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Original article

Comparative study of the protein composition of three strains of *Streptococcus thermophilus* grown either in M17 medium or in milk

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Abstract – *Streptococcus thermophilus* is a lactic acid bacterium widely used for manufacturing milk products. The protein composition of three strains of *S. thermophilus* grown in either M17 medium or milk was investigated. These conditions were chosen on the basis of the observed technological differences between these strains. Protein extracts prepared from cells harvested at the same pH (5.2 ± 0.1), were analyzed by two-dimensional gel electrophoresis. Gel comparison revealed qualitative and quantitative differences between the two growth media and between the strains. Some proteins with quantitative variation were partially identified. The most significant medium-related differences concerned the number of spots detected and the heat shock protein (GroEL, HSP70) levels which were higher in milk than in M17. On the contrary, the levels of enzymes (proton translocating ATPase, pyruvate kinase, tributyrin esterase, phospho-glycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, fructose diphosphate aldolase, superoxide dismutase) and of a putative penicillin binding protein were lower in milk than in M17. Strain-related differences were accentuated in milk for heat shock proteins, tributyrine esterase and dinitrogenase reductase.

Streptococcus thermophilus **/ milk / proteome / 2D-PAGE electrophoresis**

Résumé – **Étude comparative de la composition protéique de trois souches de *Streptococcus thermophilus* cultivées dans le milieu M17 ou dans le lait.** *S. thermophilus* est une bactérie lactique très utilisée pour la fabrication de produits laitiers. La composition protéique de trois souches de *S. thermophilus* cultivées dans le milieu M17 ou dans le lait a été étudiée. Ces conditions ont été choisies sur la base des différences technologiques observées entre les souches. Les extraits

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protéiques préparés à partir des cellules récoltées au même pH (5,2 ± 0,1), ont été analysés par électrophorèse bidimensionnelle. La comparaison des gels a révélé des différences qualitatives et quantitatives entre les deux milieux et entre les souches. Certaines protéines présentant une variation quantitative ont été partiellement identifiées. Les différences les plus significatives en fonction du milieu de croissance concernent le nombre de taches détectées et la quantité de protéines de choc thermique (GroEL, HSP70) qui sont plus élevés dans le lait que dans le M17. Les quantités d'enzymes (ATPase translocatrice de proton, pyruvate kinase, tributyrine estérase, phospho-glycérate kinase, glyceraldéhyde-3-phosphate deshydrogénase, fructose diphosphate aldolase, superoxyde dismutase) et d'une protéine putative de liaison de la penicilline sont, au contraire, plus faibles dans le lait que dans le M17. Les différences entre les souches sont accentuées dans le lait pour les protéines de choc thermique, la tributryrine estérase et la dinitrogénase réductase.

***Streptococcus thermophilus* / lait / protéome / électrophorèse 2D-PAGE**

1. INTRODUCTION

The dairy industry relies on reproducible, controlled and predictable fermentations, but variations in starter culture performance are not uncommon and require direct on-line action. Among lactic acid bacteria, *Streptococcus thermophilus* is used for yogurt production as well as starter culture for many cheeses. It is mainly used as lyophilized or frozen cultures. In Emmental cheese making, *S. thermophilus* is implicated in early events leading to initial acidification. Acidification is then completed by thermophilic lactobacilli that ferment the galactose excreted by *S. thermophilus* [1]. The properties of final products depend on the capacities of each strain making up the starter. However, the technological properties and genomic homology of strains have not been related. It has been observed that strains of *S. thermophilus* closely genetically related present various performances in cheesemaking. Consequently, cellular events taking place in milk and in dairy products are important.

2D-PAGE is widely used for studies on protein composition or proteomes of numerous cells [34]. We have previously established the 2D reference map and described the proteome of *S. thermophilus* grown in buffered M17 medium [25, 26]. However the proteome of *S. thermophilus* grown in milk has never been studied. For the first time the early protein synthesis of *Lactobacillus delbrueckii* ssp. *bulgaricus* cultured in milk was described by Rechinger et al. [29].

Recently, Chopard et al. [8] have confirmed that proteolytic enzymes from thermophilic lactobacilli are induced in milk as described by Ezzat et al. [15] and Exterkate [14]. Consequently, the performances of strains in milk have to be evaluated.

The present work compares the proteomes of three strains of *S. thermophilus* isolated from dairy starters, grown in milk and in M17 medium. The purpose of this study was to identify reliable protein indicators of the state of the strains in milk which will be useful for industry.

2. MATERIALS AND METHODS

2.1. Strains of *S. thermophilus*

The strains used were: CNRZ407, isolated from yogurt commercial starter (INRA, Jouy-en-Josas, France), ITGST80 and ITGST82, isolated from natural starters of Emmental cheese (Syndicat Interprofessionnel du Gruyère Français, SIGF, Paris, France). Identification of strains such as *S. thermophilus* was confirmed by RAPD with two primers, BO6 and B10 (URLGA, INRA Jouy-en-Josas, France) [32]. Their genomic homology was

below 90%. Strains harbored no plasmid. Glucide fermentation by strains was analyzed with API 50 CH strips (Biomérieux, Marcy l'Étoile, France). Acidifying activity was detected in milk "laitg" at 44 °C with standardized cellular suspensions, as described by Chamba and Prost [7].

2.2. Growth conditions

The milk medium, constituted by 10% milk called "laitg" (powder of skimmed milk sterilized by ionization) (Standa, Caen, France) [7] in sterilized distilled water, was centrifuged (10 min, 8000 × *g*, 10 °C). Overnight precultures in milk were prepared from stock cultures of strains and then used to inoculate (2%) M17 and milk. Cell cultures in M17 at 42 °C were made as described [19] and stopped at the extracellular (pHe) value of 5.2 ± 0.1. Cell cultures in milk with added bromocresol purple (0.016%) were made at 42 °C and stopped at the color variation of bromocresol purple (pHe value 5.2 ± 0.1). To recolt the cells, 0.33 vol of 1 mol·L^{-1} trisodium citrate and 0.13 vol of buffered saline solution (0.145 mol·L^{-1} sodium chlorure, 0.016 mol·L^{-1} sodium β-glycerophosphate, 0.1% Tween 80, pH 7.0) were added to the cultures in accordance with the protocol developed by Chopard et al. [8]. After 20 min at ambient temperature, cells were harvested by centrifugation (7 min, 4000 × *g*, 4 °C).

2.3. Preparation of soluble cytoplasmic protein samples

Cell pellets were washed three times in sterile extraction buffer pH 7.0 (0.005 mol·L^{-1} sodium phosphate, 0.001 mol·L^{-1} EDTA, 0.001 mol·L^{-1} β-mercaptoethanol) and stored at –20 °C. Raw cytoplasm was extracted from cells as described [19] and centrifuged at 20000 × *g* for 20 min at 4 °C. A_{260}/A_{280} and ribosome quantity (determined from A_{260} value) were determined in the supernatant (S20). Protein content in S20 was determined (3 measures on 3 dilutions of each sample) by the method of Bradford [5]. S20 was lyophilized and stored at –20 °C in100 μg aliquots.

2.4. 2D-PAGE and data analysis of 2D gels

2D-PAGE were performed as contract work by the GSI Proteomics division of the Genomic Solutions Society (Genomic solutions, Chelmsford, MA, USA) with the Investigator™ 2-D system. The first dimension gels were IPG strips (4–7L, Amersham Pharmacia Biosciences, Piscataway, NJ, USA) with pI range 4–7. Because there was always some dead space at the ends of any strip, effective linear range was 4.3–6.7. The second dimension gels were 10% Duracryl, tricine chemistry (22 cm × 23 cm × 1 mm) SDS polyacrylamide gels. The gels were silver-stained, scanned and their images were analyzed using the Bioimage™ 2-D analyzer software (Millipore, Ann Arbor, MI, USA) as described [26]. Background subtraction, integrated intensity calculation (volume of the spot) and normalization of spot volumes (% of the total volume scored) were determined. Gel comparisons were performed through a chosen master gel (a gel of the strain ITGST82) and manually selecting 30 common spots as anchors. Only consistent protein spots were retained for analysis. In order to account for the experimental quantitative variations, gels for each strain grown in M17 were performed with other IPG strips (pI range 3–10). The mean standard deviation on the integrated intensity of the same spots separated in 4–7 and 3–10 pI ranges was calculated and it was ± 0.23. The intensity ratio of a defined spot in the gels from two experiments indicated the change in expression level of the corresponding protein under the two conditions. A ratio of 2–3 was considered significant for silver-stained gels. The quantitative differences of the selected candidate proteins were also

verified using the Melanie II system (Biorad, Hercules, CA, USA). The standard deviation on the intensity ratio of the matched spots between the two conditions (milk/M17) was calculated and it was ± 0.17. Data analysis was performed using MS Excel. TIF images were imported into MS PowerPoint for the addition of text and a coordinate system.

2.5. Identification of proteins by peptide mass fingerprinting and N-terminal sequencing

After western blotting of the 2D gels as described [25], spots of selected proteins were excised and stored at –20 °C in eppendorf tubes. The N-terminal sequence analyses were performed as contract work by the SCSP service (Université Henri Poincaré, Nancy I, France). The tryptic digestion of spots and the MALDI-TOF mass spectrometry analysis of tryptic digests were performed as contract work by the URBSP service (INRA, Jouy-en-Josas, France).

2.6. Database searches

A database search with the N-terminal protein sequences was performed against the NCBInr (http://www.ncbi.nlm.nih.gov/) and MICADO (http://locus.jouy.inra.fr/micado) databases using the Blast and FastA programs. A database search with the monoisotopic masses of the tryptic peptides was performed against the NCBInr database using the MS-Fit search program (http://prospector.ucsf.edu/ucsfhtml3.2/msfit. htm).

3. RESULTS

3.1. Characteristics of the strains

A protocol was developed to prepare the protein samples without major milk protein contamination, because production of lactic acid during growth leads to precipitation of milk proteins. The 1-D protein profiles of the three strains did not show bands at the migration zone of the major proteins of the milk (caseins and whey proteins) used as migration control (data not shown). The ITGST80, ITGST82 and CNRZ407 strains were chosen because they have physiological and technological differences. The three strains fermented glucose, lactose and sucrose and, additionally, ITGST80 and ITGST82 fermented fructose. CNRZ407 had no urease activity. Their acidifying potential in milk at 44 °C (temperature close to the optimum growth temperature of *S. thermophilus*) was different (Fig. 1). ITGST82 had a strong acidifying activity, while that of ITGST80 was very low and that of CNRZ407 was intermediate.

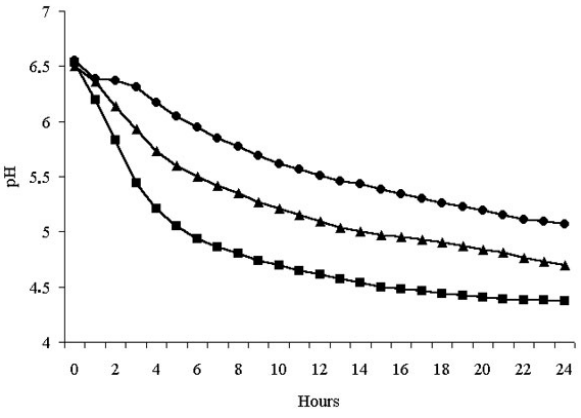

Figure 1. Acidifying activities of *S. thermophilus* strains ITGST80 (●), ITGST82 (■), CNRZ407 (▲). The acidifying activities were detected in milk ("laitg") at 44 °C with standardized cellular suspensions as described by Chamba and Prost [7].

3.2. Protein profiles of the strains grown either in M17 medium or in milk

The soluble cytoplasmic protein extracts were analyzed by 2D-PAGE with a linear gradient of pI 4–7 chosen in relation to our previous results [26]. The 2D protein profiles are given in Figures 2 and 3. Approximately 500–600 spots were counted in the gels with the image analyzer software. The average number of spots detected for the strains cultured in milk was higher (576 ± 33) than that obtained for the strains cultured in M17 (505 ± 40). ITGST82 exhibited the greatest increase in the number of spots (+34%) in milk in comparison with M17 medium. In M17, the protein profiles of the three strains were relatively homologous and the spots were focused in the pI 4.6–5.7 range (Fig. 2). In milk, the 2D protein profiles were less homologous and showed the presence of numerous proteins in the pI 5.7–6.7 zone (Fig. 3). ITGST80 (Fig. 3A) showed a characteristic profile with only few proteins in the pI 5.7–6.7 zone and a train of proteins of 30 kg·mol^{-1}. Here, we could observe a large smear in the 21 kg·mol^{-1} zone which agrees with the patterns previously obtained in stationary

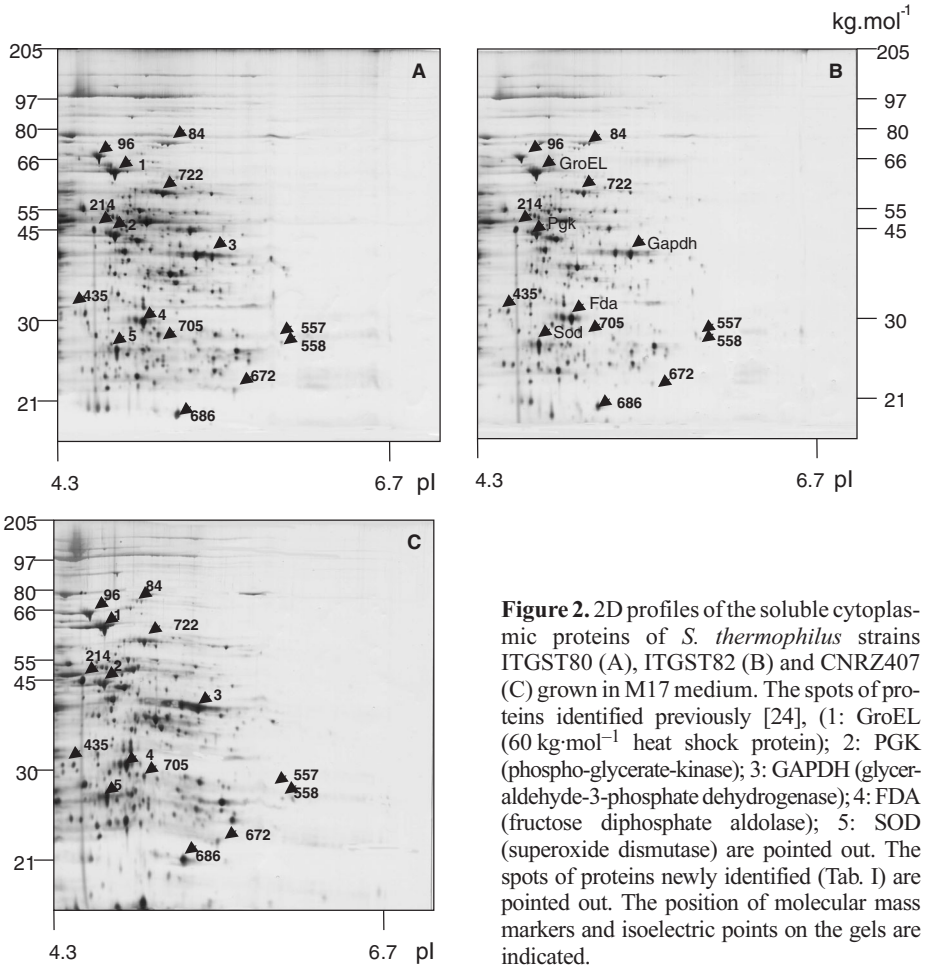

Figure 2. 2D profiles of the soluble cytoplasmic proteins of *S. thermophilus* strains ITGST80 (A), ITGST82 (B) and CNRZ407 (C) grown in M17 medium. The spots of proteins identified previously [24], (1: GroEL (60 kg·mol^{-1} heat shock protein); 2: PGK (phospho-glycerate-kinase); 3: GAPDH (glyceraldehyde-3-phosphate dehydrogenase); 4: FDA (fructose diphosphate aldolase); 5: SOD (superoxide dismutase) are pointed out. The spots of proteins newly identified (Tab. I) are pointed out. The position of molecular mass markers and isoelectric points on the gels are indicated.

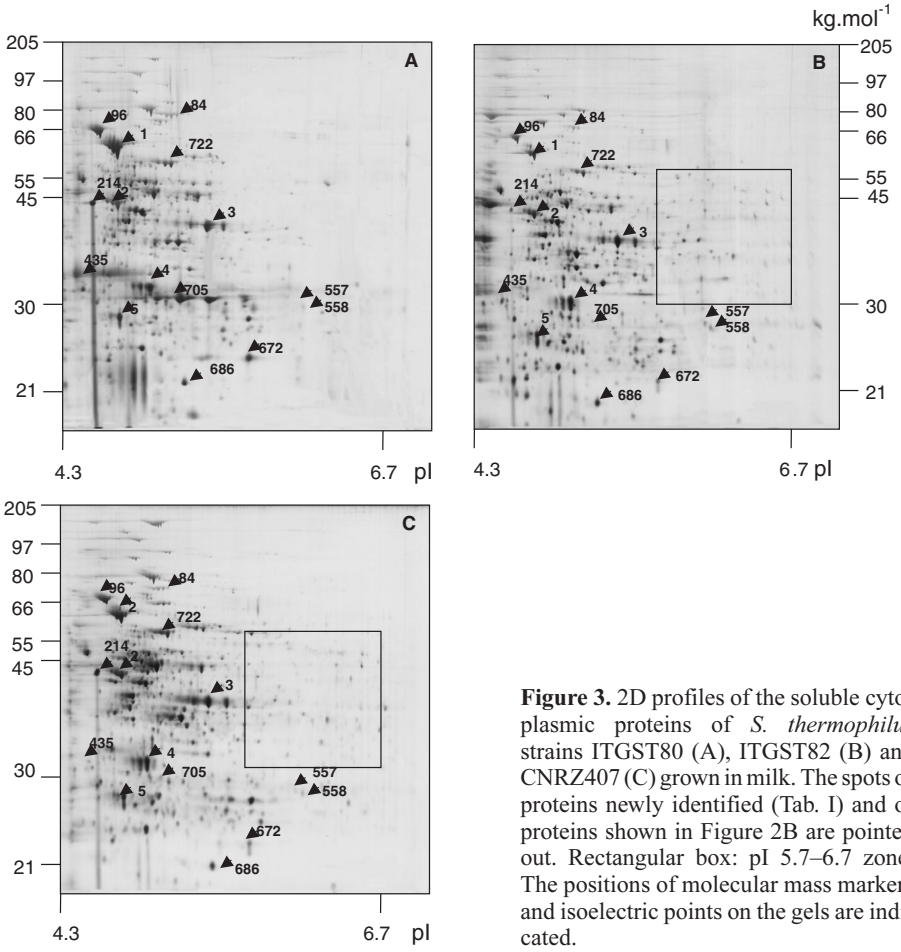

Figure 3. 2D profiles of the soluble cytoplasmic proteins of *S. thermophilus* strains ITGST80 (A), ITGST82 (B) and CNRZ407 (C) grown in milk. The spots of proteins newly identified (Tab. I) and of proteins shown in Figure 2B are pointed out. Rectangular box: pI 5.7–6.7 zone. The positions of molecular mass markers and isoelectric points on the gels are indicated.

phase [19] but also with the patterns of additional milk proteins in *L. delbrueckii* ssp *bulgaricus* cultured in milk [29]. ITGST82 had the largest number of proteins in the pI 5.7–6.7 zone with proteins of molecular mass of 60–30 kg·mol^{-1} (Fig. 3B, rectangular box).

3.3. Identification of protein spots with quantitative variation related to the growth medium

Numerous matching spots exhibited intensity variations (up or down), comparing M17 and milk culture conditions. Fifteen of them were selected for identification (Figs. 2, 3). Among these, 5 have been already identified (Fig. 2B, [25]). Identification was carried out for the others (Tab. I, Fig. 3). Table I lists all the data on the spots as well as data from the N-terminal sequencing, the peptide mass spectrometry analysis and results from protein databases. The N-terminal sequences deprived of Met residue, as previously shown [25], and a minimum of eight matching peptides were used for a protein search. The protein search considered members from all bacterial species and therefore certain proteins were identified from other species. In

Table I. Identification of proteins corresponding to spots with quantitative variation related to growth media (Fig. 3).

N-Terminal sequencing

Spot (match number)	MM/pI exp.[a]	MM/pI calc.[b]	N-terminal sequence	Identity (%)	Species	Accession [c] No	Protein name /function [d]
672	23/5.6	12/4.3	XXFENFLN ANXAYVNLD	69% (9/13)	nitrogen-fixing bacterium	AF099796	Dinitrogenase reductase
686	20/5	17/ 5	XEAIIAKKAEQVAIV	57% (8/14) 50% (7/14)	*S. sanguinis* *Ps. aeruginosa*	AF001955 M33814	Proton translocating ATPase b subunit
705	30/5.2	17/5.3	QMLVQMNXXIFVE	50% (5/10) 61% (8/13)	*L. lactis* subsp. *cremoris* Mycoplasme	AF100298 P47474	Glycosyl-transferase L21- ribosomal protein

Peptide mass finger printing

Spot (match number)	MM/pI exp.[a]	MM/pI calc.[b]	Masses matched fragments	Matched peptides coverage (%)	Species	Accession[c] N°	Protein name /function[d]
96	66/4.8	65/4.6	11/65	19%	*S. pyogenes* *S. pneumoniae*	U72719 U84387	HSP70 HSP70
722	60/5.0	54/4.8	10/58	22%	*S. thermophilus*	AF17217	Pyruvate-kinase
214	45/4.8	29/5.2	06/50	23%	*L. lactis* subsp. *cremoris*	AF157601	Tributyrine-esterase
84	70/5.0	77/9.2	4/7	7%	*S. aureus*	AF098801	PBP/Penicillin binding protein 2

Peptide sequence[e]

84	70/5.0	77/9.2	VNSTYIGYAPIDDPK	100% 15/15	*S. aureus*	AF098801	PBP/Penicillin binding protein 2
435	33/4.8	11/5.0	NHQLTQENERLTQK	78% (11/14)	*S. pyogenes*	L47325	M type 32 precursor
557	29/5.8	ni	ni	ni	ni	ni	ni
558	28/5.8	ni	ni	ni	ni	ni	ni

ni: non identified.

a) Apparent isoelectric point (pI) and molecular mass in $kg \cdot mol^{-1}$(MM) values estimated from 2-DE gels analysis.

b) Molecular mass (MM) and isoelectric point (pI) values calculated using the protein databases.

c) Accession number in the databases.

d) Protein name and function of the putative proteins.

e) Sequence of the major peptide among the few peptides delivered by the protein.

cross-species identification, wherever possible, preference went to lactic acid bacteria or *Streptococci*. For spots No 672, 686 and 705, the N-terminal sequences were identical to the internal sequences of corresponding proteins. As seen in Table I, the function of proteins may be deduced from those of known proteins with which they were partially identified. For reasons of cross-species matching, low percentages of identity and differences between experimental and calculated pI and molecular mass values were observed. In one case (spot No 84), because the percentage of protein coverage was insufficient for a reliable identity assignment, identification was also searched by another protein attribute, such as the sequence of the major peptide among the few peptides (four) delivered by the protein. Identification could be partially assigned to spot No 84. In another case (spot No 435) the same search was applied but theoretical and experimental molecular mass and pI values were not in good agreement and an identification could not really be assigned to spot No 435.

It is interesting to note the presence in Table I of proteins with known functions such as dinitrogenase reductase (DR) (spot No 672), pyruvate kinase (PK) (spot No 722), tributyrine esterase (TE) (spot No 214), glycosyl transferase (GTF) (spot No 705), penicillin binding protein (PBP) (spot No 84), proton translocating ATPase subunit (H^+-ATPase) (spot No 686) and 70 $kg \cdot mol^{-1}$ heat shock protein **(**HSP70) (spot No 96) which all together may play a role in metabolism and stress resistance.

3.4. Differences in the protein levels between medium and strains

The levels of the identified proteins were quantified and the results (as arbitrary units) are given in Figure 4. For the three strains, a small increase in the level of the heat shock proteins (HSPs) HSP70 and 60 $kg \cdot mol^{-1}$ heat shock protein (GroEL) was observed in milk. On the contrary, a decrease of 1.5 to 7 times in the mean level of the enzymes (H^+-ATPase, PK, TE, PK, glyceraldehyde-3-phosphate dehydrogenase [GAPDH], fructose-diphosphate aldolase [FDA], superoxide dismutase [SOD]) and of the putative PBP were observed in milk compared with the M17 medium (Figs. 4A, 4B). Heterogeneity in protein levels was observed between strains and inter-strain variability was seen to be accentuated in milk (Fig. 4B). In milk, ITGST82 under-expressed TE (7 times) and the HSPs (2.8 times) while CNRZ407 over-expressed PK (2 times). ITGST80 over-expressed DR (10 times), GTF (8 times), M type 32 protein precursor [M protein] (> 8 times), GroEL (3 times), and under-expressed the FDA (2 times) and the PBP (2 times). Because of the technology limitations (see Sect. 2.4) some variations seemed to be not significant.

4. DISCUSSION

The lactic acid bacteria used in the dairy industry have various technological properties, particularly an acidifying potential. The ITGST80, ITGST82 and CNRZ407 strains were chosen for their different acidifying activity. The use of culture medium such as M17 enriched in yeast extracts, vitamins, peptones, etc., enables highly demanding lactic acid bacteria such as *S. thermophilus* [10] to grow well. Since lactic acid bacteria produce lactic acid, their growth rate depends on their level of resistance to extracellular pH stress [2, 13, 24] which explains, among other things, their diverse acidification rates. The M17 is strongly buffered (β-glycerophosphate, 19 $g \cdot L^{-1}$) and therefore enables a high biomass. However, this biomass produces much more metabolites, including lactic acid, which leads to acidification of the medium. This explains the option developed here to study the proteome of *S. thermophilus* expressed in its industrial medium, which is milk. Due to

the difficulty of assessing the bacterial growth phase in milk by measuring optical density, the sampling time of cells in media was chosen to be the time where the color of the pH indicator changed (5.2 ± 0.1). This pH corresponds to the end of the exponential growth phase of the strains studied (data not shown).

The number of spots detected was higher (576 ± 33) for the cells grown in milk than for the cells grown in M17 (505 ± 40) (Figs. 2, 3). Interestingly, this increase was due to the presence of proteins in the pI range 5.7–6.7. This highlights the importance of the milk environment in the expression of more basic proteins [26] leading to

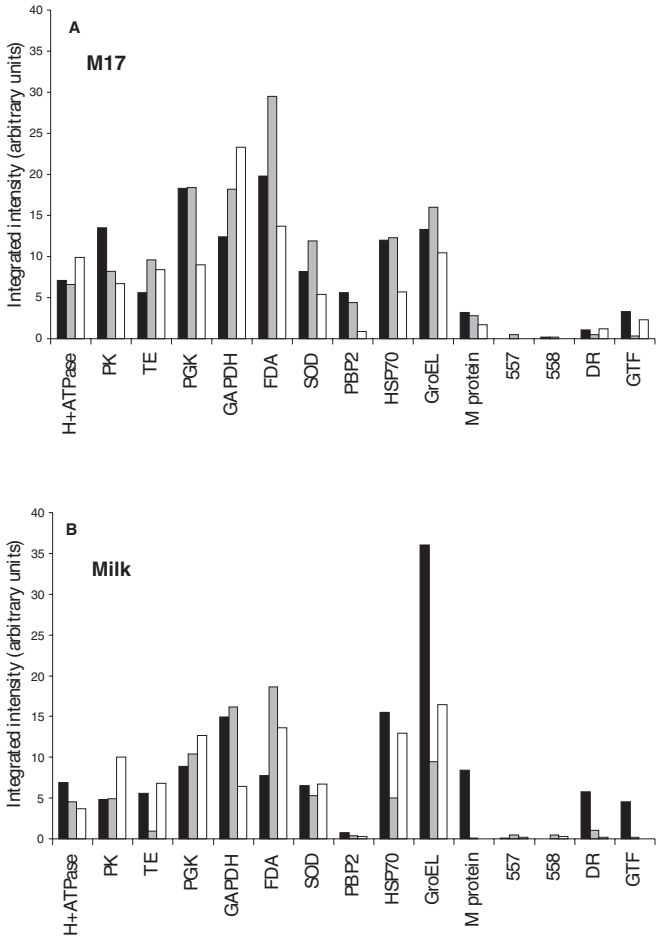

Figure 4. Differences in the levels of identified proteins of *S. thermophilus* strains ITGST80 (■), ITGST82 (▩), CNRZ407 (□) grown either in M17 (A) or in milk (B). H^+-ATPase (proton translocating ATPase), PK (pyruvate kinase), TE (tributyrin esterase), PGK (phospho-glycerate kinase), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), FDA (fructose-diphosphate-aldolase), SOD (superoxide dismutase), PBP (penicillin binding protein), HSP70 (70 kg·mol^{-1} heat shock protein), GroEL (60 kg·mol^{-1} heat shock protein), M protein (M type 32 precursor protein), 557 (spot No 557), 558 (spot No 558), DR (dinitrogenase reductase), GTF (glycosyl transferase).

the knowledge of a greater number of *S. thermophilus* proteins whose genome codes for about 1500 proteins [22]. For example, 41% of the 1500 proteins were detected with ITGST82 in milk. Proteins seemed to be resolved better into discrete spots in milk (Fig. 3) than in M17 conditions (Fig. 2) and this could be related to the non-denaturated state of these proteins. One ambiguity remains, however, about the pattern of ITGST80 cultured in milk (Fig. 3A) because a large smear in the 21 $kg \cdot mol^{-1}$ zone could be explained by additional milk proteins, as described by Rechinger et al. [29].

Elucidating the state of *S. thermophilus* in milk requires knowledge of the function and regulation of expression of proteins. Some proteins exhibiting level differences in milk culture conditions were partially identified (identity below 60%). The expression of the HSPs (GroEL, HSP70) is a characteristic marker of lactic acid bacteria to conditions of temperature [3, 28, 35] and acid stress [23, 24]. In milk, ITGST82 under-expressed GroEL and HSP70 whereas CNRZ407 and ITGST80 over-expressed them. In addition, GroEL was highly expressed whereas HSP70 was expressed at a lower level in milk. This is in agreement with the differential induction of HSPs by *Lactobacillus delbrueckii* ssp. *bulgaricus* under stress conditions [23]. The level of H^+-ATPase characteristic of acid stress response [21] was lower in milk but it was always greater for ITGST80 which already over-expressed GroEL.

Unlike ITGST82, ITGST80, which produced exopolysaccharides (Guimont, data not shown), had a high level of GTF both in M17 and in milk (Fig. 4). Stingele et al. [31], Cerning and Marschall [6] and Van Kranenburg et al. [33] have shown that the production of exopolysaccharides by *S. thermophilus* and the lactic acid bacteria is dependent on the genes of the *eps* locus coding for proteins similar to GTF. Bourgouin et al. [4] have shown the variability of the regions of the *eps* locus depending on the *S. thermophilus* strains and Faber et al. [16], De Vuyst et al. [11], Degeest and De Vuyst [12] and Petry et al. [27] have shown the influence of culture conditions on the quantity and size of exopolysaccharides produced by lactic acid bacteria. For ITGST80, the PBP putative protein, whose gene is connected to exopolysaccharide production [31], was expressed 7 times lower in milk than in M17 (Fig. 4).

Interestingly, ITGST80 overexpressed a putative dinitrogenase reductase in milk. This could be related to the active role of this type of metalloprotein in the redox potential since DR are regulated by redox conformational changes [21]. In a complex medium such as milk, numerous redox systems are present and oxydo-reduction is known to potentially modify the development growth capacity of cells [9].

TE has already been highlighted in other lactic acid bacteria (*Lactobacillus helveticus*, *Lactococcus lactis*) where it seems to be involved in the phospholipid metabolism or in vivo cell detoxification, or both [17]. For the first time, the variation in the level of this enzyme was evidenced depending on the medium and on the strains (level 7-times lower for ITGST82 in milk). Finally, protein No 435 seemed a little homologous to a precursor of the M protein precursor of *Streptococcus pyogenes*. This protein is in part homologous to the envelope proteinase (PrtS) of *S. thermophilus* [18] and also binds milk plasminogen and plasmin [20, 30].

5. CONCLUSION

This work documents that proteomics can provide new information comparing the proteins associated with growth of different strains of *S. thermophilus* either in M17 medium or in milk. However, some minor differences in the intensity and

partial identification of these proteins were limiting.

Overall these data indicate that strain-related differences were accentuated in milk. Reliable indicators (basic proteins, HSPs, DR, TE) seem to show an unstressed strain (ITGST82) and a stressed strain (ITGST80) adapted or not to dairy conditions.

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REFERENCES

[1] Accolas J.P., Veaux M., Vassal L., Mocquot G., Évolution de la flore lactique thermophile au cours du pressage des fromages à pâte cuite, Lait 58 (1978) 118–132.

[2] Alemayehu D., O'Sullivan E., Condon S., Changes in acid tolerance of *Lactococcus lactis* during growth at constant pH, Int. J. Food Microbiol. 10 (2000) 215–221.

[3] Auffray Y., Lecesne E., Hartke A., Boutibonnes P., Basic features of the *Streptococcus thermophilus* heat shock response, Curr. Microbiol. 30 (1995) 87–91.

[4] Bourgoin F., Pluvinet A., Gintz B., Decaris B., Guedon G., Are horizontal transfers involved in the evolution of the *Streptococcus thermophilus* exopolysaccharide synthesis loci, Gene 233 (1999) 151–161.

[5] Bradford M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding, Anal. Biochem. 72 (1976) 248–254.

[6] Cerning J., Marshall V.M., Exopolysaccharides produced by the dairy lactic acid bacteria, Recent Res. Dev. Microbiol. 3 (1999) 195–209.

[7] Chamba J.F., Prost F., Mesure de l'activité acidifiante des bactéries lactiques thermophiles utilisées pour la fabrication des fromages à pâte cuite, Lait 69 (1989) 417–431.

[8] Chopard M.A, Schmitt M., Perreard E., Chamba J.F., Aspect qualitatif de l'activité protéolytique des lactobacilles thermophiles utilisés en fabrication de fromage à pâte pressée cuite, Lait 81 (2001) 183–194.

[9] Dave R., Shah N., Effect of cysteine on the viability of yogurt and probiotic bacteria in yogurts made with commercial starter cultures, Int. Dairy J. 7 (1997) 537–545.

[10] De Roissart H., Luquet F.M., Bactéries Lactiques, Lorica, Uriage, France 1994.

[11] De Vuyst L., Vanderveken F., Van de Ven S., Degeest B., Production and isolation of exopolysaccharides from *Streptococccus thermophilus* grown in a milk medium and evidence for their growth-associated biosynthesis, J. Appl. Microbiol. 84 (1998) 1059–1068.

[12] Degeest B., De Vuyst L., Indication that the nitrogen souce influences both amount and size of exopolysaccharides produced by *Streptococcus thermophilus* LY03 and modelling of the bacterial growth and exopolysaccharide production in a complex medium, Appl. Environ. Microbiol. 65 (1999) 2863–2870.

[13] Duwat P., Ehrlich S.D., Gruss A., Effects of metabolic flux on stress response patways in *Lactococcus lactis*, Mol. Microbiol. 31 (1999) 845–858.

[14] Exterkate F.A., A dual directed control of cell wall proteinase in *Streptococcus cremoris* AM1: a possible mechanism of regulation during growth in milk, J. Dairy Sci. 68 (1985) 562–571.

[15] Ezzat N., El Soda M., Desmazeaud M.J., Ismail A., Peptide hydrolases from the *Thermobacterium* group of lactobacilli. II. Physiological factors and enzyme production, Milchwissenschaft 37 (1982) 666–668.

[16] Faber E.J., Kamerling J.P., Vliegentharet F.F., The exopolysaccharides produced by *Streptococcus thermophilus* Rs and Sts have the same repeating unit but differ in viscosity of their milk cultures, Carbohydr. Res. 310 (1998) 269–276.

[17] Fernandez L., Beerthuyzen M.M., Brown J., Siezen R.J., Coolbear T., Holland R., Kuipers O.P., Cloning, characterization, controlled overexpression and inactivation of the major tributyrin esterase gene of *Lactococcus lactis,* Appl. Environ. Microbiol. 66 (2000) 1360–1368.

[18] Fernandez-Espla M.D., Garault P., Monnet V., Rul F., *Streptococcus thermophilus* cell wall-anchored proteinase: release, purification and biochemical and genetic characterization, Appl. Environ. Microbiol. 66 (2000) 4772–4778.

[19] Gonzalez-Marquez H., Perrin C., Bracquart P., Guimont C., Linden G., A 16 kDa protein family overexpressed by *Streptococcus thermophilus* PB18 in acids environments, Microbiology 143 (1997) 1587–1592.

[20] Grufferty M.B., Fox P.F., Milk alkaline proteinase, J. Dairy Res. 55 (1988) 609–630.

[21] Halbleib C.M., Zhang Y., Ludden P.W., Regulation of dinitrogenase reductase ADP-ribosyltransferase and dinitrogenase reductase-activating glycohydrolase by a redox-dependent

conformational change of nitrogenase Fe protein, J. Biol. Chem. 275 (2000) 3493–3500.

[22] Le Bourgeois P., Mata M., Ritzenthaler P., Genome comparison of *Lactococcus* strains by pulse field gel electrophoresis, FEMS Microbiol. Lett. 50 (1989) 65–69.

[23] Lim E.M., Ehrlich S.D., Maguin E., Identification of stress inducible proteins in *Lactobacillus delbrueckii* ssp. *bulgaricus,* Electrophoresis 21 (2000) 2557–2561.

[24] O'Sullivan E., Condon S., Intracellular pH is a major factor in the induction of tolerance to acid and other stresses in *Lactococcus lactis,* Appl. Environ. Microbiol. 63 (1997) 4210–4215.

[25] Perrin C., Gonzalez-Marquez H., Gaillard J.L., Bracquart P., Guimont C., Reference map of soluble proteins from *Streptococcus thermophilus* by two-dimensional electrophoresis, Electrophoresis 21 (2000) 949–955.

[26] Perrin C., Poirson C., Bracquart P., Gaillard J.L., Guimont C., Établissement de l'empreinte protéique de base de *Streptococcus thermophilus* par 2D-PAGE, Sci. Aliments 20 (2000) 97–104.

[27] Petry S., Furlan S., Crepeau M.J., Cerning J., Desmazeaud M., Factors affecting exocellular polysaccharide production by *Lactobacillus delbrueckii* ssp. *bulgaricus* grown in a chemically defined medium, Appl. Environ. Microbiol. 66 (2000) 3427–3431.

[28] Rallu F., Gruss A., Ehrlich D., Maguin E., Acid– and mutistress-resistant mutants of *Lactococcus lactis*: identification of intracellular stress signals, Mol. Microbiol. 35 (2000) 517–528.

[29] Rechninger K.B., Siegumfeldt H., Svendsen I., Jakobsen M., Early protein synthesis of *Lactobacillus delbrueckii* ssp *bulgaricus* in milk revealed by ^{35}S methionine labeling and two-dimensional gel electrophoresis, Electrophoresis 21 (2000) 2660–2669.

[30] Richardson B.C., Elston P.D., Plasmin activity in commercial caseins and caseinates, J. Dairy Sci. Technol. 19 (1984) 63–66.

[31] Stingele F., Mollet B., Disruption of the gene encoding penicillin-binding protein 2b (pbp2b) causes altered cell morphology and cease in exopolysaccharide production in *Streptococcus thermophilus* Sfi6, Mol. Microbiol. 22 (1996) 357–366.

[32] Tailliez P., Quénée P., Chopin A., Estimation de la diversité parmi les souches de la collection des lactobacilles, Lait 76 (1996) 147–158.

[33] Van Kranenburg R., Vos H.R., Van Swam I.I., Kleerebezem M., De Vos W.M., Functional analysis of glycosyltransferase genes from *Lactococcus lactis* and other gram-positive cocci: complementation, expression and diversity, J. Bacteriol. 181 (1999) 6347–6353.

[34] Wilkins M.R., Sanchez J.C., Gooley A., Appel R.D., Humphrey Smith I., Hochstrasser D.F., Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it, Biotechnol. Gen. Eng. Rev. 13 (1995) 19–50.

[35] Zink R., Walker C., Schmidt G., Elli M., Pridmore D., Reniero R., Impact of multiple stress factors on the survival of dairy lactobacilli, Sci. Aliments 20 (2000) 119–126.