between the allele 1 and 4. N: New allele.
[a] Strains isolated in our laboratory.