ORIGINAL ARTICLE

Sensitivity of capsular-producing *Streptococcus thermophilus* strains to bacteriophage adsorption

C. Rodríguez[1], R. Van der Meulen[2], F. Vaningelgem[2], G. Font de Valdez[1], R. Raya[1], L. De Vuyst[2] and F. Mozzi[1,2]

1 Centro de Referencia para Lactobacilos (CERELA)-CONICET, San Miguel de Tucumán, Argentina
2 Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Department of Applied Biological Sciences and Engineering, Vrije Universiteit Brussel, Brussels, Belgium

Keywords
bacteriophages, capsular polysaccharides, exopolysaccharides, lactic acid bacteria.

Correspondence
F. Mozzi, CERELA. Chacabuco 145, 4000 San Miguel de Tucumán, Argentina.
E-mail: fmozzi@cerela.org.ar

2007/0868: received 4 June 2007, revised 28 November 2007 and accepted 8 January 2008

doi:10.1111/j.1472-765X.2008.02341.x

Abstract

Aims: To determine whether the presence and type of exopolysaccharides (EPS), slime-EPS or capsular, and the structural characteristics of the polymers produced by *Streptococcus thermophilus* strains could interfere with or be involved in phage adsorption.

Methods and Results: Phage–host interactions between eight EPS-producing *Strep. thermophilus* strains (CRL419, 638, 804, 810, 815, 817, 821, 1190) and five streptococcus specific phages (ϕYsca, ϕ3, ϕ5, ϕ6, ϕ8) isolated from Argentinean faulty fermentation failed yoghurts were evaluated. No relationship was found between the EPS chemical composition and the phage sensitivity/resistance phenotype. In general, the capsular-producing strains were more sensitive to phage attacks than the noncapsular-producing strains. *Streptococcus thermophilus* CRL1190 (capsular-producing) was the only strain sensitive to all bacteriophages and showed the highest efficiency of plating. Phage adsorption to a capsular-negative, EPS low-producing mutant of strain CRL1190 was reduced, especially for ϕYcsa and ϕ8.

Conclusions: The presence of capsular polysaccharide surrounding the cells of *Strep. thermophilus* strains could play a role in the adsorption of specific phages to the cells.

Significance and Impact of the Study: Capsular-producing *Strep. thermophilus* strains should be evaluated for their bacteriophage sensitivity if they are included in starter cultures for the fermented food industry.

Introduction

Streptococcus thermophilus is commonly used in the manufacture of yoghurt and some cheeses, playing an essential role in the organoleptic properties of these fermented dairy foods (Hassan and Frank 2001). Certain *Strep. thermophilus* strains produce exopolysaccharides (EPS), both capsular polysaccharides (CPS) associated with the cell surface and slime-EPS secreted into the cellular environment (Vaningelgem *et al.* 2004; Mozzi *et al.* 2006). Most lactic acid bacteria (LAB) synthesize heteropolysaccharides (HePS) composed of repeating units of galactose, glucose and/or rhamnose. A large biodiversity of HePS with respect to monomer composition and ratio, structure, molecular mass and yield has been observed for *Strep. thermophilus* strains (Vaningelgem *et al.* 2004; Mozzi *et al.* 2006). When produced *in situ,* these EPS decrease the syneresis, increase the viscosity and improve the texture of dairy products (Duboc and Mollet 2001). Concerning their physiological role, EPS from LAB have been claimed to protect the cells from detrimental environmental conditions, such as dehydration, macrophages, antibiotics or bacteriophages, to sequester essential cations and to be involved in adhesion and biofilm formation (Looijesteijn *et al.* 2001).

Lytic phages are the most significant cause of fermentation failures in the dairy industry worldwide (Moineau *et al.* 1996). Phage contamination can dramatically affect

the acidifying and enzymatic capacity of LAB and may result in the lysis of the starter strains in the fermentation vat (Suárez *et al.* 2002). The first step in the bacteriophage lytic cycle is a highly specific interaction between phage proteins located at the tip of the phage tail and specific binding sites (receptors) on the host cell wall (Lindberg 1973). In Gram-positive bacteria, phage receptors are usually cell-surface carbohydrates such as galactose and rhamnose (Forde and Fitzgerald 1999; Quiberoni *et al.* 2004). Controversial reports exist regarding the involvement of EPS in bacteriophage protection. It has been suggested that EPS from LAB could mask the phage receptors and hence protect LAB against bacteriophage attacks (Forde and Fitzgerald 1999; Looijesteijn *et al.* 2001; Deveau *et al.* 2002). Nevertheless, the presence of EPS does not seem to exert such protection, as Brüssow *et al.* (1994) isolated phages from yoghurt factories active on ropy strains of *Strep. thermophilus.* Other authors (Forde and Fitzgerald 1999; Looijesteijn *et al.* 2001; Deveau *et al.* 2002; Lévesque *et al.* 2005) described several phages infecting the EPS-producing strain *Strep. thermophilus* RD534 and EPS-producing lactoccocal strains. The involvement of CPS on phage attack, however, is limited to a few reports (Broadbent *et al.* 2003).

The aim of this study was to determine whether the presence and type of HePS (slime-EPS or CPS) produced by *Strep. thermophilus* strains as well as the polymers structural characteristics could interfere with or be involved in specific bacteriophage adsorption.

Materials and methods

Bacterial strains, bacteriophages, media and culture conditions

Streptococcus thermophilus strains, ability for ropiness and yield and structural characteristics (chemical composition and molecular mass) of their EPS used in this study (Table 1) have been determined previously (Mozzi *et al.* 2006). The strains were obtained from the CERELA Culture Collection (Tucumán, Argentina) and were subcultured at 37°C twice in LAPTg broth [(peptone, 1·5%; tryptone, 1·0%; yeast extract, 1·0%; glucose, 1·0%) (w/v); and Tween 80, 0·1% (v/v)] before experimental use.

The bacteriophages ϕYcsa, ϕ5, ϕ6 and ϕ8, propagated on *Strep. thermophilus* Cc1, and the bacteriophage ϕ3, propagated on *Strep. thermophilus* Bcch2, were isolated from Argentinean faulty yoghurts and belong to the *pac* type phages (Suárez *et al.* 2002; Quiberoni *et al.* 2003), except for ϕYcsa, which has not been characterized yet. All phages were kindly provided by Dr J. Reinheimer (PROLAIN, Santa Fe, Argentina). Cells of *Strep. thermophilus* at an optical density at 600 nm (OD_{600}) = 0·05–0·08 were infected with a phage (2%, v/v) and incubated at 37°C for 4–6 h until lysis or a decrease of the OD_{600} was observed. Phage titration was performed as described by Quiberoni *et al.* (2003) and lysis plaques were expressed as plaque forming units (PFU) ml^{-1}.

Table 1 *Streptococcus thermophilus* strains used in this study

Streptococcus thermophilus strain	R*	CPS	MM†	Monomer ratio (on a molar basis)‡ Glc	Gal	Rha	GlcN	GalN
CRL419	–	–	Low	2·0	1·0	–	1·0	–
CRL638	+	–	High	1·0	2·0	–	–	2·0
CRL804	–	–	Low	–	2·5	1·0	–	–
CRL810	–	–	Low	1·0	2·5	–	1·0	2·0
CRL815	–	+	Low	–	4·0	1·0	1·0	1·0
CRL817	–	–	Low	1·0	2·0	–	1·0	1·5
CRL821	–	+	Low	2·5	2·5	–	1·0	1·5
CRL1190	+	+	High	1·0	1·5	–	–	–
M16	–	–	High	1·0	1·5	–	–	–

*Ropiness.
†MM: molecular mass; high- and low-MM: arbitrarily defined as $>1 \times 10^6$ Da and $<1 \times 10^6$ Da respectively.
‡Glc, glucose; Gal, galactose; Rha, rhamnose, GlcN, glucosamine; GalN, galactosamine (Mozzi *et al.* 2006).

Sensitivity of CPS/EPS-producing *Strep. thermophilus* strains to phages

The sensitivity of the CPS/EPS-producing *Strep. thermophilus* strains to bacteriophages was evaluated by the spot-test. Briefly, 200 μl of a log phase culture (OD_{600} = 0·3) in LAPTg medium were mixed with 3 ml LAPTg-Ca (LAPTg plus 10 mmol l^{-1} $CaCl_2$) soft agar (0·65% w/v agar) and poured as a thin-top layer on LAPTg-Ca agar (1·5% w/v agar). Five microliter of appropriate 10-fold dilutions of each phage suspension were spotted on the cell lawn. Lysis zones or plaques were observed after overnight incubation of the plates at 37°C. The efficiency of plating (EOP) for each phage on the different sensitive *Strep. thermophilus* strains was obtained by dividing each phage titre, determined by the standard double-layer technique, by the highest titre obtained. Assays were performed in triplicate and mean values with standard deviations are shown.

Isolation and characterization of a noncapsular (CPS^-) and low EPS-producing mutant strain

Chemical mutagenesis of *Strep. thermophilus* CRL1190 was performed as described previously (Mozzi *et al.* 2001a). Briefly, *Strep. thermophilus* CRL1190 (CPS^+/EPS^+) was grown in LAPTg broth at 37°C until an OD_{600} = 0·7, harvested by centrifugation (10 000 ***g*** for 10 min),

resuspended in LAPTg broth containing nitrosoguanidine (400 μg ml^{-1}) and caffeine (1·0 g l^{-1}), and incubated for two generation times. Diluted samples were spread on LAPTg agar (1·5% w/v) and incubated at 37°C for 48 h. Individual colonies were screened for ropiness and CPS formation by using the Indian Ink negative staining technique described by Mozzi *et al.* (2001b) and further checked grown in milk and LAPTg broth. EPS production was evaluated in milk cultures according to Mozzi *et al.* (2006) and expressed as milligram of polymer dry mass per liter. The EPS monomer composition and molecular mass determinations were performed as described earlier (Mozzi *et al.* 2006). The maximum specific growth rate (μ_{max}) of a mutant (named M16) and parental strains was determined by following the OD_{600} in LAPTg broth at 37°C. RAPD-PCR assays using the primer pairs RAPD2 (sequence 5′-3′AGC AGC GTC G) and M13 (sequence 5′-3′ GAG GGT GGC GGT TCT), singly employed as described by Fontana *et al.* (2005), were used to confirm that strain M16 was derived from strain CRL1190. RAPD products were electrophoresed at 80 V on 2·0% (w/v) agarose gel and stained with ethidium bromide. For comparison, all *S. thermophilus* strains used in this study were included. Chromosomal DNA isolation was performed according to Pospiech and Neumann (1995).

The sensitivity of the mutant strain M16 to phages was determined as described above.

Phage adsorption

Phage adsorption assays were conducted with cells of *Strep. thermophilus* CRL1190 and the mutant M16 grown in LAPTg-Ca (OD_{600} = 0·3) at 37°C. Cells (500 μl) were incubated with each phage at 37°C for 15 min, samples were then centrifuged (8000 ***g*** for 5 min), and the phage concentration in the supernatant was titrated using the appropriate sensitive host strain. Assays were performed in triplicate and mean values of the percentage of phage adsorption on strain M16/phage adsorption on strain CRL1190 with standard deviations are shown.

Results

Sensitivity of CPS/EPS-producing *Strep. thermophilus* strains to phages

The host range of five lytic streptococcus-specific phages was determined on eight EPS-producing *Strep. thermophilus* strains (Table 2). Most of these strains produced EPS in amounts lower than 100 mg l^{-1} (Mozzi *et al.* 2006) and showed a varied ability to form CPS or ropy cultures; *Strep. thermophilus* CRL1190 displayed both properties (Table 1). No correlation between the EPS monomer composition and the phage sensitivity/resistance phenotype of the strains was observed. *Streptococcus thermophilus* CRL1190 was the only strain sensitive to the five phages used and showed the highest titer (2·7–4·0 × 10^{10} PFU ml^{-1}) for all of them. The EOP values of the phages on the remaining strains were 10^{1}- to 10^{3}-fold lower when compared with *Strep. thermophilus* CRL1190. Furthermore, the strains *Strep. thermophilus* CRL638 and CRL815 were only resistant to phages ϕ3 and ϕYcsa, respectively, while *Strep. thermophilus* CRL419 and CRL821 showed resistance to both phages ϕ3 and ϕYcsa. In contrast, three CPS^{-} strains, *Strep. thermophilus* CRL804, CRL810 and CRL817, were resistant to all phages (Table 2).

Phage infections experiments were performed on early exponential cells. At this growth stage, the CPS^{+} strains showed evidence of CPS formation, i.e. a clear capsule

Table 2 Sensitivity of CPS^{+} and CPS^{-} EPS-producing *Streptococcus thermophilus* strains to specific phages

Strep. thermophilus strain	CPS	ϕYcsa	ϕ3	ϕ5	ϕ6	ϕ8
CRL419	–	–	–*	+(6·3 × 10^{-1})† ± 0·30	+(2·6 × 10^{-1}) ± 0·12	+(9·0 × 10^{-2}) ± 0·05
CRL638	–	+(3·9 × 10^{-1}) ± 0·18	–	+(1·5 × 10^{-1}) ± 0·08	+(3·6 × 10^{-1}) ± 0·19	+(3·0 × 10^{-1}) ±0·15
CRL804	–	–	–	–	–	–
CRL810	–	–	–	–	–	–
CRL815	+	–	+(2·4 × 10^{-1}) ± 0·12	+(2·0 × 10^{-3}) ± 0·002	+(1·9 × 10^{-1}) ± 0·13	+(3·5 × 10^{-1}) ± 0·21
CRL817	–	–	–	–	–	–
CRL821	+	–	–	+(4·1 × 10^{-1}) ± 0·21	+(4·0 × 10^{-1}) ± 0·18	+(5·3 × 10^{-1}) ± 0·25
CRL1190	+	+(1·0)	+(1·0)	+(1·0)	+(1·0)	+(1·0)
M16	–	+(1·1 × 10^{-3}) ± 0·001	ND‡	+(8·4 × 10^{-4}) ± 0·0003	+(5·7 × 10^{-4}) ± 0·0003	+(1·7 × 10^{-3}) ± 0·001

*Negative means EOP values <3 × 10^{-9}.
†EOP values, mean of three independent experiments.
‡ND, not determined.
Values are mean ± SD.

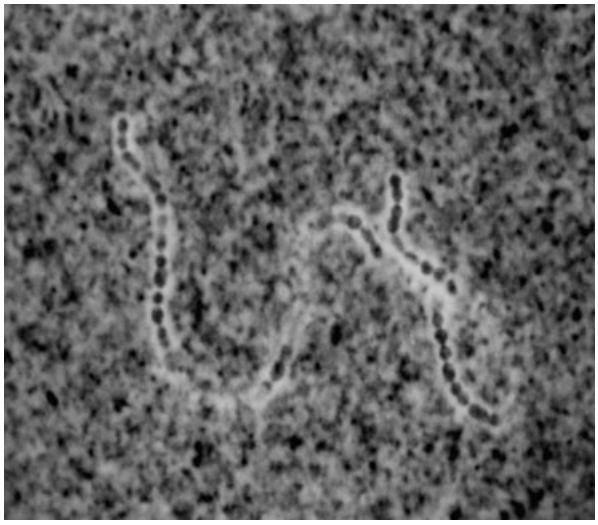

Figure 1 Capsular polysaccharide formation by early exponential cells of *Streptococcus thermophilus* CRL1190 grown in LAPTg medium at 37°C, as revealed by the Indian Ink negative staining technique (Mozzi *et al.* 2001b). Magnification used: 1000×.

became visible after 3 h of incubation for *Strep. thermophilus* CRL1190 (Fig. 1), while no synthesis of slime-EPS was detected yet.

Host range on a CPS$^-$ mutant strain

Isolation of a CPS$^-$ mutant using the insertion-inactivation technique as described by O' Sullivan and Fitzgerald (1999) failed as *Strep. thermophilus* CRL1190 cells were not amenable to electroporation. An isogenic CPS$^-$/EPS$^+$ mutant strain M16 producing lower EPS amount than the parental strain, was obtained from *Strep. thermophilus* CRL1190 by chemical mutagenesis (mutation frequency: $3{\cdot}6 \times 10^{-4}$). The identical RAPD-PCR profiles obtained for the strains M16 and CRL 1190 (Fig. 2) using the primers RAPD2 and M13 indicated that the mutant M16 was a derivative of CRL1190. The mutant M16 produced 60% less EPS than the parental strain but showed a similar maximal growth rate (μ_{max} = 0·81 h^{-1}) and same final pH in milk fermentations (data not shown). The EPS from the strain M16 showed the same monomer composition and similar molecular mass to the EPS produced by the parental strain (Table 1).

Strains CRL1190 and M16 were submitted to phage infection under similar growth conditions and multiplicity of infection (MOI: 1–4). The strain M16 showed the same host range with EOP values ($1{\cdot}1 \times 10^{-3}$ to $8{\cdot}4 \times 10^{-4}$) lower than those on the parental strain (Table 2).

Phage adsorption

Phage adsorption assays with the wild type and mutant strains were performed to determine whether the presence of the capsule in the parental strain could be involved in phage adsorption. In general, the percentage adsorption values were lower for the mutant strain (Fig. 3); i.e., adsorption of ϕYcsa and ϕ8 decreased 75 and 67%, respectively.

Discussion

The physiological role of EPS for LAB is still unclear. It is assumed that these biopolymers have some kind of biological function but are not essential for bacterial growth; as they are not used as energy reserve, enzymatic and

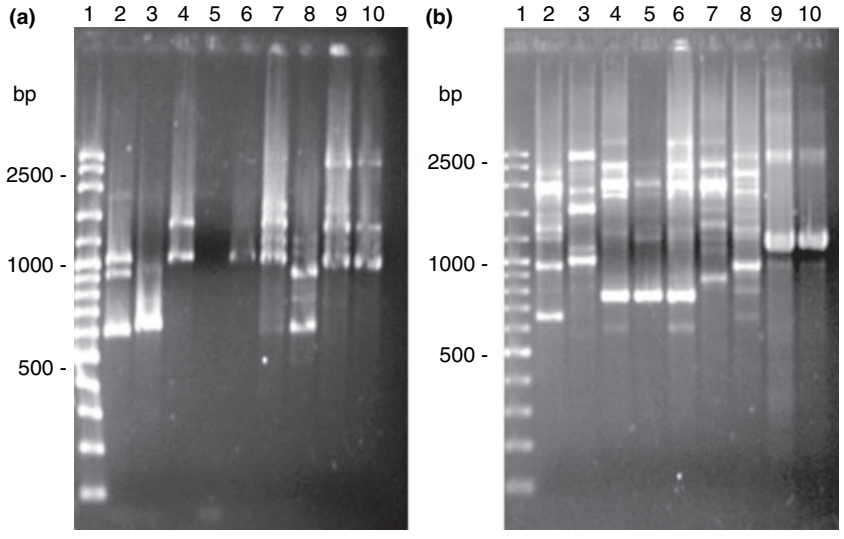

Figure 2 Comparison of RAPD profiles with primers RAPD2 (a) and M13 (b) from purified DNA of the *Streptococcus thermophilus* strains: lane 1, 100 bp molecular weight ladder; lane 2, CRL638; lane 3, CRL419; lane 4, CRL810; lane 5, CRL817; lane 6, CRL821; lane 7, CRL804; lane 8, CRL815; lane 9, CRL1190 and lane 10, M16.

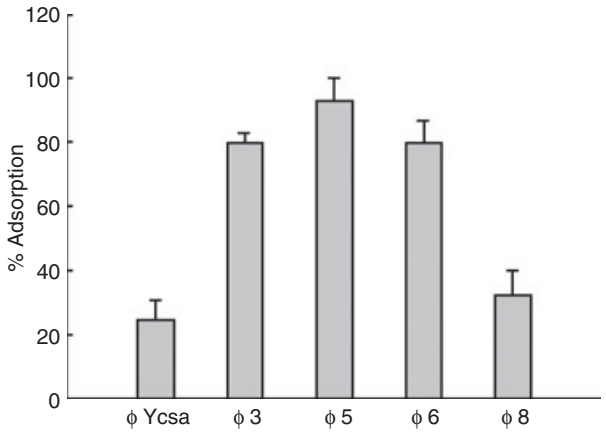

Figure 3 Percentage of relative phage adsorption onto *Streptococcus thermophilus* M16 (CPS^-) and *Strep. thermophilus* CRL1190 (CPS^+) cells calculated as PFU ml^{-1} on strain M16 × 100/PFU ml^{-1} on strain CRL 1190.

physical removal of EPS does not negatively affect cell growth and mutants unable to produce EPS appear spontaneously.

Among the different attributes assigned to these biopolymers, it has been claimed that EPS could protect the cells from bacteriophage attack by acting as a physical barrier probably masking the cell receptors (Forde and Fitzgerald 1999). Looijesteijn *et al.* (2001) showed that cell-associated EPS protected *Lactococcus lactis* strains against bacteriophages, while addition of loose EPS to a nonEPS-producing strain did not confer any phage adsorption resistance. In our study, five of eight slime-EPS-producing strains were sensitive to phage attack. Although four different host range profiles were observed (Table 2), no relationship between the HePS chemical composition from the *Strep. thermophilus* strains tested and the phage sensitivity/resistance phenotypes was found. Phages that attacked *Strep. thermophilus* CRL821 were not active against *Strep. thermophilus* CRL810, although both strains produced HePS with a similar chemical composition. The presence of either rhamnose or galactose had no significant effect on phage adsorption. *Streptococcus thermophilus* CRL804, for instance, insensitive to all phages, produced a HePS composed of rhamnose and galactose, while *Strep. thermophilus* CRL815, which showed a HePS containing rhamnose and galactose in addition to galactosamine and glucosamine, was sensitive to four bacteriophages (Tables 1 and 2). Moreover, galactose, present in all HePS from the *Strep. thermophilus* strains assayed, was not sufficient for phage adsorption, as revealed by the insensitivity to phage adsorption of the strains *Strep. thermophilus* CRL804, CRL810 and CRL817. Also, the sugar composition of the HePS had no effect on phage sensitivity of *L. lactis* strains (Deveau *et al.* 2002). It is interesting to highlight, however, that the EPS from *Strep. thermophilus* CRL1190, the most sensitive strain to phage attack, was composed of galactose and glucose as the EPS from strain *Strep. thermophilus* RD534 that could be infected by 12 different phages (Lévesque *et al.* 2005).

Broadbent *et al.* (2003) evaluated the sensitivity of a CPS-producing *Strep. thermophilus* strain and its CPS-negative derivative towards three specific phages and found no relationship between CPS and phage attack. However, in Gram-negative bacteria, slime-EPS and/or CPS may be directly involved in phage–host interactions. In *Vibrio cholerae*, phages that specifically lyse CPS^+ strains have been isolated (Albert *et al.* 1996).

In our work, although both CPS^+ and CPS^- *Strep. thermophilus* strains tested were sensitive to phage infection (i.e. strains CRL1190 and CRL638) all CPS^+ *Strep. thermophilus* strains were sensitive to phage infection with EOP values similar or higher than those of CPS^- strains. The phage infection assays were performed on early exponential cells, where production of CPS but not of slime-EPS was observed; at this growth stage slime-EPS could not be a relevant physical barrier for phages but instead the CPS might interact with the phage. Kinetics studies on EPS production by *Strep. thermophilus* CRL1190 showed that slime-EPS synthesis starts after 8 h of incubation (data not shown). Similar results were reported by Degeest and De Vuyst (1999) for the strain *Strep. thermophilus* LY03.

Phage adsorption assays with *Strep. thermophilus* CRL1190 (CPS^+) and its derivative strain M16 (CPS^-) demonstrated that phage infection, mainly of ϕYcsa and ϕ8, was more effective in the presence of CPS. The mutant M16 was confirmed to be a derivative from the CRL1190 strain by SDS-PAGE analysis of the total cell protein profile (supplemental data) and by RAPD-PCR fingerprints profiles using primers M13 and RAPD2, previously applied to differentiate strains within other LAB species (Fontana *et al.* 2005). The mutant M16 showed similar growth behaviour, EPS and host range than the parental strain, but the EOP determined for each phage showed a 10^3–10^4-fold decrease when compared with the parental strain. The similar EPS chemical composition and molecular mass found for the mutant strain indicated that these characteristics had no influence on phage adsorption. The reduced adsorption values observed for all phages could explain the decrease in the EOP values. Also, the presence of multiple mutations in M16 could have affected, besides the phage adsorption event, other steps of phage multiplication. It is possible that the reduced ability of phages to adsorb to the cell surface may be simply related to the absence of specific phage

receptors on the cell surface of these cells (Moineau *et al.* 1996), which could be carbohydrate components such as galactose or rhamnose. As these sugars are commonly present in HePS from LAB, both slime-EPS and CPS could actually favour phage adsorption (Broadbent *et al.* 2003).

Our results suggest that, the presence of CPS but not slime-EPS surrounding the cells of *Strep. thermophilus* strains could play a role in the adsorption of specific phages to the cells. The strain specificity for phage attack underlines the importance of rational screening for high performant *Strep. thermophilus* starter cultures.

Acknowledgements

We thank to CONICET, ANPCyT, CIUNT and SECyT from Argentina, FWO-Flanders from Belgium (project FW/PA/02-BIX/011), and the VUB-CONICET agreement for financial support. We are thankful to Dr Jorge Reinheimer for providing the phages and to Dr Elvira Hébert for her valuable contribution.

References

Albert, M.J., Bhuiyan, N.A., Rahman, A., Ghosh, A.N., Hultenby, K., Weintraub, A., Nahar, S., Kibriya, A.K.M.G. *et al.* (1996) Phage specific for *Vibrio cholerae* O139. *Bengal J Clin Microbiol* **34**, 1842–1845.

Broadbent, J.R., McMahon, D.J., Welker, D.L., Oberg, C.J. and Moineau, S. (2003) Biochemistry, genetics, and applications of exopolysaccharide production in *Streptococcus thermophilus*: a review. *J Dairy Sci* **86**, 407–423.

Brüssow, H., Frémont, M., Bruttin, A., Sidoti, J., Constable, A. and Fryder, V. (1994) Detection and classification of *Streptococcus thermophilus* bacteriophages isolated from industrial milk fermentation. *Appl Environ Microbiol* **60**, 4537–4543.

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

Deveau, H., Van Calsteren, M.-R. and Moineau, S. (2002) Effect of exopolysaccharides on phage-host interactions in *Lactococcus lactis*. *Appl Environ Microbiol* **68**, 43–64.

Duboc, P. and Mollet, B. (2001) Applications of exopolysaccharides in the dairy industry. *Int Dairy J* **11**, 759–768.

Fontana, C., Cocconcelli, P.S. and Vignolo, G. (2005) Monitoring the bacterial population dynamics during fermentation of artisanal Argentinean sausages. *Int J Food Microbiol* **103**, 131–142.

Forde, A. and Fitzgerald, E.G.F. (1999) Analysis of exopolysaccharide (EPS) production mediated by the bacteriophage adsorption blocking plasmid, pCI658, isolated from *Lactococcus lactis* ssp. *cremoris* HO2. *Int Dairy J* **9**, 465–472.

Hassan, A.N. and Frank, J.F. (2001) Starter cultures and their use. In *Applied Dairy Microbiology* ed. Marth, E.H. and Steele, J.L. pp. 151–206. New York, NY: Marcel Dekker.

Lévesque, C., Duplessis, M., Labonté, J., Labrie, S., Fremaux, C., Tremblay, D. and Moineau, S. (2005) Genomic organization and molecular analysis of virulent bacteriophage 2972 infecting an exopolysaccharide-producing *Streptococcus thermophilus* strain. *Appl Environ Microbiol* **71**, 4057–4068.

Lindberg, A.A. (1973) Bacteriophage receptors. *Ann Rev Microbiol* **27**, 205–241.

Looijesteijn, P.J., Trapet, L., de Vries, E., Abee, T. and Hugenholtz, J. (2001) Physiological function of exopolysaccharides produced by *Lactococcus lactis*. *Int J Food Microbiol* **64**, 71–80.

Moineau, S., Borkaev, M., Holer, B.J., Walker, S.A., Kondo, J.K., Vedamuthu, E.R. and Vanderbergh, P.A. (1996) Isolation and characterization of lactococcal bacteriophages from cultured buttermilk plants in the United States. *J Dairy Sci* **79**, 377–382.

Mozzi, F., Rollán, G., Savoy de Giori, G. and Font de Valdez, G. (2001a) Effect of galactose and glucose on the exopolysaccharide production and the activities of biosynthetic enzymes in *Lactobacillus casei* CRL 87. *J Appl Microbiol* **91**, 160–167.

Mozzi, F., Torino, M.I. and Font de Valdez, G. (2001b) Identification of exopolysaccharide-producing lactic acid bacteria. A method for the isolation of polysaccharides in milk cultures. In *Food Microbiology Protocols. Methods in Biotechnology* ed. Spencer, F. and Ragout, A. pp. 183–190 Totowa: Humana Press Inc.

Mozzi, F., Vaningelgem, F., Hébert, E.M., Van der Meulen, R., Foulquié-Moreno, M.R., Font de Valdez, G. and De Vuyst, L. (2006) Diversity of heteropolysaccharides-producing lactic acid bacterium strains and their biopolymers. *Appl Environ Microbiol* **72**, 4431–4435.

O' Sullivan, T.F. and Fitzgerald, G.F. (1999) Electrotransformation of industrial strains of *Streptococcus thermophilus*. *J Appl Microbiol* **86**, 275–283.

Pospiech, A. and Neumann, B. (1995) A versatile quick-prep of genomic DNA from gram-positive bacteria. *Trends Genet* **11**, 217–218.

Quiberoni, A., Auad, L., Binetti, A.G., Suárez, V.B., Reinheimer, J.A. and Raya, R. (2003) Comparative analysis of *Streptococcus thermophilus* bacteriophages isolated from a yogurt industrial plant. *Food Microbiol* **20**, 461–469.

Quiberoni, A., Guglielmotti, D., Binetti, A. and Reinheimer, J. (2004) Characterization of three *Lactobacillus delbrueckii* subsp. *bulgaricus* phages and the physicochemical analysis of phage adsorption. *J Appl Microbiol* **96**, 340–351.

Suárez, V.B., Quiberoni, A., Binetti, A.G. and Reinheimer, J.A. (2002) Thermophilic lactic acid bacteria phages isolated

from Argentinian dairy industries. *J Food Prot* **65**, 1597–1604.

Vaningelgem, F., Zamfir, M., Mozzi, F., Adriany, T., Vancanneyt, M., Swings, J. and De Vuyst, L. (2004) Biodiversity of exopolysaccharides produced by *Streptococcus thermophilus* strains is reflected in their production and their molecular and functional characteristics. *Appl Environ Microbiol* **70**, 900–912.

