The specificity of oligopeptide transport by *Streptococcus thermophilus* resembles that of *Lactococcus lactis* and not that of pathogenic streptococci

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**INTRODUCTION**

*Streptococcus thermophilus* is able to synthesize several amino acids (Garault et al., 2000; Limauro et al., 1996). This bacterium is therefore much less nutritionally demanding than other lactic acid bacteria (Bellengier et al., 1997; Chervaux et al., 2000; Hebert et al., 2000; van Niel & Hahn-Hagerdal., 1999). In fact, a chemically defined medium containing only three to six free amino acids as the nitrogen source (depending on the strain under study) is able to sustain a significant growth of *S. thermophilus* (Letort & Juillard, 2001). However, the growth of this lactic acid bacterium in milk largely depends on the utilization of exogenous amino acids. For instance, the ability to use caseins as a source of amino acids results in an increase of the maximal population (Shahbal et al., 1993). Similarly, addition of peptides to milk enhances the growth of this micro-organism (Desmazeaud & Hermier, 1972, 1973). The use of these amino acid sources requires the presence of a complex proteolytic system. The main components of this system have now been identified and characterized (Fernandez-Esla et al., 2000; Garault et al., 2002; Neviani et al., 1995; Rul & Monnet, 1997). Among them, peptide transport represents a key step: inactivation of the oligopeptide transport function resulted in a growth defect of *S. thermophilus* in milk (Garault et al., 2002). A similarly crucial role for oligopeptide transport for growth in milk has also been shown in *Lactococcus lactis* (Juillard et al., 1995a; Kunji et al., 1995; Lamarque et al., 2004).

The oligopeptide transport system Opp of *L. lactis* has been extensively studied over the last 10 years. It belongs to the superfamily of ABC transporters (Higgins, 1992) and consists of five proteins (OppA, B, C, D and F). Among them, OppA is devoted to the binding of the substrate. The Opp system of *L. lactis* presents atypical substrate specificity, compared to other micro-organisms. Intact cells of *L. lactis* are able to transport peptides containing up to at least 18 amino acid residues (Detmers et al., 1998). Moreover, the Opp system functionally reconstituted in OppA artificial membranes has the (up to now) unique capacity to bind and transport peptides containing up to at least 35 amino acid residues (Doeven et al., 2004). Recently, a second oligopeptide transport system, Opt, has been characterized in *L. lactis* IL1403 (Lamarque et al., 2004). The capability of this second system to bind and to transport peptides has not yet been studied in great detail. Nevertheless, this Opt system is also able to take up some long peptides, up to at least nine amino acid residues. The lactococcal oligopeptide transport systems therefore seem to be especially well adapted to...
lactocepin, which mainly releases peptides of large molecular mass from caseins (Juillard et al., 1995b; Kunji et al., 1996).

Much less attention has been devoted to the oligopeptide transport system of *S. thermophilus*. The presence of a functional oligopeptide transport system (Ami) has been reported only recently in *S. thermophilus* ST18 (Garault et al., 2002). The Ami system also belongs to the superfamily of ABC transporters. In *S. thermophilus* ST18, three highly homologous binding proteins (AmiA1, AmiA2 and AmiA3) are associated to a single translocon AmiCDEF. The presence of three distinct binding proteins has also been reported in other streptococci such as *Streptococcus gordonii* and *Streptococcus pneumoniae* (Alloing et al., 1994; Jenkinson et al., 1996). Nevertheless, these pathogenic streptococci are reported to transport peptides containing up to nine amino acid residues only. In contrast, the ability of *S. thermophilus* ST18 to consume a 23-mer peptide during its growth suggests that at least this strain has the capability to transport larger peptides than can other streptococci (Garault et al., 2002). The aim of the present study was therefore to analyse the specificity of the oligopeptide transport system of *S. thermophilus*.

**METHODS**

**Strains and culture conditions.** Twelve Prt* strains of *Streptococcus thermophilus* were used in the present study. Ten of them (ST3, ST4, ST5, ST6, ST7, ST8, ST9, ST10, ST13, ST21) were from industrial sources. *S. thermophilus* CNRZ 302 was from the CNRZ collection (URLGA, INRA, Jouy-en-Josas, France). The construction of ST18Prt* has been described elsewhere (Garault et al., 2002). The proteinase-negative phenotype of the strains was checked by plating cultures on Fast-Slow-Differentiation-Agar medium (Huggins & Sandine, 1984) and by estimating the amount of labelled peptides released by resting cells from G14-methylated casein, as described by Monnet et al. (1987). Strains were stored at −80 °C in M17 broth (Terzaghi & Sandine, 1975) supplemented with 10 g lactose l−1 and 10% (w/v) glycerol.

Precultures were grown in M17 broth containing 5 g lactose l−1 at 37 °C and were centrifuged (5000 g, 20 °C, 10 min) in the exponential stage of growth. Cells were washed twice in 50 mM KH2PO4/K2HPO4, pH 6.8, and inoculated at ~5 × 10^5 cfu. ml⁻¹ in chemically defined medium (CDM) (Letort & Juillard, 2001) containing different mixtures of peptides as the sole source of amino acids. CDM was overlaid with paraffin oil and incubated at 37 °C.

**Bacterial enumeration.** Cell populations were determined either by spiral plating on M17 agar using a spiral plater (WASP, Don Whitley Scientific Ltd), or by OD_{600} measurement using a Microbiology Reader Bioscreen C (Labsystems). In the case of spiral plating, colonies were counted after incubation at 37 °C for 24 h in anaerobic jars (Anaerocult, Merck).

**Preparation of peptide fractions.** Reconstituted milk (10%, w/v, low heat milk powder, Nilac, Netherland Dairy Research Institute), was acidified to pH 4.6 by addition of 0.1 M HCl. Precipitated caseins were removed by centrifugation (10000 g, 4 °C, 10 min), the supernatant was ultrafiltered through a 10000 Da-cut-off membrane (YM10, Amicon, Millipore). Peptides were then extracted and concentrated by solid-phase extraction on a C18 reverse-phase cartridge (Sep-Pack tC18, Waters). This first pool of peptides is called PP10 throughout the text. Additional fractionation of PP10 was performed using 3000 and 1000 Da-cut-off membranes (YM3 and YM1, respectively; Amicon), resulting in two peptide fractions called PP3 and PP1, respectively. The mean chain length of each peptide fraction was estimated as previously described (Helinck et al., 2003). The peptide concentration of each fraction was estimated by measuring the amino acid concentrations by cation-exchange chromatography and ninhydrin post-column derivatization (LC5000, Biotronic, Biochrom) after acidic hydrolysis.

The tryptic digest of zcta-casein was obtained as previously described (Juillard et al., 1998). The hydrophobicity of the peptides was computed using the normalized consensus hydrophobicity scale of amino acids (Eisenberg et al., 1984).

**Peptide analysis.** Peptides were separated by reverse-phase HPLC (RP-HPLC) at 40 °C by a gradient system (Waters 625) fitted with a Nucleosil C18, 5 μm, 300 Å, 4.6 × 250 mm column (Machery Nagel). Solvent A was 0-115% trifluoroacetic acid (TFA), solvent B was 0-1% TFA in 60% CH3CN. The flow rate was 1 ml min⁻¹. A 5 min isocratic phase of solvent A was followed by a linear gradient from 0 to 80% of solvent B within 80 min. Peptides were either detected simultaneously by UV measurement (214 nm) and fluorescence measurement (excitation 340 nm and emission 425 nm) after on-line post-column derivatization by o-phthalaldehyde (Herraz et al., 1994), or collected after UV detection for mass spectrometry analysis.

Peptides collected from HPLC after UV detection were concentrated with C18-ZipTip (Millipore), and analysed by mass spectrometry on an HP G20225A MALDI-TOF System (Hewlett Packard). Equal volumes (1 μl) of peptide solution and z-cyano-4-hydroxyphenylalanine matrix solution (HP G2054A) were mixed, spotted onto the sample probe surface and crystalized under vacuum. Spectra were acquired in the reflector mode with the following parameters: 20 kV accelerating voltage, a 337 nm laser and 100 ns delayed extraction.

**Peptide transport.** The procedure for peptide transport analysis was adapted from Kunji et al. (1995). Cells were grown in CDM to an A_{560} of 0.8 and washed twice in 50 mM KH2PO4/KHPO4, pH 6.8. Depletion on the intracellular pool of amino acids was achieved by incubating cells (A_{650} ~25) for 30 min at 30 °C in the presence of 10 mM 2-deoxy-D-glucose. After two washing steps, depleted cells (A_{650} ~2) were re-energized for 5 min in the presence of glucose (25 mM) and MgSO4 (2 mM). The time-course of peptide transport by energized cells (A_{650} ~1) was performed at 30 °C. Peptide transport was followed by measuring both the disappearance of the peptide from the external medium by RP-HPLC analysis, as indicated above, and the simultaneous increase in the intracellular pool of amino acids by the method of Charbonnel et al. (2003).

**Estimation of cell lysis.** The possible lysis of cells during peptide transport was estimated by measuring the liberation of the intracellular aminopeptidase PepC in the external medium, using arginine p-nitroanilide (0-15 mM) as the substrate. Quantification of the lysis was obtained by comparing the PepC activity measured in the external medium to that obtained with a cell-free extract.

**RESULTS**

*S. thermophilus* ST8 is able to use peptides larger than 3 kDa as an amino acid source

The capability of the proteinase-negative strain *S. thermophilus* ST8 to grow in CDM containing a mixture of peptides
as the sole source of amino acids was analysed. The peptides were isolated from milk and separated into three size classes by ultrafiltration. The extent of the growth of *S. thermophilus* ST8 depended on the peptide pool (Table 1).

Despite a high peptide concentration (approx. 200 μg ml⁻¹, i.e. ~300 μM), *S. thermophilus* ST8 grew only poorly in CDM-PP1. The acidic hydrolysis of the pool of peptides revealed the presence of all amino acids in the peptide sequences. In particular, the four amino acids required for the growth of *S. thermophilus* ST8, namely His, Met, Glu and Pro, were present at 55, 30, 203 and 244 μM, respectively. According to the nutritional requirements of the strain, these concentrations are expected to sustain growth up to a final population of at least 10⁸ c.f.u. ml⁻¹ (Letort & Juillard, 2001). Addition of free His (1 mM) to CDM-PP1 enhanced the growth to 9 × 10⁷ c.f.u. ml⁻¹, whereas CDM supplementation with free Pro, Met or Gln had no detectable effect. The strain under study therefore failed to use most of the His-containing peptides of PP1, whereas PP1 is able to furnish Pro, Met and Gln, at least to some extent.

The ability of *S. thermophilus* ST8 to grow to a higher population level in CDM-PP3 than in CDM-PP1 indicated that peptides larger than 1000 Da could fulfil the amino acid requirements of the strain. The final population obtained in CDM-PP3 was in the same range as that obtained in CDM-PP10 (Table 1). As (i) the strain under study is proteinase-negative (PrtS⁻) and (ii) the peptide concentration was similar in the two media (300 μM), it indicates that the strain presumably used peptides larger than 3000 Da during growth in CDM-PP10.

To establish this hypothesis, the consumption of peptides was evaluated by RP-HPLC analysis during the growth of *S. thermophilus* ST8 in CDM-PP10 enriched with free His, Pro, Gln and Met (1-0, 5-9, 2-6 and 0-8 mM, respectively). At the end of the growth, the population of ST8 was about 2 × 10⁸ c.f.u. ml⁻¹. Most of the peptides were no longer detected (Fig. 1), including peptides eluting between 52 and 70 min. These last peptides are specific to the PP10 pool, since they are not detected in the PP3 pool. They were submitted to mass spectrometry analysis. This 52–70 min region of the chromatogram contained 73 different masses, ranging from 1 to 5-5 kDa. Fifty-two of them were no longer detected after the growth of *S. thermophilus* ST8. The masses of these consumed peptides ranged from 1 to 3.5 kDa (Fig. 2). Since strain ST8 lacks a cell envelope proteinase, these peptides are most likely transported across the cell membrane. In contrast, no consumption of peptides larger than 4 kDa was observed.

**Peptide utilization by *S. thermophilus* ST8 as a function of biochemical properties**

To analyse more precisely the preferences of *S. thermophilus* ST8 for peptide utilization, the strain was grown in CDM containing a tryptic digest of αs₂-casein as the source of amino acids, instead of milk peptides of unknown sequence. The peptides resulting from the tryptic hydrolysis of αs₂-casein were identified by mass spectrometry analysis and N-terminal sequencing. The rate of peptide consumption (*T₅₀*) was defined as the time at which 50% of the peptide disappeared from the culture medium, as estimated by HPLC measurement. Note that the duration of exponential growth was 3 h only (inoculation level ~4 × 10⁶ c.f.u. ml⁻¹).

It was not possible to define a very clear effect of the size, charge and hydrophobicity of a peptide on its rate of utilization by *S. thermophilus* ST8 (Table 2). Nevertheless, some preferences could be detected. First of all, large acid peptides (24 amino acids or more; pI below 4·0) were not consumed. A group of nine peptides was consumed very rapidly, with the *T₅₀* value lower than 1·5 h. All these peptides, except one (TKLTEEKNR) had a pI value higher than 8·0. Most of them (78%) contained fewer than 12 amino acid residues. Moreover, only two peptides were highly hydrophilic. A second group of eight peptides was characterized by a low consumption rate (*T₅₀* >1·5 h). Three of them had a pI value lower than 8·0; most of them (75%) contained at least 8 amino acid residues. Last, only two of these peptides had a positive hydrophobicity index.

Therefore, it can be concluded that low molecular mass, high pI value and high hydrophobicity favour a high consumption rate of the peptide. Nevertheless, these biochemical features of the peptide are apparently not the only ones that determine the rate of consumption.

**Table 1.** Growth parameters of *S. thermophilus* ST8 in CDM, as a function of the molecular mass of the peptides used as the source of amino acids

<table>
<thead>
<tr>
<th>Growth medium*</th>
<th>Mean chain length of the peptides</th>
<th>Growth rate (h⁻¹)</th>
<th>Maximal population (c.f.u. ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDM-PP1</td>
<td>6</td>
<td>0.3 ± 0.3</td>
<td>(3.7 ± 0.5) × 10⁶</td>
</tr>
<tr>
<td>CDM-PP3</td>
<td>9</td>
<td>1.3 ± 0.4</td>
<td>(6.0 ± 0.4) × 10⁷</td>
</tr>
<tr>
<td>CDM-PP10</td>
<td>18</td>
<td>1.0 ± 0.2</td>
<td>(7.1 ± 0.5) × 10⁷</td>
</tr>
</tbody>
</table>

*CDM containing the PP1, PP3 and PP10 pools of peptides (300 μM) as the sole source of peptides, respectively.
S. thermophilus efficiently transports peptides containing 23 amino acids

To unequivocally attribute the peptide consumption by S. thermophilus ST8 to its transport across the cell membrane, transport experiments were performed by using resting cells and pure peptides. Peptide transport was estimated by measuring both the disappearance of the peptide from the external medium and the intracellular accumulation of the constituting amino acids as a result of the instantaneous hydrolysis of the transported peptide by intracellular peptidases (Kunji et al., 1995).

No significant intracellular accumulation of free amino acids could be detected when cells were incubated in the presence of KNTMEHVZZZEESIIZQETYKQEK, although a control experiment confirmed that the peptide was cleaved by a cell-free extract of S. thermophilus. (data not shown). Moreover, the external concentration of the peptide remained constant during the duration of the experiment (Fig. 3). These results indicate the inability of S. thermophilus ST8 to transport KNTMEHVZZZEESIIZQETYKQEK.

In contrast, incubation of resting cells in the presence of FPQYLQYLYQGPIVLNPWDQVKR resulted in the disappearance of the peptide from the external medium (Fig. 3). No peptidase activity could be detected in the external medium, indicating that no detectable cell lysis occurred. The disappearance of the peptide was accompanied by the internal accumulation of several amino acids constitutive of the peptide (Fig. 4). The initial rate of FPQYLQYLYQGPIVLNPWDQVKR uptake was calculated to be approx. 50 nmol min\(^{-1}\) (mg protein)\(^{-1}\) (mean of two experiments).

Extension of the study to other S. thermophilus strains

To evaluate whether the peptide transport preferences of S. thermophilus ST8 were representative of the species, ten additional proteinase-negative S. thermophilus strains were incubated in CDM containing a tryptic digest of \(\alpha\)-\(s_2\)-casein as the sole source of amino acids. The growth rates varied among the strains under study, ranging from 0-4 h\(^{-1}\) (CNRZ 302) to 1-2 h\(^{-1}\) (ST18Pr\(^+\)). The final OD\(_{600}\) varied from 0-45 (ST9) to 1-00 (ST5). Despite these variations in growth, no clear differences in the peptide composition of the growth media could be shown at the end of growth. In particular, none of the strains consumed the large anionic peptides KNTMEHVZZZEESIIZQETYKQEK and NAVPI-TPLNREQZTZENSKKTMESTEVTFTK during their growth, whereas the largest cationic peptides KNTMEHVZZZEESIIZQETYKQEK and NAVPI-TPLNREQZTZENSKKTMESTEVTFTK were systematically absent from the culture media at the end of the growth.
DISCUSSION

The preferences for peptide utilization by *S. thermophilus* were determined by analysing (i) the capability of one strain to grow in a chemically defined medium containing a mixture of peptides, (ii) the consumption of a mixture of peptides during growth, and (iii) the transport of pure peptides. These different approaches yielded the same conclusion, namely the ability of the strain under study to take up large non-acidic peptides (up to at least 23 amino acids). This unique capacity, as compared to non-lactic streptococci, seems to be a common feature of the species.

The specificity of peptide transport by *S. thermophilus* appears to be very broad. No clear rule could be shown, despite the fact that (i) large acidic peptides were not

### Table 2. Consumption rate of a tryptic digest of *β*-casein by *S. thermophilus* ST8 during growth in CDM as a function of net charge, molecular mass and hydrophobicity of the peptides

The rate of peptide consumption is defined as the time at which 50% of the peptide has disappeared from the culture medium ($T_{50}$), as estimated by HPLC analysis. NC, no consumption; Z, phosphoserine.

<table>
<thead>
<tr>
<th>Oligopeptide</th>
<th>pI</th>
<th>Molecular mass (Da)</th>
<th>Hydrophobicity</th>
<th>$T_{50}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMAINPSKENLCSFTCK</td>
<td>8.3</td>
<td>1899.9</td>
<td>-0.003</td>
<td>&lt;1</td>
</tr>
<tr>
<td>NMAINPZKENLCSFTCK</td>
<td>8.3</td>
<td>1979.8</td>
<td>-0.003</td>
<td>&lt;1</td>
</tr>
<tr>
<td>LNFLK</td>
<td>10.4</td>
<td>634.4</td>
<td>0.041</td>
<td>&lt;1</td>
</tr>
<tr>
<td>VIPYVR</td>
<td>10.5</td>
<td>746.5</td>
<td>0.039</td>
<td>0.88</td>
</tr>
<tr>
<td>FALPQYLK</td>
<td>10.3</td>
<td>979.6</td>
<td>0.031</td>
<td>0.98</td>
</tr>
<tr>
<td>NAVPITPTLNR</td>
<td>11.3</td>
<td>1195.7</td>
<td>-0.002</td>
<td>0.99</td>
</tr>
<tr>
<td>AMKPIQPK</td>
<td>10.9</td>
<td>1098.6</td>
<td>-0.002</td>
<td>1.20</td>
</tr>
<tr>
<td>TKLTEKKNR</td>
<td>7.4</td>
<td>1247.3</td>
<td>-0.084</td>
<td>1.31</td>
</tr>
<tr>
<td>TVYQHQK</td>
<td>10.3</td>
<td>903.5</td>
<td>-0.330</td>
<td>1.33</td>
</tr>
<tr>
<td>HYQK</td>
<td>10.3</td>
<td>573.3</td>
<td>-0.156</td>
<td>1.69</td>
</tr>
<tr>
<td>ALNEINQFYQK</td>
<td>7.1</td>
<td>1367.7</td>
<td>-0.006</td>
<td>1.76</td>
</tr>
<tr>
<td>EVVR</td>
<td>11.3</td>
<td>502.3</td>
<td>-0.069</td>
<td>1.77</td>
</tr>
<tr>
<td>FPOYLOLYQGPIVLNPWDQVK</td>
<td>10.1</td>
<td>2865.5</td>
<td>0.003</td>
<td>1.86</td>
</tr>
<tr>
<td>FPOYLOLYQGPIVLNPWDQVK</td>
<td>8.0</td>
<td>2709.3</td>
<td>0.008</td>
<td>2.16</td>
</tr>
<tr>
<td>LTEEKNRLNFLK</td>
<td>7.4</td>
<td>1633.0</td>
<td>-0.029</td>
<td>2.25</td>
</tr>
<tr>
<td>TKVIPYVR</td>
<td>11</td>
<td>975.6</td>
<td>-0.003</td>
<td>2.45</td>
</tr>
<tr>
<td>LTEEKNR</td>
<td>4.7</td>
<td>1018.5</td>
<td>-0.094</td>
<td>2.57</td>
</tr>
<tr>
<td>NAVPITPLNEQRLZEEKNKTVMDMSTEVFTKK</td>
<td>5.0</td>
<td>4294.0</td>
<td>-0.006</td>
<td>NC</td>
</tr>
<tr>
<td>KNTMEHVVZZZEISIQETYQKEK</td>
<td>4.5</td>
<td>3132.3</td>
<td>-0.350</td>
<td>NC</td>
</tr>
</tbody>
</table>

**Fig. 3.** Peptide transport by *S. thermophilus* ST8: time-course study of the external concentration of FPOYLOLYQGPIVLNPWDQVKR (●) and KNTMEHVVZZZEISIQETYQKEK (▲).

**Fig. 4.** Transport of FPOYLOLYQGPIVLNPWDQVKR by *S. thermophilus* ST8: internal accumulation rate of the corresponding amino acids.

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transported and (ii) a short length (1000–3500 Da), a high hydrophobicity, and a positive net charge of the peptide favoured a rapid transport. This broad specificity renders \textit{S. thermophilus} especially well adapted to its natural habitat, milk, and extremely competitive when associatively grown with \textit{Lactobacillus delbrueckii} subsp. \textit{bulgaricus} (Courtin \textit{et al}, 2002). Nevertheless, peptide transport has been reported to limit the growth rate of the strain in media containing casein-derived peptides (Letort \textit{et al}, 2002). It therefore suggests that this limitation results from kinetic considerations, presumably the rate of peptide donation from the binding protein(s) to the transmembrane complex (see below), as is the case for \textit{L. lactis} (Lanfermeijer \textit{et al}, 1999), rather than from a limited range of peptide preference.

Peptide transport by \textit{S. thermophilus} results from the activity of (at least) two distinct transport systems, but only the Ami system is capable of transporting peptides containing more than three amino acid residues (Garault \textit{et al}, 2002). Consequently, the preferences for peptide utilization we describe reflect the specificity of Ami. Peptide transport by Ami first requires its interaction with the binding protein(s). In \textit{S. thermophilus} ST18, only the inactivation of AmiA3 impairs the capability of the strain to grow on peptide-containing media (Garault \textit{et al}, 2002). Surprisingly, the number of oligopeptide-binding AmiA proteins in \textit{S. thermophilus} seems to vary between strains. \textit{S. thermophilus} ST18 has three binding proteins (Garault \textit{et al}, 2002). \textit{S. thermophilus} LMG 18311 and CNRZ 1066, whose genome sequences have just been published (Bolotin \textit{et al}, 2004), have only two oligopeptide-binding proteins. This variability is in apparent disagreement with our results, which suggest that the preferences for peptide utilization by \textit{S. thermophilus} are rather constant. This apparent discrepancy questions the role of the different oligopeptide-binding AmiA proteins of \textit{S. thermophilus}.

Substrate specificities of oligopeptide transport systems have been studied in detail in \textit{Salmonella typhimurium} and \textit{L. lactis}. \textit{S. typhimurium}. \textit{Sal. typhimurium} transports peptides of from two to five residues with little regard for sequence. Three positively charged residues (R_{139}, H_{397} and K_{331}) interact with the C-terminal carboxyl group of the liganded tri-, tetra- and pentapeptide, respectively, whereas a negatively charged residue (D_{445}) forms a salt bridge with the N-terminal \textit{z}-amino group of the bound peptide (Tame \textit{et al}, 1994, 1995). The Opp system of \textit{L. lactis} MG1363 transports oligopeptides containing from four to 35 amino acids (Doeven \textit{et al}, 2004), with preference for hydrophobic, cationic peptides (Juillard \textit{et al}, 1998). The Opp system of \textit{L. lactis} transports peptides of up to nine amino acids at least, depending on their amino acid sequence (Lamarque \textit{et al}, 2004). In that respect, the preferences for peptide utilization by \textit{S. thermophilus} resemble those of \textit{L. lactis}. Moreover, none of the positively charged residues of \textit{Sal. typhimurium} that are involved in the binding of the C-terminal part of the peptide are present in the AmiA proteins of \textit{S. thermophilus}.

In contrast, the aspartate residue is conserved in all AmiA proteins, as also in lactococcal OppA, whereas it is not conserved in lactococcal OppA.

Despite this similarity in the preferences for oligopeptide utilization, oligopeptide transport systems from \textit{S. thermophilus} (Ami) and \textit{L. lactis} (Opp) are differently organized. The transport system from \textit{S. thermophilus} consists of several different binding proteins, as in pathogenic streptococci (Alloing \textit{et al}, 1994; Jenkinson \textit{et al}, 1996), whereas that of \textit{L. lactis} has only one binding protein. In each case, the presence of the binding protein is necessary for the transport function. Nevertheless, the proposed models for the initial steps of peptide translocation by some pathogenic streptococci and lactococci are different. In \textit{L. lactis}, the peptide binds to the open form of the binding protein. Fixation of the peptide induces a conformational change of the binding protein, resulting in the partial entrainment of the peptide. The partially entrapped peptide is transferred to the transmembrane complex. In \textit{S. gordonii}, it has been proposed that peptide binding and subsequent uptake require the interaction between two binding proteins, HppA and HppP (Jenkinson \textit{et al}, 1996). In contrast, interaction between binding proteins is not required for the peptide-binding function in \textit{S. pneumoniae}: binding proteins are presumably acting independently (Alloing \textit{et al}, 1994). This second model also applies to the Opt system of \textit{L. lactis}, as the inactivation of optS does not impair the transport function of Opt (Lamarque \textit{et al}, 2004). So far, it is unclear which of these models applies to \textit{S. thermophilus}.

On the basis of both structural organization and overlapping specificities of the binding proteins, \textit{S. thermophilus} resembles \textit{S. pneumoniae}. Nevertheless, on the basis of substrate specificity, \textit{S. thermophilus} resembles \textit{L. lactis}. However, sequence comparison between the three binding proteins of \textit{S. thermophilus} and those of \textit{L. lactis} Opp and Opt reveals different percentages of homology (Table 3). In particular, the identity between AmiA of \textit{S. thermophilus} and OppA of \textit{L. lactis} is clearly not high enough to explain the close similarities of preference for peptide utilization. From these data, it is clear that peptide transport specificity cannot be deduced from the overall sequence similarity of peptide-binding proteins. However, the role of OppA in the determination of the specificity of peptide transport is clearly established (Charbonnel \textit{et al}, 2003; Doeven \textit{et al}, 2004).

<table>
<thead>
<tr>
<th>\textit{S. thermophilus}</th>
<th>\textit{S. pneumoniae}</th>
<th>\textit{L. lactis}</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmiA1</td>
<td>65-6</td>
<td>58-1</td>
</tr>
<tr>
<td>AmiA2</td>
<td>65-6</td>
<td>59-0</td>
</tr>
<tr>
<td>AmiA3</td>
<td>67-4</td>
<td>58-2</td>
</tr>
</tbody>
</table>

Table 3. Identity values (%) of AmiA proteins from \textit{S. thermophilus} ST18 with various oligopeptide-binding proteins
possible explanation could be that the binding protein(s) of S. thermophilus is not the sole determinant of the specificity of peptide transport. This hypothesis has been proposed in the case of the lactococcal Opp system (Charbonnel et al., 2003), although this point is controversial (Doeven et al., 2004). Extensive analysis of specificities of both peptide binding and peptide transport displayed by single and multiple negative mutants for the binding proteins is required to solve this intriguing question.

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