African Journal of Biotechnology Vol. 6 (19), pp. 2280-2286, 4 October 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Inhibition of phage infection in capsule-producing *Streptococcus thermophilus* using concanavalin A, lysozyme and saccharides

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Accepted 16 July, 2007

Lactic cultures that produce capsular polysaccharides are widely used in the dairy industry. However, little information is available on their phage-cell interactions. Concanavalin A (Con A), lysozyme, and saccharides were investigated for their ability to modify phage-cell interactions in such a manner as to inhibit phage infection. The ability of phage to infect cells was determined by measuring acid production in Elliker broth. Acid production by capsule-producing *Streptococcus thermophilus* was inhibited less by bacteriophage when cells were pretreated with Con. A than was acid production by a capsule-free variant. The presence of 0.5 mg/ml lysozyme in Elliker broth significantly reduced phage infection. However, there was no increased effect when lysozyme and Con A were combined in the growth medium. The addition of 5 g/L of glucosamine to Elliker broth also inhibited phage infection. The results of this study indicate that it is possible to reduce phage infection of capsule-forming *S. thermophilus* by blocking or modifying phage adsorption sites.

Key words: Capsular polysaccharides, Concanavalin A, bacteriophage adsorption, adsorption sites, phage inhibition.

INTRODUCTION

The use of capsule-producing starter cultures can improve the physical properties of low fat yogurt and some reduced fat cheeses (Hassan and Frank, 1997; Hassan et al., 1996; Hassan et al., 1996). Capsules produced by lactic acid bacteria (LAB) are of similar size to fat globules and presence in sufficient quantity imparts a texture in reduced fat dairy products similar to that in their full fat counterpart (Hassan et al., 2002; Hassan et al., 2004; Hassan et al., 2002). LAB is normally exposed to phages that are naturally present in milk processed under non sterile environments (Carpa et al., 2006). Therefore, the increasing use of these cultures by the dairy industry has produced a need for information on controlling phage infection during their production. Bacterial capsules are exopolysaccharides (EPS) that remain attached to the cells. This EPS coat can delay phage infection, perhaps by providing a physical barrier to phage adsorption (Cerning, 1990; De Vuyst and Degeest, 1999; Looijesteijn et al., 2001; Valyasevi et al., 1990). Lindberg (1977) found that cell-associated EPS provided *Lactococcus* strains with only weak protection against phages, and Forde et al. (1999) observed that loosely-bound EPS produced by some lactococcal strains only slightly inhibited phage adsorption. The susceptibility of a bacterial strain to bacteriophage infection is dependent on the recognition of specific binding sites on the cell wall (primary receptors) and, if a capsule is present, recognition of capsular EPS binding sites (secondary receptors) (Leiman et al., 2000). Since the capsule may act as both a physical barrier to phage infection and as an attachment site for specialized bacteriophages (Scholl et al., 2005) treatments that enhance its barrier property or that alter specific binding sites may lead to a delay in phage infection.

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In this study we investigated various means to alter potential phage binding sites associated with the capsule to determine their effect on phage infection. These treatments included the use of a lectin, concanavalin A, that binds to EPS sugar residues, low concentrations of lysozyme to alter cell wall associated binding sites, and selected saccharides that may block binding sites on the phage particle. The aim of this investigation is to increase understanding of phage infection of capsule-producing lactic acid bacteria with ultimate application in the formulation of phage-inhibitory media for these cultures.

MATERIALS AND METHODS

Cultures and phage strains

The capsule-producing (CPS^+) strain *Streptococcus thermophilus* CHCC 3534, its capsule-free mutant (CPS^-) strain *S. thermophilus* CHCC 5842, and the associated lytic phage (CHPC 577) were provided by Chr. Hansen, Denmark. The wild type and mutant strains were genetically compared using Rep-PCR (Bacterial BarCodes, Inc., Houston, TX) as described by Prazak et al. (2002).

Chemicals and reagents

Concanavalin A lyophilized powder (Con A from *Canavalia ensiformis* (Jack Bean) type III was purchased from Sigma-Aldrich Inc., St Louis, Mo., USA. Lysozyme lyophilized powder (E. C. 3.2.1.17.) from chicken egg white of 48, 800 units/ mg protein, 46,400 units/ mg solid was purchased from Sigma-Aldrich Inc., St Louis, Mo., USA. Saccharides to be tested for blocking bacteriophage adsorption sites included; D-galactose (Fischer chemicals, NJ, USA.), D-glucosamine, D-mannose, *N*-Acetyl-D-galactosamine (galNac), *N*- Acetyl-D-glucosamine (glcNac), *N*-Acetyl-D-mannosamine (manNac), α-L-rhamnose (Sigma- Aldrich Inc., St Louis, Mo., USA.) and D-ribose (Calbiochem Co., LA, USA.).

Culture preparation

Cultures were maintained in M17 (Difco Brand, Becton Dickenson, Sparks, MD) with lactose (5 g/L) as the sole sugar. Stock cultures were prepared by growing cultures for 12 h at 38°C. Cells were then separated by centrifugation at 3,743 x g using a Beckman Allegra 21R centrifuge, and resuspended in 1 ml of fresh M17 broth. The suspended cells were transferred as 0.5 ml portions to 2 ml beads-containing cryogenic vials (Pro lab diagnostics TX, USA). Vials were stored at –80°C until needed. Working cultures were prepared by aseptically transferring 1 - 2 beads to 10 ml of either M17 or Elliker (Difco) media and incubating for 16 h at 38°C.

Preparation of phage stocks and phage titer determination

High titer phage lysate stock suspensions were produced by the broth method when large quantities (>100 ml) were required and the plate method when small quantities were needed (Yokokura, 1971). For the broth method, 1 ml of the phage lysate was aseptically transferred to 10 ml of M17 broth containing 10 mM $CaCl_2.6H_2O$ and 10 g/L lactose as the sole carbon source, inoculated with 0.1 ml of early exponential phase host cell culture to achieve about 10^7 cfu/ml. The phage/culture mixture was incubated at 38°C until clearing (usually within 3 - 5 h). The phage was separated by centrifugation at 3,743 x g at 4°C for 10 min to remove bacterial cells and cell debris. The supernatant fluid (~10 ml) was added to 100 ml of early exponential phase culture (1 ml per 100 ml) containing 10 mM $CaCl_2.6H_2O$ and incubated at 38°C until clearing occurred. The phage-containing supernatant fluid was again collected as previously described and then sterilized using 0.2 µm pore size Nuclepore filters. A few drops of chloroform were then added to the phage stock lysates, followed by vigorous shaking for few minutes to eliminate viable cells (Adams, 1959). The phage lysate was stored at –80°C for a maximum period of 2 - 3 weeks, and periodically tested for the phage titer using the plaque assay technique. The phage lysate preparation did not significantly decline in titer over 3 weeks of storage.

Small amounts of high titer phage stocks were prepared as described by Svenson and Cristianson (1991). Three or four plaques were transferred to 2 ml of chilled (4°C) M17 broth contained in a sterile test tube, which was refrigerated overnight. The entire contents of the tube were added to 10 ml of a 2 h M17 liquid culture of the host cells grown at 38°C. With continued incubation at the same temperature, lysis occurred within 2 to 4 h. After lysis, the phage culture was centrifuged and sterilized as previously described. The titer of the phage stock suspensions was determined by using the double-layer plaque titration method reported by Stummeyer et al. (2006).

Inhibition of phage activity by concanavalin A

Stock solutions of Con A were prepared by dissolving 1.75 g of lyophilized powder in 25 ml of phosphate buffered saline (PBS, 10 mM potassium phosphate in 150 mM NaCl adjusted to pH 7.2) to give a final concentration of 0.007 g/ml. The powder was dissolved by stirring at room temperature for 3 h, then filter sterilized, and refrigerated at 4°C for further use. The effect of Con A on phage activity was determined by monitoring pH changes produced by the capsule-forming *S. thermophilus* in the presence of phage. The phage infection test employed Elliker broth supplemented with 10 mM $CaCl_2.6H_2O$ as the growth medium. An inoculum of 0.5 ml of each test strain was transferred separately to 5 ml of Elliker broth dispensed in sterile test tubes, to which 0.5 ml of Con A stock solution was added to give a final concentration of 0.007 g/ml. Tubes were incubated at 38°C for 2 h to allow Con A binding to the cells of both strains, after which 1 ml of the phage lysate was added. The initial pH of the culture media of both test strains was recorded. The acid production was monitored by measuring pH every 3 h over a period of 12 h. Control tests were prepared following the above procedure with the exclusion of Con A and/or phage.

Use of lysozyme to modify bacteriophage binding sites

The ability of lysozyme to reduce phage infection of capsule-forming *S. thermophilus* was determined. The culture was inoculated (1 ml per 100 ml) into Elliker broth containing 10 mM $CaCl_2.6H_2O$ and 0.5 mg/ml lysozyme. The culture was incubated at 38°C, till reaching the early exponential phase (2 - 3 h). 1 ml of purified phage lysate was added, the initial pH was recorded, and the culture was then incubated at 38°C for 12 h. Acid production was monitored every 3 h by recording pH values. The effect of lysozyme was determined by comparing the pH drop of the test culture to that of control cultures with lysozyme (no phage) and cultures with phage (no lysozyme).

Evaluation of sugars for the blocking of phage adsorption

Selected sugars were evaluated for their ability to delay phage infection in the capsule-forming *S. thermophilus* using a modifica-

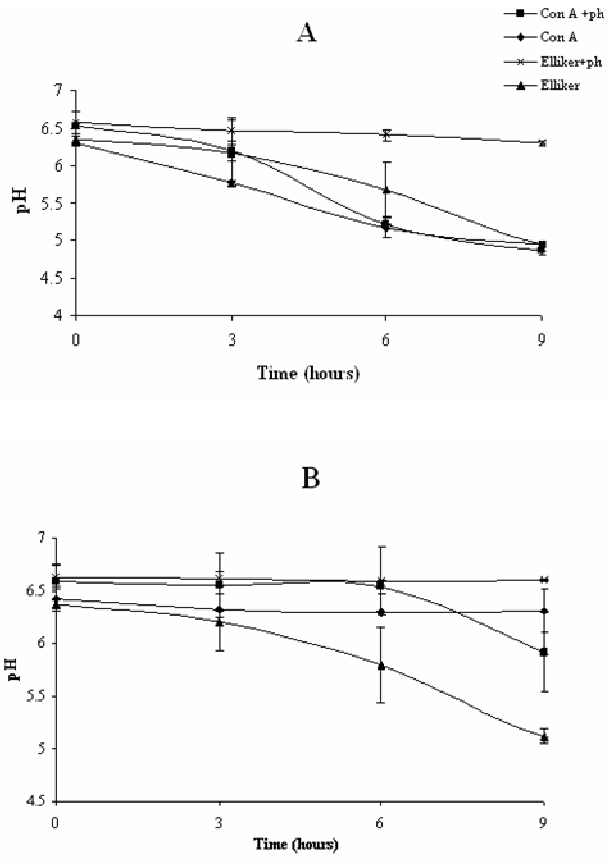

Figure 1. Acid production by capsule-producing *S. thermophilus* (A) and capsule-free variant (B) in Elliker broth at 37 EC in the presence of 7 mg/g Con A with (■) and without phage (♦). The controls included culture with phage (×), and culture with no phage (▲). Error bars represent the standard error of the mean pH values of each culture (n= 5). ph = phage.

tion of the procedure described by Terzaghi and Sandine (1975). Sugars were selected based on their potential involvement in binding site composition. They included: D- galactose, D-glucosamine, galNac, glcNac, manNac, D-mannose, α- L-rhamnose, and D-ribose. Sugars (5 g/L) were added to Elliker broth containing 10 mM $CaCl_2.6H_2O$. The capsule-forming culture was propagated at 38°C in Elliker broth supplemented with the selected sugar for sufficient time to obtain cells in early exponential phase. Once early exponential growth was obtained, 1 ml of the phage lysate was added, and the initial pH was determined. Tubes were again incubated at 38°C and pH was monitored every 3 h for up to 12 h. Similarly, another set of cultures were prepared for the purpose of determining the effect of Con A on phage infection when combined with each saccharide. These experiments employed the same methodology as described, but culture/phage mixture included Con A at a concentration of 0.007 g/ml. Acid production in Con A-free control cultures was determined for each test sugar.

Statistical analysis

All experiments were replicated three times. Standard deviations were calculated and included in the graphical representation of the data.

RESULTS AND DISCUSSION

The capsule-free variant used in this study was obtained from the wild type by selection of a spontaneously formed colony type variant. This variant exhibited the same RepPCR pattern as the wild type and similar phage susceptibility and acid producing ability in Elliker broth (data not shown).

Inhibition of phage infection by Con A

The wild-type and capsule-free cultures acidified Elliker broth to pH 5.0 in 9 h in the absence of phage and failed to acidify in presence of the phage (Figure 1). Incubation of cells with Con A lectin before exposure to phage completely counteracted phage infection for the capsule-producing strain (pH 5.0 in 9 h) and only partially for the capsule-free strain (pH of 6.0 in 9 h). These data are consistent with lectin binding to the capsule producing a barrier to phage infection. Archibald and Coapes (1972) observed lectin blocking of cell wall receptor sites for phage in *Bacillus subtilis*, but there are no previous reports of a lectin blocking effect in the presence of a capsule. The ability of Con A in inhibiting phage infection in the capsule-producing strain could be due to either blocking of phage receptor sites on the capsule or the cell wall.

Phage may adsorb to more than one receptor on the surface of the host (Geller et al., 2005). Con A binds to the glucose and N-acetyl glucosamine residues of the cell wall of gram-positive bacteria (Birdsell and Doyle, 1973). Phage adsorption relies on specific interactions with cell wall polysaccharides (Moineau et al., 1996). Cell wall polysaccharides are distinct from the capsular polysaccharide primarily in that they are bound to the peptidoglycan or loosely associated with the cell envelope, while the capsular polysaccharide forms a thick outermost shell that may be covalently bound to the cell wall (Gopal and Crow, 1993). The capsular and cell wall polysaccharides share structural saccharide constituents (glucose, N-acetyl glucosamine, galactose, rhamnose) that can form phage receptor and Con A binding sites (Quiberoni et al., 2000; Vedamuthu and Neville, 1986; Wilson and Ratcliffe, 2000).

Phage that infect capsule-producing bacteria contain a polysaccharide depolymerase that binds to the capsular material and degrades the polymer allowing the phage access to the surface of the outer membrane, where they bind to a secondary receptor site (Schleifer and Kilpper-Bälz, 1987).

Inhibition of phage infection by lysozyme and Con A

The addition of a low level of lysozyme to Elliker broth partially inhibited phage infection of the capsule-producing culture (Figure 2). The level of lysozyme was

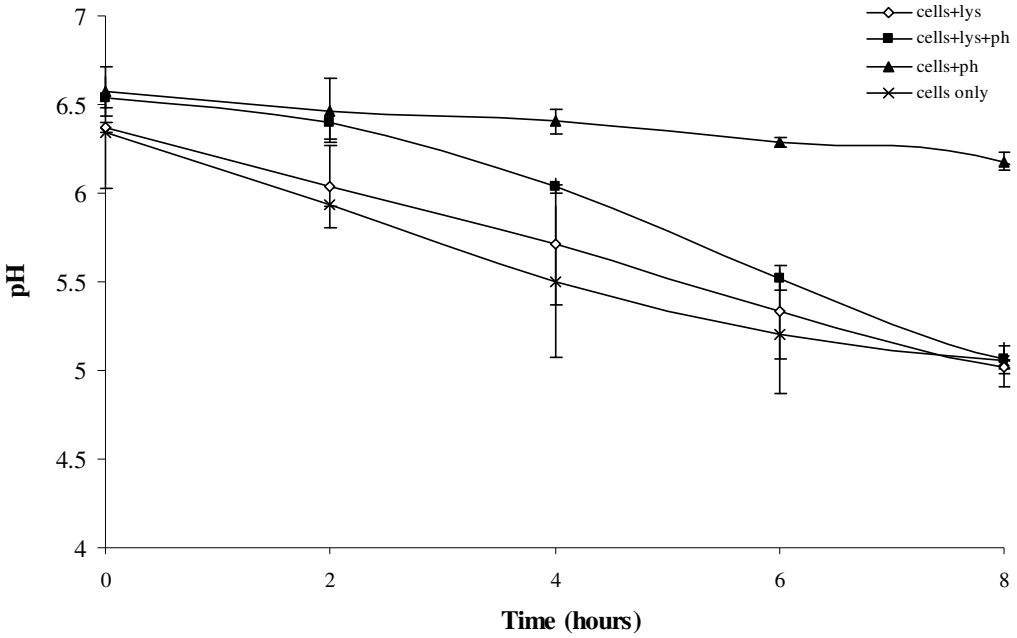

Figure 2. Acid production by capsule-producing *S. thermophilus* in the presence of bacteriophage and lysozyme. Growth was in Elliker broth for 12 hours at 37 EC. lysozyme (◊), lysozyme and phage (■), phage only (▲), and cells only (×). Results represent the mean pH values of each culture mixture (n = 4). lys = lysozyme.

selected so as not to significantly reduce acid production by the culture (Figure 2). Lysozyme hydrolyses 1,-4 linkages between *N*-acetylmuramic acid and N-acetyl glucosamine. Monteville et al. (1994) and Yoon et al. (2002) reported that carboxyl groups on the peptide-glycan may be removed or modified through the action of lysozyme. This suggests that acetyl groups in the capsular polysaccharide may be altered by lysozyme resulting in inhibiton of phage adsorption. Wicken et al. (1983) observed that lysozyme treatment of *Bacillus cereus* reduced the number of binding sites for glcNac-specific lectins, and also reduced susceptibility to phage infection. Lysozyme could also inhibit phage adsorption by direct binding to phage adsorption sites.

Data on the combined effect of adding lysozyme and Con A on phage infection is presented in Figure 3. The addition of lysozyme to a ConA-containing broth did not increase the rate of acidification and in fact slightly reduced it. It is apparent from this data that the phage inhibitory effects of ConA and lysozyme do not complement each other when applied to the capsule-forming strain of *S. thermophilus*, and that Con A more completely blocks the action of the phage than does lysozyme.

Inhibition of phage infection by combining saccharides with ConA

The major monomers constituting the capsular polysaccharide of the strain used in this study are glucose, galactose, rhamnose, glcNac (data not shown). The addition of these monomers to growth media may be able to block adsorption sites on the phage thereby reducing the ability of the phage to bind to the capsule or cell wall. The capsule-producing culture was grown with various saccharides in presence and absence of Con A. The phage was then added to the culture in early log phase and acid production was compared to that of cultures incubated with the saccharides in the absence of phage. The resulting acidification data is presented in Figure 4. For most of the saccharides, acidification was delayed for the initial 3 h indicating no significant effect on initial phage absorption (In Figure 1, one can see a delay in acidification during the first 3 h even in media containing no phage) . Glucosamine was the only saccharide that allowed initial acidification (Figure 4D). This observation is consistent with that of Nagao et al. (1995) who found that glucosamine interfered with adsorption of phage to *S. thermophilus* BJ15, although it is not known if this strain produces a capsule. Gabig et al. (2002) concluded that carbohydrates can sequester cations or deplete or exclude them from the cell surface and thereby prevent effective phage adsorption. Also, since glucosamine is cationic it could therefore attract the negatively charged carboxyl groups of the phage tails (Leiman et al., 2000). However, the addition of ConA to the medium counteracted the phage inhibitory effect of the saccharide. This indicates that ConA and glucosamine interact with the effect of reducing their ability to bind to phage adsorption sites.

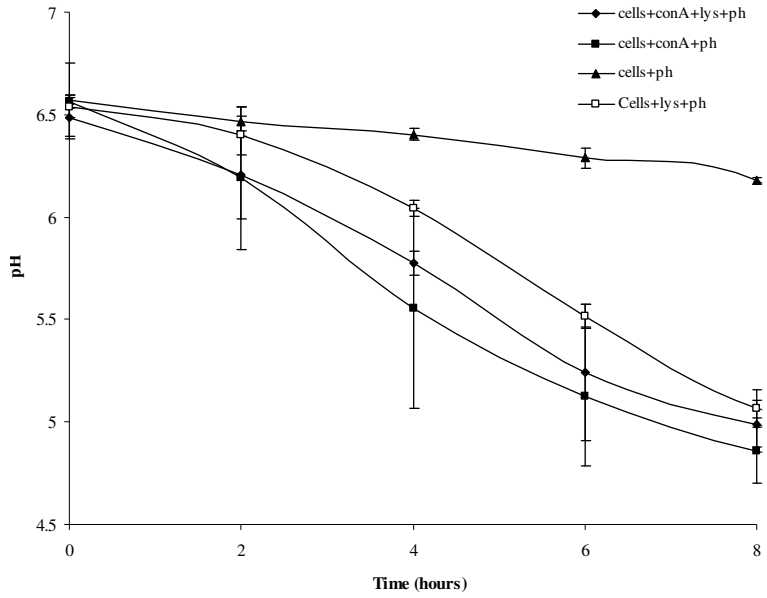

Figure 3. Acid production by capsule-producing *S. thermophilus* in the presence of bacteriophage, lysozyme and Con A. Growth was in Elliker broth for 12 hours at 37 EC.
phage (▲), lysozyme with phage (□), ConA with phage (■) and lysozyme and ConA with phage (♦). Results represent the mean pH values of each culture mixture (n= 4). lys=lysozyme

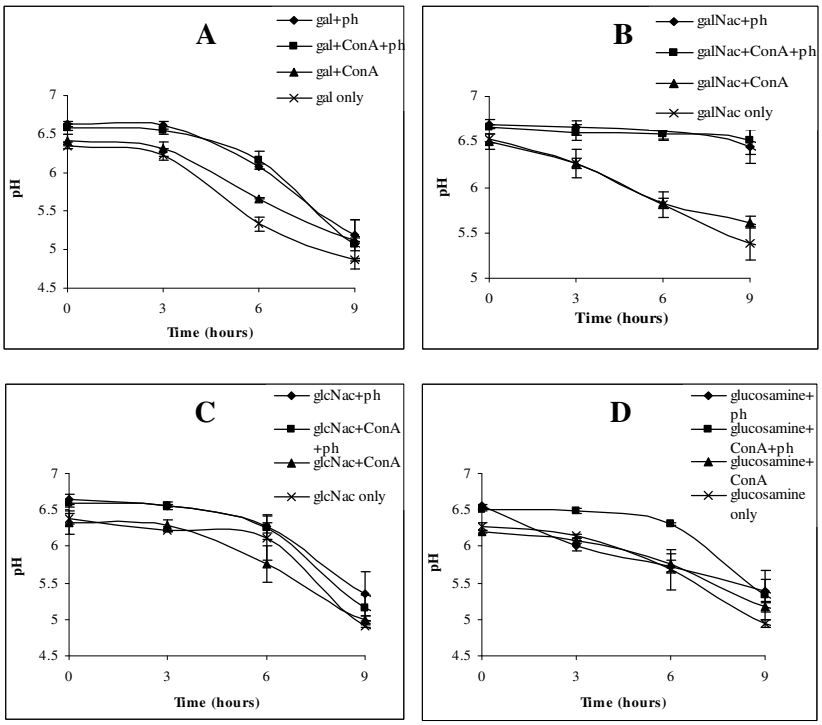

Figure 4a. Acid production by capsule-producing *S. thermophilus* in the presence of bacteriophage, various saccharides and Con A. A, B, C, D designate data for galactose, galNac, glcNac, and glucosamine, respectively. Growth was in Elliker broth for 12 hours at 37 EC. Key: saccharide only (×), saccharide with Con A (▲), saccharide with Con A and phage were added (■), and saccharide and phage (♦). Each value represents the mean of three replications.

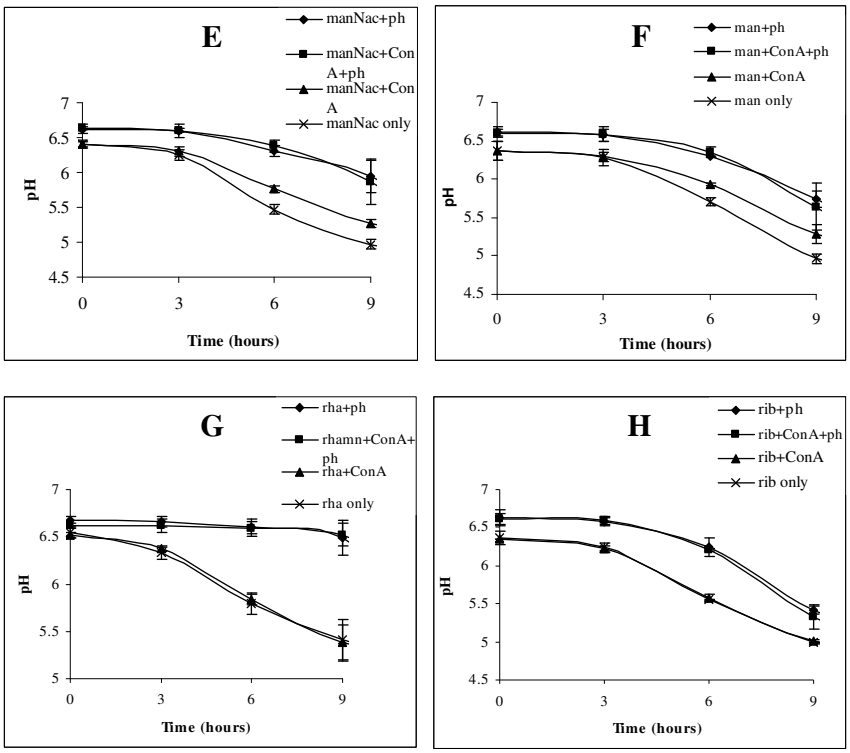

Figure 4b. Acid production by capsule-producing *S. thermophilus* in the presence of bacteriophage, various saccharides and Con A. E, F, G, H designate data for manNac, mannose, rhamnose, and ribose, respectively. Growth was in Elliker broth for 12 hours at 37 EC. Key: saccharide only (×), saccharide with Con A (▲), saccharide with Con A and phage were added (■), and saccharide and phage (♦). Each value represents the mean of three replications.

Other saccharides used in this study have been previously observed to inhibit phage infection. Rhamnose inactivated phages of *S. thermophilus* CYM (Nagao et al., 1995) and *Lactococcus lactis* subsp. *cremoris* KH (Valyasevi et al., 1990). The phage used in this study apparently does not have rhamnose binding sites. This indicates a difficulty in practical application of saccharides as phage inhibitory agents, since phage types may differ in their susceptibility to various blocking agents.

In conclusion, the presence of a capsule on *S. thermophilus* presents an opportunity for enhanced protection against phage protection. Since phage must bind to the capsule and be transported to the cell surface, treatments that interfere with this process, such as binding capsular polysaccharides with lectins, lysozyme, and saccharides can reduce the ability of the phage to rapidly infect the cell.

ACKNOWLEGMENTS

This research was undertaken with the financial support and grants provided by the Egyptian government through a channel program between Alexandria University (Egypt) and the University of Georgia (USA). We thank Dr. Thomas Jansen for providing the bacterial strains and the bacteriophage.

REFERENCES

Adams M (1959). Stages in phage multiplication. In: Anderson E, Gots J, Jacob F, and Wollman E-L. (eds.) Bacteriophages, Interscience publishers, pp. 161-187.

Archibald A, Coapes H (1972). Blocking of bacteriophage receptor sites by Concanavalin A. J. Gen. Microbiol. 73: 581- 585.

Birdsell D, Doyle R (1973). Modification of bacteriophage 25 adsorption to *Bacillus subtilis* by concanavalin Afr. J. Bacteriol. 113: 198-202.

Carpa M, Quiberoni A, Ackermann H, Moineau S, Reinheimer J (2006). Characterization of new virulent phage (MLC-A) of *Lactobacillus paracasei*. J. Dairy Sci. 89: 2414-2423.

Cerning J (1990). Exocellular polysaccharides produced by lactic acid bacteria. FEMS Microbiol. Rev. 87: 113-130.

De Vuyst L, Degeest B (1999). Heteropolysaccharides from lactic acid bacteria. FEMS Microbiol. Rev. 23: 153-177.

Forde A, Daly C, Fitzgerald G (1999). Identification of four phage resistance plasmids from *Lactococcus lactis* ssp. *cremoris* HO2. Appl Environ. Microbiol. 65: 1540-1547.

Gabig M, Antosiewicz H, Kwiatkowska M, Los M, Thomas M, Wegrzyn G (2002). The cell surface protein Ag43 facilitates phage infection of Escherichia coli in the presence of bile salts and carbohydrates. Microbiol. 148: 1533-1542.

Geller B, Ngo H, Mooney D, Su P, Dunn N (2005) Lactococcal 936-species phage attachment to surface of *Lactococcus lactis*. J. Dairy Sci. 88: 900-907.
Gopal P, Crow V (1993). Characterization of loosely associated material from the cell surface of *Lactococcus lactis* subsp. *cremoris* E8 and its phage resistant variant strain 398. Appl. Environ. Microbiol. 59: 3177-3182.
Hassan A, Corredig M, Frank J (2002). Capsule formation by non-ropy cultures affects the viscoelastic properties of yogurt during structure formation. J. Dairy Sci. 85: 716-720.
Hassan A, Corredig M, Frank J, ElSoda M (2004). Microstructure and rheology of an acid coagulated cheese (karish) made with an exopolysaccharide-producing *Streptococcus thermophilus* strain and its exopolysaccharide non-producing genetic variant. J. Dairy Res. 71: 116-120.
Hassan A, Frank J (1997). Modification of microstructure and texture of rennet curd by using a capsule-forming non ropy lactic culture, J. Dairy Res. 64: 115-121.
Hassan A, Frank J, Corredig M (2002). Microstructure of feta cheese made using different cultures as determined by confocal scanning laser microscopy. J. Food Sci. 67: 2750-2753.
Hassan A, Frank J, Schmidt K, Shalabi S (1996). Rheological properties of yogurt made with encapsulated nonropy lactic cultures. J. Dairy Sci. 79: 2091-2097.
Hassan AN, Frank JF, Schmidt KA, Shalabi SI (1996). Textural properties of yogurt made with encapsulated nonropy lactic cultures, J. Dairy Sci. 79: 2098-2103.
Leiman P, Kostyuchenko V, Schneider M, Kurochkina L, Mesyanzhinov V, Rossmann M (2000). Structure of bacteriophage T4 gene product 11, the interface between the baseplate anf short tail fibers. J. Mol. Biol. 301: 975- 985.
Lindberg A (1977). Bacterial surface carbohydrates and bacteriophage adsorption. In: Sutherland IW (ed.) Surface Carbohydrates of the Prokaryotic Cell, Academic Press, London, UK, pp. 289-356.
Looijesteijn P, Trapet L, de Vries E, Abee T, Hugenholtz J (2001). Physiological function of exopolysaccharides produced by *Lactococcus lactis*. Int. J. Food. Sci. 64: 71- 80.
Moineau S, Borkaev M, Holler B, Walker S, Kondo J, Vedamuthu E, Vaderbergh P (1996). Isolation and characterization of lactococcal bacteriophages from cultured buttermilk plants in the United States, J. Dairy Sci. 79: 2104-2111.
Monteville M, Ardestani B, Geller B (1994). Lactococcal bacteriophages require a host cell wall carbohydrate and a plasma membrane protein for adsorption and enjection of DNA. Appl. Environ. Microbiol. 60: 3204-3211.
Nagao P, Silva-Filho F, Benchetrit L, Barrucand L (1995). Cell surface hydrophobicity and the net electric surface charge of group B streptococci: the role played in the micro-organism-host cell interaction. Microbios. 82: 207-216.
Prazak A, Murano E, Mercado I, Acuff G (2002). Prevalence of *Listeria monocytogenes* during production and post harvest processing on cabbage. J. Food Prot. 65: 1728-1734.
Quiberoni A, Stiefel J, Reinheimer J (2000). Characterization of phage receptors in *Streptococcus thermophilus* using purified cell walls obtained by a simple protocol. J. Appl. Microbiol. 89: 1059-1065.
Schleifer K, Kilpper-Bälz R (1987). Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. Syst. Appl. Microbiol. 10: 1-19.
Scholl D, Adhya S, Merril C (2005). *Escherichia coli* K1's capsule is a barrier to bacteriophage T7. Appl. Environ. Microbiol. 71: 4872-4874.
Stummeyer K, Schwarzer D, Claus H, Vogel U, Gerardy-Schahn R, Muhlenhoff M (2006). Evolution of bacteriophages infecting encapsulated bacteria: lessons fron *Escherichia coli* K1-specific phages. Mol. Microbiol. 60: 1123-1135.
Svenson V, Cristianson A (1991). Methods for phage monitoring, FIL-IDF Bull. 263: 29- 39.
Terzaghi B, Sandine W (1975). Improved medium for lactic streptococci and their bacteriophages. Appl. Environ. Microbiol. 29: 807- 813.
Valyasevi R, Sandine W, Geller B (1990). The bacteriophage kh of *Lactococcus lactis* subsp. *cremoris* KH is the rhamnose of the extracellular wall polysaccharide. Appl. Environ. Microbiol. 56: 1882-1889.
Vedamuthu E, Neville J (1986). Involvement of a plasmid in production of ropiness (mucoidness) in milk cultures by *Streptococcus cremoris* MS. Appl. Environ. Microbiol. 51: 677-682.
Wicken A, Ayres A, Campbell L, Knox K (1983). Effect of growth conditions on production of rhamnose-containing cell wall and capsular polysaccharides by strains of *Lactobacillus casei* subsp. *rhamnosus*. J. Bacteriol. 153: 84-92.
Wilson R, Ratcliffe N (2000). Effect of lysozyme on the lectin-mediated phagocytosis of *Bacillus cereus* by haemocytes of the cockroach, *Blaberus discoidalis*. J. Insect Physiol. 46: 663-670.
Yokokura T (1971). Phage receptor material in *Lactobacillus casei* cell wall. Jpn. J. Microbiol. 15: 457-463.
Yoon S, Barrangou-Poueys R, Breidt F, Klaenhammer T, Fleming H (2002). Isolation and characterization of bacteriophages from fermenting sauerkraut. Appl. Environ. Microbiol. 68: 973- 976.