Genetic and Biochemical Characterization of the Oligopeptide Transport System of *Lactococcus lactis*

SOILE TYNKKYNEN,[1]* GIRBE BUIST,[2] EDMUND KUNJI,[3] JAN KOK,[2] BERT POOLMAN,[3] GERARD VENEMA,[2] AND ALFRED HAANDRIKMAN[2]

Research and Development Centre, Valio Ltd., P.O. Box 390, SF-00101 Helsinki, Finland,[1] and Department of Genetics[2] and Department of Microbiology,[3] University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

The nucleotide sequence of a chromosomal DNA fragment of *Lactococcus lactis* subsp. *lactis* SSL135, previously implicated in peptide utilization, has been determined. The genes *oppDFBCA*, encoding the oligopeptide transport system (Opp), and that encoding the endopeptidase PepO were located on this 8.9-kb DNA fragment. The *oppDFBCA* and *pepO* genes are probably organized in an operon. Analysis of the deduced amino acid sequences of the genes indicated that the oligopeptide transport system consists of two ATP-binding proteins OppD and OppF, two integral membrane proteins OppB and OppC, and a substrate-binding protein OppA. On the basis of the homology of OppF and OppD of *L. lactis* with other ABC (ATP-binding cassette) transporter proteins, the *L. lactis* Opp system can be classified as a member of this group. Two integration mutants, one defective in OppA and the other defective in PepO, were constructed. Growth of these mutants in a chemically defined medium with oligopeptides showed that the transport system, but not the endopeptidase, is essential for the utilization of peptides longer than three residues. Uptake of the pentapeptide Leu-enkephalin in glycolyzing lactococcal cells was followed by rapid hydrolysis of the peptide intracellularly. Importantly, extracellular hydrolysis of Leu-enkephalin is not observed. The OppA-deficient mutant was unable to transport Leu-enkephalin. Growth experiments with pasteurized milk revealed that transport of oligopeptides forms an essential part of the proteolytic system in lactococci.

For growth in milk, lactococci depend on the presence of a proteolytic system composed of a cell envelope-associated proteinase (PrtP), several peptidases, and amino acid and peptide transport systems (17, 19, 24, 42). The first step in the utilization of milk proteins (caseins) is their degradation by a proteinase to (oligo-) peptides of various sizes (32, 33, 56). All lactococcal proteinases are capable of degrading β-casein, while some strains produce a proteinase that also degrades α- and κ-casein. The size limit for peptide uptake in lactococci was suggested to be 5 or 6 amino acid residues (23, 40); longer peptides produced by PrtP are believed to be subject to further degradation by extracellular peptidases before transport into the cell can take place. Several peptidases from lactococci have been described, and most of these have been purified to homogeneity and characterized biochemically (19). The genes encoding aminopeptidase C (*pepC*) (5), aminopeptidase N (*pepN*) (45, 47, 53), X-prolyl-dipeptidyl-aminopeptidase (*pepXP*) (30, 34), endopeptidase PepO (*pepO*) (31), and tripeptidase PepT (*pepT*) (30a) have been cloned and characterized. The nucleotide sequence analyses, together with immunological studies (46) strongly suggest that PepN, PepC, PepXP, PepO, and PepT are located intracellularly.

Biochemical evidence shows that separate transport systems for amino acids, a single di- and tripeptide transporter and an oligopeptide transport system, are operative in lactococci (40, 42). The di- and tripeptide transport system is driven by the proton motive force and is essential for growth of lactococci on casein-containing media (43, 44). The gene (*dtpT*) for the di- and tripeptide transport system encodes a protein typical of secondary polytopic membrane proteins (11). The transport of oligopeptides is, most likely, coupled to ATP hydrolysis (21).

A spontaneous mutant of *Lactococcus lactis* subsp. *lactis* MG1614, MG1614V, is not capable of growth in milk, despite the presence of a functional proteinase gene. We have previously described the cloning of a chromosomal DNA fragment of *L. lactis* subsp. *lactis* SSL135 in plasmid pVS8 which is needed for rapid growth and acid production of strain MG1614V in milk (58). The chromosomal fragment in pVS8 enabled strain MG1614V to also grow on tryptic peptides of casein. Since the presence of pVS8 in MG1614V did not result in the enhanced release of amino acids from casein, it was proposed that pVS8 specified a combined peptidase-peptide transport system (51).

In this article, we present the nucleotide sequence of part of the chromosomal insert in pVS8 and demonstrate that it, indeed, encodes an endopeptidase and a functional oligopeptide transport system, as was demonstrated in peptide uptake experiments. Moreover, the genes encoding the oligopeptide-binding protein and the endopeptidase were disrupted in the chromosome of *L. lactis* to assess their roles in the utilization of casein and oligopeptides.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture media. The bacterial strains and plasmids used in this study are listed in Table 1. Lactococcal strains were grown at 30°C in M17 broth (49) supplemented with 0.5% glucose or lactose. Growth in milk was tested in pasteurized low-fat milk as described previously (58). *Escherichia coli* was grown in Luria-Bertani broth (27) at 37°C. When needed, erythromycin (5 μg/ml for *L. lactis* and 100 μg/ml for *E. coli*), chloramphenicol and rifampin (5 and 50 μg/ml, respectively, for *L. lactis*), and ampicillin (100 μg/ml for *E. coli*) were added.

---

* Corresponding author.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant description[a]	Reference or source
L. lactis subsp. *lactis*		
ML3	Lac$^+$ Prt$^+$ Opp$^+$ PepO$^+$	21, 44
MLDT1	Di- and tripeptide transport-deficient mutant of ML3	21, 44
MG1363	Lac$^-$ Prt$^-$ Opp$^+$ PepO$^+$; plasmid-free derivative of NCDO 712	8
MG1614V	Lac$^-$ Prt$^-$ PepO$^+$; plasmid-free derivative of NCDO 712; a spontaneous oligopeptide transport deficient-mutant of MG1614 (8)	51, 52, 58
VS230	Cm^r; MG1614V transformed with pVS8	58
VS772	MG1363*oppA*::pLS19A OppA$^-$ PepO$^-$	This study
VS798	MG1363*pepO*::pLS19B Opp$^+$ PepO$^-$	This study
E. coli JM109	*endA1 recA1 gyrA96 thi hsdR17* ($r_K{}^-$ $m_K{}^+$) *relA1 supE44* λ$^-$ Δ(*lac-proAB*) [F' *traD36 proAB lacIqZ*ΔM15]	61
Plasmids		
pVS8	Cm^r; contains *oppDFBCApepO*	58
pLS19	Amp^r Em^r; pUC19 carrying the *erm* gene of pE194 in the *Nde*I site	K. Leenhouts, University of Groningen
pLS19A	1,130-bp *Xba*I-*Eco*RV fragment of *oppA* ligated to pLS19	This study
pLS19B	800-bp *Pst*I-*Eco*RI fragment of *pepO* ligated to pLS19	This study

[a] Lac$^+$, lactose-fermenting phenotype; Prt$^+$, ability to produce proteinase; Opp$^+$, functional oligopeptide transport system; OppA$^-$, deficiency in the substrate-binding protein of the oligopeptide transport system; PepO$^+$ and PepO$^-$, production and lack of production of the endopeptidase PepO, respectively; Cm^r, Amp^r, and Em^r, resistance to chloramphenicol, ampicillin, and erythromycin, respectively.

General DNA techniques and DNA sequencing. Plasmid DNA and chromosomal DNA from *L. lactis* were isolated by the methods of Anderson and McKay (3) and Leenhouts et al. (25), respectively. Plasmid DNA from *E. coli* was isolated as described by Maniatis et al. (27). *L. lactis* was electroporated as described previously (15). Conjugation of lactococci was done according to the method of Gasson and Davies (9). DNA modification enzymes were obtained from Boehringer GmbH (Mannheim, Germany) and New England Biolabs Inc. (Beverly, Mass.). Fragments of the chromosomal DNA insert in pVS8 were cloned into pGEM4Z (Promega Corp., Madison, Wis.) for plasmid sequencing. Both strands were sequenced by the dideoxy chain termination method using SP6 or T7 promoter sequencing primers and the Sequenase version 2.0 DNA sequencing kit (United States Biochemical Corp., Cleveland, Ohio). Part of the sequencing was also done with synthetic oligonucleotide primers (Applied Biosystems, Inc., Foster City, Calif.) designed on the basis of known sequences. Homology searches in the EMBL Swiss-Prot protein sequence data base (release 23) were done by using the FASTA algorithm of Pearson and Lipman (35). Free energies of ribosome binding sites were calculated by the method of Tinoco et al. (50) using the lactococcal 16S-rRNA sequence (26).

Plasmid pLS19, carrying the origin of replication of pBR322, was used as an integration vector in *L. lactis* (25). The 1,130-bp *Xba*I-*Eco*RV fragment of pVS8 was ligated into pLS19 digested with *Xba*I and *Sma*I, resulting in plasmid pLS19A. Plasmid pLS19B contains the 800-bp *Pst*I-*Eco*RI fragment of pVS8 ligated to the *Pst*I-*Eco*RI fragment of pLS19. *E. coli* was used as a host for the plasmid constructions. Southern hybridizations were performed by using the Dig DNA labelling and detection kit (Boehringer GmbH) according to the instructions of the manufacturer.

Growth on leucine-containing peptides. Growth of *L. lactis* was tested in the chemically defined medium described by Poolman and Konings (38), except that cysteine and tyrosine were added at final concentrations of 250 and 287 mg/liter, respectively. Growth on leucine-containing peptides was tested in chemically defined medium lacking leucine. Leucine-containing peptides were added to final concentrations of 100 μM.

Leu-Gly, Leu-Gly-Gly, Gly-Leu-Gly-Leu, and Tyr-Gly-Gly-Phe-Leu (Leu-enkephalin) were purchased from Bachem (Bubendorf, Switzerland). Tyr-Gly-Gly-Phe-Leu-Lys (Leu-enkephalin-Lys), Ser-Ile-Gly-Ser-Leu-Ala-Lys, and Val-His-Leu-Thr-Pro-Val-Gly-Lys were purchased from Sigma Chemical Co. (St. Louis, Mo.). Growth was checked visually after an 18-h incubation at 30°C.

Transport studies. Transport of Leu-enkephalin was monitored as described by Kunji et al. (21). Prior to transport, cells were washed twice with 100 mM potassium phosphate (pH 6.5) and resuspended to a final A_{660} of approximately 25. To de-energize the cells, 2-deoxyglucose was added to a final concentration of 10 mM and the mixture was incubated for 20 min at 30°C. Cells were subsequently washed twice and resuspended in 100 mM potassium phosphate, pH 6.5. For transport assays, cells (A_{660}, ~4) were preincubated for 5 min in the presence of 0.5% (wt/vol) glucose, after which Leu-enkephalin was added to a final concentration of 1 mM. At various time intervals, cells (1 ml) were collected on a 0.45-μm-pore size cellulose acetate filter (Schleicher & Schuell GmbH, Dassel, Germany) by using a manifold filtration apparatus (Hoefer, San Francisco, Calif.) with vacuum applied by a Divac 2.4 L pump (Leybold AG, Köln, Germany). The cells were washed three times with ice-cold potassium phosphate (100 mM; pH 6.5). The filter was subsequently transferred to a vial (20 ml) (Packard, Canberry Industries, Meriden, Conn.) which contained 300 μl of 5% (vol/vol) perchloric acid and 10 mM Na-EDTA. After 30 min of incubation, 110 μl of acidic cell extract was pipetted into an Eppendorf tube containing 100 μl of 1 M KOH–1 M KHCO$_3$ to adjust the pH to 9.5. Samples were stored at −20°C. Amino acids and peptides were analyzed after derivatization with dansyl chloride, essentially by methods described by Tapuhi et al. (48) and Wiedmeier et al. (60). The dansylated neutralized cell extracts were separated by high-performance liquid chromatography as described previously (39).

Nucleotide sequence accession number. The *oppDFBCApepO* sequence has been assigned the GenBank accession number L18760.
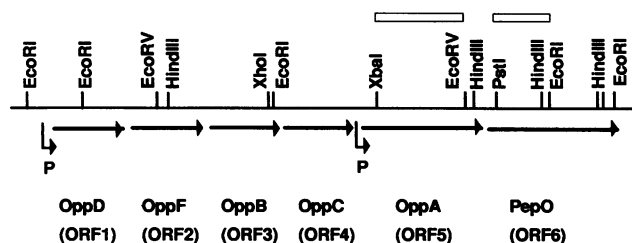
FIG. 1. Schematic representation of the sequenced chromosomal DNA region from *L. lactis* subsp. *lactis* SSL135, present in plasmid pVS8, encoding the oligopeptide transport system Opp and the endopeptidase PepO. Arrows indicate the lengths and orientations of the various genes. The positions of the putative promoter regions are indicated (P). Only relevant restriction endonuclease sites are indicated. Bars, DNA fragments used for the gene disruption studies and as probes in hybridization experiments.

RESULTS

Nucleotide sequence analysis of the chromosomal insert in pVS8. The nucleotide sequence of an 8,918-bp region of chromosomal DNA of *L. lactis* in pVS8 revealed the presence of six open reading frames (ORFs), which are schematically drawn in Fig. 1. The entire sequence is presented in Fig. 2. With the exception of ORF2 (GTG codon), all ORFs start with an ATG codon. All ORFs are preceded by possible ribosome binding sites with $\Delta G°$s ranging from -9.4 to -20.4 kcal/mol (ca. $-39,000$ to $-85,400$ J/mol). ORF1 contains 338 codons, has the potential to specify a 37.4-kDa protein, and is preceded by potential lactococcal -35 and -10 promoter sequences (54, 55). The start codon of ORF2 and the stop codon of ORF1 overlap. ORF2 contains 319 codons and could encode a 35.9-kDa protein. ORF2 and ORF3 are overlapping for seven codons. ORF3, containing 319 codons (putative protein, 35.9 kDa), and ORF4, with 294 codons (32.8-kDa protein), are separated by a 9-bp intercistronic region. ORF4 and ORF5 are separated by 109 nucleotides. A second putative promoter is found directly upstream of the fifth ORF, ORF5, which comprises 600 codons. The 65.8-kDa protein putatively encoded by ORF5 contains a signal sequence typical for prokaryotic prolipoproteins (Fig. 2) (57). ORF5 and ORF6 are separated by a 124-bp intercistronic region. ORF6 contains 627 codons (71.5-kDa putative protein). In the 370 bp downstream of ORF6, two weak stem-loop structures that could act as rho-independent terminators (-2.3 and -9.4 kcal/mol [ca. $-9,600$ and $-39,000$ J/mol, respectively]) were found (Fig. 2).

Plasmid pVS8 encodes the lactococcal oligopeptide transport system and the endopeptidase PepO. The EMBL Swiss-Prot protein sequence data base (release 23) was searched for proteins homologous to those deduced from ORF1 to ORF6. The proteins specified by ORF1 and ORF2 have significant homology with several ATP-binding proteins. The highest similarity scores were found with proteins that are part of oligopeptide transport systems. Identical amino acids were found throughout the protein sequences. The sequences conserved include the Walker A motif, the P-loop or glycine-rich loop involved in phosphoryl transfer, and the Walker motif B that is associated with many nucleotide-binding proteins (Fig. 3) (12, 59). On the basis of the similarity to the ATP-binding proteins of the oligopeptide transport system of *Salmonella typhimurium*, ORF1 and ORF2 were named OppD and OppF, respectively. Part of the alignment of OppD and OppF of *L. lactis* with the ATP-binding proteins of oligopeptide transport systems of *S. typhimurium* (14), *Bacillus subtilis* (36, 41), the

dipeptide transport system of *B. subtilis* (28), and the *ami* locus of *Streptococcus pneumoniae* (1) is shown in Fig. 3. When OppD is aligned with these four proteins, 25% identical and 48% similar residues are found. The corresponding values in the alignment for OppF are 25 and 57%, respectively. The lactococcal OppD and OppF proteins share 34.5% identical residues.

Hydropathy analyses by the method of Kyte and Doolittle (22) revealed that ORF3 and ORF4 each have six potential membrane-spanning segments (not shown). The polypeptides are homologous to OppB and OppC from *S. typhimurium* (14), AmiC and AmiD from *S. pneumoniae* (1), OppB (SpoOKB) and OppC (SpoOKC) from *B. subtilis* (36, 41), and DciAB and DciAC from the *B. subtilis* dipeptide transport system (28). On the basis of its similarity at the amino acid level with OppB, SpoOKB, AmiC, and DciAB (ranging from 27 to 32%), ORF3 was named OppB. Also, on the basis of its similarity with OppC, SpoOKC, AmiD, and DciAC (ranging from 29 to 39%), ORF4 was named OppC. Lactococcal OppB and OppC share only 21% identical residues.

Upon screening of the data base for proteins homologous to OppC, it was found that a protein identical to OppC from *L. lactis* had been cloned and sequenced by Petzel and McKay (37). These authors characterized a 1.65-kb *Xbal-Xhol* fragment from the *L. lactis* subsp. *cremoris* lactose plasmid pSK11L, which facilitated the Campbell-like integration of the thermosensitive replicating plasmid pSK11L into the lactococcal chromosome (7, 16). It appears that this entire *Xbal-Xhol* fragment is present in the lactococcal piece of DNA cloned in pVS8 (Fig. 1).

ORF5 is homologous to the substrate-binding proteins of oligopeptide transport systems. The deduced amino acid sequence of ORF5 shares 24 and 26% identical residues with the OppA proteins of *S. typhimurium* (14) and *B. subtilis* (36, 41), respectively, and 23% identical residues with AmiA from *S. pneumoniae* (1). On the basis of this homology, ORF5 was designated OppA.

The deduced amino acid sequence of ORF6 is identical to that of the lactococcal neutral endopeptidase PepO (31). Mierau et al. (31) cloned the gene encoding PepO from *L. lactis* subsp. *cremoris* P8-2-47 using antibodies raised against the purified enzyme. Although the *pepO* genes of both strains are virtually identical, the nucleotide sequences immediately downstream of the genes diverge. It was demonstrated that the expression of PepO in *E. coli* depends on the presence of a 2.5-kb region upstream of *pepO*, and Mierau et al. (31) suggested that *pepO* is part of an operon. As indicated above, a consensus lactococcal -35 and -10 region immediately upstream of *oppA* could be identified (Fig. 2). Apparently, this promoter is operative in *E. coli*, but its functionality in *L. lactis* remains to be established.

Construction of OppA- and PepO-deficient mutants of *L. lactis*. Plasmid pVS8, which contains all the genes for oligopeptide transport (*opp*) and for the endopeptidase PepO, is able to complement the deficiency of the ability of the spontaneous mutant *L. lactis* MG1614V to grow in milk. To establish whether *opp*, *pepO*, or both are critical for casein utilization, two integration mutants were constructed. Plasmid pLS19A, carrying an internal fragment of *oppA*, and plasmid pLS19B, containing an internal fragment of *pepO*, were inserted in the chromosome of the plasmid-free wild-type strain *L. lactis* MG1363. One of the erythromycin-resistant transformants obtained with each integration plasmid was analyzed by Southern hybridization. Chromosomal DNAs of strains VS772 (*oppA* disruption) and VS798 (*pepO* disruption) and that of the recipient strain MG1363 were digested with *Eco*RI or *Hind*III.

```
  1 AAAAATAAAACAATAATAAAAGCAGTTTTTAGTATGATTACTGCTTTTATTATTTCCTCCAAAACTTTTGCTTTACCTTTATTTCGCGTAATGTTCAGAA
                                                                                                     M
101 AATTCATGAACATACCTAAAATAGTAAATTTTTGCAAATATGCAGAAAAAGTAGTATACTTTTATTAAGTCTATTTAGAAAGATTTTATTGAGGTAAATA
                             -35                     -10                               RBS   OppD
      E  S  E  N  I  L  E  A  K  Q  V  S  V  A  F  R  I  A  G  K  F  Q  K  A  I  Y  D  I  D  L  S  L  K
201 TGGAAAGTGAAAATATTTTGGAAGCAAAACAAGTGAGTGTTGCTTTTCGGATTGCTGGTAAATTTCAAAAAGCAATTTATGATATTGATTTAAGTCTTAA

      R  G  E  V  L  A  I  V  G  E  S  G  S  G  K  S  T  F  A  T  A  V  M  G  L  H  N  P  N  Q  T  Q  I
301 ACGTGGTGAAGTTTTAGCTATTGTTGGGGAATCAGGTTCTGGGAAGTCAACTTTTGCAACTGCTGTTATGGGATTACATAATCCAAATCAAACTCAAATT

      T  G  S  I  L  L  D  D  E  E  V  I  G  K  T  G  D  S  M  A  S  I  R  G  S  K  V  G  M  I  F  Q  N  P
401 ACAGGCTCCATTTTATTGGATGATGAAGAAGTGATTGGTAAAACGGGTGATTCCATGGCAAGTATTCGAGGAAGTAAAGTTGGAATGATTTTTCAAAATC

      L  T  A  L  N  P  L  M  K  I  G  Q  Q  I  K  E  M  L  A  V  H  D  V  Y  P  E  N  Q  Y  E  S  R  I
501 CACTCACTGCGCTTAATCCATTGATGAAAATTGGGCAGCAAATCAAGGAAATGTTGGCCGTGCATGATGTTTATCCAGAAAATCAGTATGAAAGTAGAAT

      F  Q  L  L  E  Q  V  G  I  P  N  P  K  R  V  V  N  Q  F  P  H  Q  L  S  G  G  M  R  Q  R  V  M  I
601 CTTTCAACTTTTAGAACAAGTAGGAATTCCAAATCCTAAAAGAGTCGTTAATCAATTTCCTCACCAACTTTCAGGCGGGATGAGACAAAGAGTAATGATT

      A  I  A  I  A  N  D  P  D  L  I  I  A  D  E  P  T  T  A  L  D  V  T  I  Q  A  Q  I  L  D  L  I  L  E
701 GCAATAGCCATTGCCAATGACCCAGATTTGATTATTGCTGATGAGCCAACGACTGCTTTAGACGTTACCATTCAAGCTCAGATTTTAGACTTGATTTTAG

      I  Q  K  K  K  N  A  G  V  I  L  I  T  H  D  L  G  V  V  A  E  V  A  D  T  V  A  V  M  Y  A  G  Q
801 AAATACAAAAGAAGAAGAATGCTGGGGTTATTCTAATTACTCATGATTTAGGGGTTGTTGCTGAAGTTGCTGATACAGTGGCGGTGATGTATGCCGGACA

      L  V  E  K  T  S  V  E  E  L  F  Q  N  P  K  H  P  Y  T  R  S  L  L  R  S  N  P  S  A  E  T  V  S
901 ACTCGTCGAAAAAACTTCTGTTGAAGAGCTTTTTCAAAATCCCAAACATCCATACACCCGGTCACTTTTGCGTTCAAATCCATCTGCCGAAACAGTTTCG

       D  D  L  Y  V  I  P  G  S  V  P  S  L  S  K  I  E  Y  D  K  D  L  F  L  A  R  V  P  W  M  K  E  E  A
1001 GATGATTTATATGTGATTCCAGGGTCTGTGCCTTCTCTGTCAAAAATTGAGTATGACAAAGATTTATTTCTCGCTCGAGTGCCTTGGATGAAAGAAGAAG

       Q  K  V  I  S  E  K  M  T  E  I  S  S  N  H  F  V  R  G  Q  A  W  K  K  F  E  F  P  D  Q  K  L  K
1101 CTCAAAAAGTAATTTCGGAAAAAATGACTGAAATTTCTTCAAATCATTTTGTTCGAGGTCAGGCTTGGAAAAAATTTGAATTTCCAGATCAAAAATTGAA
       G  G  E  K  *    M  S  E  I  L  N  L  K  D  L  K  V  Y  Y  P  I  R  S  G  F  F  N  R  V  T  D  N  V  L  A
1201 AGGGGGTGAAAAGTGAGCGAAATTCTTAATTTAAAGGACTTAAAAGTTTATTATCCTATTCGATCTGGTTTCTTTAATCGAGTGACAGATAATGTTTTAG
      RBS         OppF

       V  D  G  V  D  L  T  I  H  E  G  E  T  V  G  L  V  G  E  S  G  S  G  K  S  T  I  G  K  T  I  V  G
1301 CTGTTGATGGAGTAGATTTAACGATTCATGAAGGAGAAACTGTCGGTTTAGTTGGTGAATCTGGCTCTGGAAAATCAACGATTGGCAAAACAATTGTTGG

       L  E  Q  M  T  S  G  Q  L  I  Y  K  G  Q  D  V  S  K  K  K  I  R  N  Q  L  K  Y  N  K  D  V  Q  M
1401 TTTAGAACAAATGACATCAGGACAATTGATTTACAAAGGACAAGATGTCAGCAAAAAAAAGATAAGAAACCAGCTCAAATACAATAAAGATGTTCAAATG

       I  F  Q  D  A  F  S  S  L  N  P  R  K  T  I  Y  D  I  I  A  E  P  I  R  N  F  E  K  I  D  A  N  T  E
1501 ATTTTTCAAGATGCATTTTCGAGTTTGAATCCACGGAAAACAATTTACGATATCATTGCGGAGCCTATTCGAAATTTTGAAAAATAGATGCTAATACGG

       N  K  R  I  H  E  L  L  D  I  V  G  L  P  K  Q  A  L  E  Q  Y  P  F  Q  F  S  G  G  Q  Q  Q  R  I
1601 AAAATAAACGGATTCATGAATTATTGGATATTGTTGGACTACCTAAACAAGCTTTAGAACAGTATCCTTTCCAATTTTCTGGAGGTCAACAACAAAGAAT

       G  I  A  R  A  V  A  T  N  P  K  L  I  V  A  D  E  P  V  S  A  L  D  L  S  V  Q  A  Q  V  L  N  F
1701 TGGAATCGCACGGGCAGTTGCTACTAATCCTAAGTTAATTGTGGCAGATGAACCAGTTTCAGCATTAGACTTATCTGTTCAAGCTCAGGTTTTGAATTTC

       M  K  L  I  Q  K  D  L  G  I  A  F  L  F  I  S  H  D  L  G  V  V  R  H  M  T  D  N  I  A  V  M  H  N
1801 ATGAAGCTCATTCAAAAGGACTTGGGAATTGCATTTCTTTTTATTTCTCATGATTTAGGGGTTGTCCGCCATATGACTGATAATATTGCAGTCATGCACA

       G  R  I  V  E  K  G  T  R  R  D  I  F  D  E  P  Q  H  I  Y  T  K  R  L  L  S  A  I  P  S  I  D  V
1901 ACGGTCGAATTGTCGAAAAAGGAACACGAAGAGATATTTTTGATGAGCCACAGCACATTTATACTAAACGACTCTTATCTGCTATCCCTTCGATTGATGT

       T  R  R  A  E  N  R  K  N  R  L  K  V  E  Q  D  F  E  D  K  K  A  N  F  Y  D  K  D  G  H  A  L  P
2001 TACAAGAAGAGCGGAAAATCGGAAAAACCGTCTAAAAGTTGAACAAGACTTTGAGGATAAAAAAGCAAATTTTTATGACAAAGATGGACACGCTTTGCCC
       L  K  K  L  S  E  S  H  W  A  A  L  P  K  G  G  E  N  V  E  S  N  Y  *    M  W  K  V  I  I  R  R  I  L  L  M  I  P  Q  L
2101 TTAAAAAAATTAAGTGAAAGTCATTGGGCTGCTTTGCCAAAAGGAGGGGAAAATGTGGAAAGTAATTATTAGACGTATTTTATTGATGATCCCTCAATTA
                              RBS         OppB

       F  I  L  S  I  L  V  F  F  F  A  K  L  M  P  G  D  P  F  S  G  L  I  G  P  H  T  D  P  H  E  V  E  A
2201 TTTATCTTGAGTATTCTTGTTTTCTTCTTTGCTAAATTGATGCCTGGTGATCCTTTTTCAGGGTTGATTGGTCCTCATACCGACCCACATGAAGTTGAAG

       L  R  R  A  A  G  L  Y  D  P  W  W  E  Q  Y  L  R  W  L  G  N  A  S  L  P  I  A  S  N  F  G  F
2301 CATTAAGACGGGCAGCAGGTTTATATGACCCTTGGTGGGAGCAGTATCTCAGGTGGCTAGGGAATGCCATACATGGGAATTTAGGAATGTCCTATAATCT

       K  E  P  V  M  T  V  I  G  H  R  A  I  N  T  F  W  M  S  L  L  S  V  I  L  T  Y  L  F  A  I  P  M
2401 CAAAGAGCCTGTGATGACTGTCATTGGACATAGAGCGATTAATACTTTTTGGATGTCACTTTTGTCAGTTATTTTAACTTACTTATTTGCTATTCCGATG

       S  I  V  A  A  R  N  E  G  K  W  Q  D  Q  L  W  L  T  Y  N  S  I  T  F  G  I  P  P  Y  V  F  Y  L  L
2501 TCGATAGTTGCAGCTCGAAATGAAGGAAAATGGCAAGACCAATTGTGGTTGACCTATAATTCAATTACTTTTGGTATTCCACCTTACGTATTCTATCTCT

       I  I  F  G  Y  S  L  N  W  F  P  T  G  G  T  V  S  P  D  A  M  G  I  I  P  V  F  F  S  K  I
2601 TGATTATCTTTATCTTTGGTTATAGTCTTAATTGGTTTCCGACAGGTGGGACGGTAAGTCCAGATGCGATGGGAATAATTCCTGTTTTCTTTAGTAAGAT

       Y  H  M  I  L  P  A  F  S  L  A  V  F  G  T  V  G  I  F  T  Y  F  R  S  G  I  L  D  E  Q  T  Q  D
2701 TTATCACATGATTCTTCCGGCTTTTAGTTTGGCGGTCTTTGGAACAGTTGGAATCTTTACTTACTTCCGCTCAGGAATTTTAGATGAACAAACACAAGAT

       Y  V  R  T  A  R  A  K  G  V  K  E  K  V  I  F  R  R  H  I  R  N  A  S  L  P  I  A  S  N  F  G  F
2801 TATGTACGAACGGCTCGAGCCAAAGGGGTTAAGGAAAAAGTGATTTTTAGACGTCATATTTTGAGAAATGCCTCATTACCAATTGCTTCTAATTTTGGAT

       V  I  T  G  L  L  G  G  A  I  F  A  E  T  I  F  G  Y  P  G  L  G  Q  L  F  I  T  S  I  S  G  R  D
2901 TTGTGATTACTGGACTCTTGGGAGGAGCAATCTTTGCTGAAACAATTTTCGGGCTATCCTGGCTTAGGACAACTTTTTATTACTTCAATATCTGGGCGAGA

       Y  S  M  I  T  A  L  I  L  L  N  G  F  S  G  L  L  G  A  L  L  P  D  I  I  M  A  M  V  D  P  R  I
3001 TTATTCAATGATTACGGCTTTGATTTTATTAAATGGTTTTTCGGGACTTCTTGGAGCCCTCCTGCCGGATATTATCATGGCAATGGTTGACCCACGAATT
       R  I  Q  *    M  T  E  K  K  H  K  N  S  L  S  L  V  H  S  I  K  E  E  L  K  K  D  K  L  A  M
3101 CGGATTCAATAAGGAGTGAAATGACAGAAAAAAAAACACAAAAATTCTTTATCATTAGTTCACTCAATCAAAGAAGAACTAAAAAAAGATAAATTAGCGA
                      RBS         OppC

       I  S  T  I  F  L  V  A  V  F  L  I  V  Y  I  Y  S  M  F  L  K  Q  S  N  Y  V  D  V  N  I  M  D  Q
3201 TGATTTCAACAATTTTTCTAGTTGCTGTCTTTCTAATCGTTTATATTTACTCGATGTTTTTAAAACAATCAAATTATGTTGATGTGAATATCATGGACCA

       Y  L  A  P  L  T  N  G  H  L  L  G  T  D  N  G  G  R  D  I  I  M  M  L  M  I  S  A  R  N  S  F  N
3301 ATATCTGGCACCGCTGACGAATGGACATTTACTGGGAACTGATAATGGTGGACGAGATATTATCATGATGTTAATGATTTCTGCACGAAACTCTTTCAAT

       I  A  F  A  V  T  L  I  T  L  V  V  G  N  I  L  G  V  I  T  G  Y  F  G  G  R  F  D  L  I  F  M  R  F
3401 ATCGCTTTTGCAGTTACACTCATTACTTTAGTTGTAGGAAATATCTTAGGGGTAATTACGGGCTACTTTGGCGGAAGGTTTGATTTAATCTTCATGCGTT

       T  D  F  V  M  I  L  P  S  M  M  I  I  I  V  F  V  T  I  I  P  R  F  N  S  W  S  L  I  G  I  I  S
3501 TCACTGATTTTGTCATGATTTTACCATCAATGATGATCATTATTGTTTTTGTAACTATCATCCCACGGTTTAATTCTTGGTCTTTGATTGGGATTATCAG
```

FIG. 2. Nucleotide and deduced amino acid sequences of the L. lactis subsp. lactis SSL135 oligopeptide transport system and the endopeptidase PepO. The putative ribosome binding sites (RBS) and promoter regions are boldfaced and underlined, respectively. The consensus lipoprotein signal peptidase cleavage site in OppA is also underlined. Possible stem-loop structures are indicated (dashed arrows).

The 1,130-bp XbaI-EcoRV fragment of pVS8 (Fig. 1) was used as a probe in the analysis of the oppA mutant VS772 (Fig. 4), whereas the 800-bp PstI-EcoRI fragment (Fig. 1) was used as a probe to analyze the pepO mutant VS798 (Fig. 5). The hybridizing restriction fragments of the chromosomal DNAs of both mutants have sizes expected when the oppA and pepO genes are disrupted by integration. Both mutants were used to assess the role of OppA and PepO in peptide transport, peptide degradation, and growth in milk (see below).

opp genes encode a functional oligopeptide transport system. The uptakes of Leu-enkephalin in different strains of L. lactis were compared to demonstrate that opp is involved in oligopeptide uptake. Addition of Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) to glucose-metabolizing cells of the wild-type strain L. lactis ML3 resulted in the accumulation of the corresponding amino acid residues inside the cell (Fig. 6A). Apparently, Leu-enkephalin is rapidly hydrolyzed by high intracellular aminopeptidase, endopeptidase, and/or other peptidase activities. Changes in internal concentrations of other amino acids were not observed (data not shown). Accumulation of amino acid residues from Leu-enkephalin was observed neither in L. lactis MG1614V (Fig. 6B), which lacks

```
      I  F  S  W  I  G  T  T  R  L  I  R  A  R  T  M  T  E  V  N  R  D  Y  V  R  A  S  K  T  S  G  T  S
3601 TATCTTTAGTTGGATAGGGACAACGCGTCTGATTCGGGCAAGAACAATGACGGAAGTCAATCGAGATTATGTTCGAGCATCAAAAACTTCGGGAACCTCT

      D  F  K  I  M  F  R  E  I  W  P  N  L  S  T  L  V  I  A  E  A  T  L  V  F  A  G  N  I  G  L  E  T  G
3701 GATTTTAAAATTATGTTTCGGGAAATATGGCCCAACTTGTCCACCTTAGTCATTGCTGAAGCAACGCTTGTTTTTGCTGGAAATATTGGTTTAGAAACAG

      L  S  F  L  G  F  G  L  P  A  G  T  P  S  L  G  T  M  I  N  E  A  T  N  P  E  T  M  T  D  K  P  W
3801 GGCTTTCTTTCTTAGGTTTTGGACTTCCAGCGGGGACACCATCTTTGGGAACAATGATAAATGAAGCGACTAATCCAGAAACAATGACTGATAAGCCTTG

      T  W  V  P  A  T  V  V  I  L  I  V  V  L  A  I  I  F  I  G  N  A  L  R  R  V  A  D  Q  R  Q  A  T
3901 GACTTGGGTTCCAGCAACAGTGGTCATCCTAATTGTGGTGCTTGCTATTATCTTTATCGGAAATGCACTAAGACGAGTGGCTGACCAAAGACAAGCTACA

      R  *
4001 AGATAAACAACATTCAAGAGTGATAAAAATGTTTATCTATTAAAAATGGATATGATAAATTAAATATTATATTGAAGCAAGCAAGAAATAAAAAGAAATT
                                               -35                    -10

            M  N  K  L  K  V  T  L  L  A  S  S  V  V  L  A  A  T  L  L  S  A  ↓C  G  S  N  Q  S  S
4101 ATTGGAGAAACTAAGATGAACAAATTAAAAGTAACTTTATTGGCAAGTAGTGTAGTTTTAGCAGCTACACTCCTAAGTGCTTGTGGTTCTAATCAAAGCT
     RBS            OppA

      S  T  S  T  K  K  L  K  A  G  N  F  D  V  A  Y  Q  N  P  D  K  I  K  G  N  L  K  V  A  Y  Q
4201 CAAGTACAAGTACAAAAAAATTAAAAGCGGGGAACTTTGACGTTGCTTATCAAAATCCAGACAAGGCAATCAAAGGTGGAAATTTAAAAGTCGCATATCA

      S  D  S  P  M  K  A  Q  W  L  S  G  L  S  N  D  A  T  F  A  T  M  S  G  P  G  G  G  Q  D  G  L  F
4301 AAGTGATTCTCCAATGAAAGCACAATGGTTATCGGGACTTTCTAATGATGCAACTTTTGCTACAATGTCTGGTCCTGGTGGAGGTCAAGATGGTTTGTTC

      F  T  D  S  G  F  K  F  I  K  G  G  A  A  D  V  A  L  D  K  E  S  K  T  A  T  I  T  L  R  K  D  L  K
4401 TTCACAGACAGTGGATTCAAATTTATTAAGGGAGGAGCTGCCGATGTTGCTCTAGATAAAGAGTCTAAAACGGCAACGATTACCCTTCGTAAAGATTTGA

      W  S  D  G  S  E  V  T  A  K  D  Y  E  F  T  Y  E  T  I  A  N  P  A  Y  G  S  D  R  W  T  D  S  L
4501 AATGGTCTGATGGTTCAGAAGTAACGGCTAAAGATTATGAATTTACCTATGAAACGATTGCTAATCCTGCTTATGGTTCCGACCGTTGGACTGATTCTCT

      A  N  I  V  G  L  S  D  Y  H  T  G  K  A  K  T  I  S  G  I  T  F  P  D  G  E  N  G  K  V  I  K  V
4601 GGCAAATATTGTTGGCTTGAGTGATTATCATACGGGTAAAGCTAAAACAATCTCAGGGATCACTTTCCCTGATGGTGAAAATGGAAAAGTCATCAAGGTT

      Q  F  K  E  M  K  P  G  M  T  Q  S  G  N  G  Y  F  L  E  T  V  A  P  Y  Q  Y  L  K  D  V  A  P  K  D
4701 CAATTTAAAGAAATGAAACCGGGGATGACTCAATCAGGGAATGGTTACTTCCTTGAAACGGTAGCGCCTTATCATATTGAAAGATGTCGCTCCTAAAG

      L  A  S  S  P  K  T  T  T  K  P  L  V  T  G  P  F  K  P  E  N  V  V  A  G  E  S  I  K  Y  V  P  N
4801 ATTTGGCTTCTAGTCCAAAAACGACAACGAAACCATTAGTTACAGGGCCTTTTAAACCGGAAAATGTTGTTGCTGGTGAATCTATTAAATATGTCCCAAA

      P  Y  Y  W  G  E  K  P  K  L  N  S  I  T  Y  E  V  V  S  T  A  K  S  V  A  A  L  S  S  S  K  Y  D
4901 TCCTTACTATTGGGGTGAAAAACCAAAACTAAATTCAATTACTTATGAAGTTGTATCAACAGCAAAATCAGTCGCAGCACTTTCATCAAGTAAATATGAT

      I  I  N  G  M  V  S  S  Q  Y  K  Q  V  K  N  L  K  G  Y  K  V  L  G  Q  Q  A  M  Y  I  S  L  M  Y  Y
5001 ATTATTAACGGCATGGTTAGTAGCCAGTATAAGCAAGTGAAAAACTTAAAGGGATACAAGGTTTTAGGACAACAAGCGATGTATATTTCCTTAATGTACT

      N  L  G  H  Y  D  A  K  N  S  I  N  V  Q  D  R  K  T  P  L  Q  D  Q  N  V  R  Q  A  I  G  Y  A  R
5101 ATAACTTAGGGCACTATGATGCTAAAAATTCAATTAATGTCCAAGATCGTAAAACGCCATTGCAAGACCAAAATGTTCGTCAAGCCATTGGTTATGCTCG

      N  V  A  E  V  D  N  K  F  S  N  G  L  S  T  P  A  N  S  L  I  P  P  I  F  K  Q  F  T  S  S  V
5201 AAATGTCGCAGAAGTGGATAATAAGTTCTCAAACGGACTTTCAACTCCTGCAAATAGTTTGATTCCACCTATCTTTAAACAATTTACGAGTTCATCAGTT

      K  G  Y  E  K  Q  D  L  D  K  A  N  K  L  L  D  E  D  G  W  K  L  N  K  S  T  G  Y  R  E  K  D  G  K
5301 AAAGGATATGAAAAACAAGATCTTGATAAAGCTAATAAAACTTTTGGATGAAGACGGATGGAAATTGAATAAATCTACAGGCTACCGTGAAAAAGATGGAA

      E  L  S  L  V  Y  A  A  R  V  G  D  A  N  A  E  T  I  A  Q  N  Y  I  Q  Q  W  K  K  I  G  V  K  V
5401 AAGAATTATCGCTTGTTTATGCGGCTCGTGTAGGTGATGCAAACGCTGAAACCATTGCCCAAAACTATATCCAACAATGGAAGAAAATTGGGGTAAAAGT

      S  L  Y  N  G  K  L  M  E  F  N  S  W  V  D  H  M  T  T  P  P  G  A  N  D  W  D  I  T  D  G  S  W
5501 GAGTCTTTATAACGGTAAATTGATGGAATTTAATTCTTGGGTCGACCATATGACGACTCCTCCAGGAGCAAATGACTGGGATATCACTGACGGTTCTTGG

      S  L  A  S  E  P  S  Q  Q  D  L  F  S  A  A  A  P  Y  N  F  G  H  F  N  D  S  E  I  T  K  D  L  N  D
5601 TCATTGGCATCTGAACCTTCTCAACAAGATTTGTTCTCAGCAGCAGCACCTTATAACTTTGGTCACTTTAATGATTCAGAAATTACTAAGGATTTAAATG

      I  D  S  A  K  S  E  N  P  T  Y  R  K  A  A  F  V  K  Y  Q  E  D  M  N  K  K  A  Y  V  I  P  T  N
5701 ATATTGATTCTGCCAAGTCTGAAAATCCAACTTACCGTAAAGCAGCTTTCGTTAAATATCAAGAAGATATGAATAAAAAAGCTTATGTGATTCCAACTAA

      F  M  L  N  Y  T  P  V  N  K  R  V  V  G  M  T  L  D  Y  G  A  M  N  T  W  S  E  I  G  V  S  S  A
5801 CTTTATGTTGAATTATACTCCTGTTAACAAACGTGTAGTTGGAATGACGCTTGATTATGGTGCAATGAATACTTGGTCAGAAATTGGTGTTTCTTCTGCT

      K  L  A  T  K  *
5901 AAGTTGGCCACCAAATAGTTTTACTTAACTTTTGTTTACAAGTTTTTTTGTAAAAATACCCTCACTTTAGTTTTCGCAATTCTAAAGTCACAGAAGTTCA

                                                      M  T  R  I  Q  D  D  L  F  A  T  V  N  A  E  W  L  E  N  A
6001 AATTTATGATTTGAGCTTCTTTTATTAAAAAAGGAGTTTGATATGACAAGGATTCAAGATGATTTATTCGCTACTGTTAATGCTGAATGGTTAGAAAATG
                                    RBS        PepO

      E  I  P  A  D  K  P  R  I  S  A  F  D  E  L  V  L  K  N  E  K  N  L  A  K  D  L  A  D  L  S  Q  N
6101 CAGAAATTCCTGCAGACAAACCAAGAATTTCAGCTTTTGATGAATTAGTACTAAAAAATGAGAAAAATTTGGCTAAAGATTTAGCTGATTTATCACAAAA

      L  P  T  D  N  P  E  L  L  E  A  I  K  F  Y  N  K  A  G  D  W  Q  A  R  E  K  A  D  F  S  A  V  K
6201 CCTACCTACTGATAATCCAGAGTTGCTTGAAGCAATCAAATTTTATAATAAAGCAGGAGATTGGCAAGCAAGAGAAAAAGCGGATTTTTCTGCCGTAAAA

      N  E  L  A  K  V  E  T  L  N  T  F  E  D  F  K  N  N  L  T  Q  L  V  F  H  S  Q  A  P  L  P  F  S  F
6301 AATGAACTTGCTAAAGTTGAAACTTTAAATACTTTTGAAGATTTTAAAAATAATTTGACTCAACTCGTTTTCCATTCACAGGCTCCTCTTCCATTTTCTT

      S  V  E  P  D  M  K  D  A  I  H  Y  S  L  G  F  S  G  P  G  L  I  L  P  D  T  T  Y  Y  N  D  E  H
6401 TTAGTGTTGAACCTGATATGAAAGATGCTATTCACTATTCTCTTGGATTCTCTGGTCCGGGCTTGATTTTACCTGATACCACTTATTATAATGATGAACA

      P  R  K  K  E  L  L  D  F  W  A  K  N  T  S  E  I  L  K  T  F  D  V  E  N  A  E  E  I  A  K  S  A
6501 CCCTAGAAAAAAAGAATTACTTGATTTTTGGGCTAAAAATACAAGCGAAATTTTAAAGACATTTGATGTTGAAAATGCAGAAGAGATTGCAAAGTCTGCC

      L  K  F  D  A  L  L  V  P  S  A  N  T  S  E  E  W  A  K  Y  A  E  L  Y  H  P  I  S  T  D  N  F  V  S
6601 CTTAAATTCGATGCTGTCTTTTAGTTCCATCAGCAAATACTTCTGAAGAGTGGGCAAAATATGCTGAACTTTATCATCCAATTTCTACTGACAACTTTGTCA

      K  V  K  N  L  D  L  K  S  L  I  K  D  L  V  K  T  E  P  D  K  V  I  V  Y  E  D  R  F  Y  E  S  F
6701 GTAAAGTTAAAAACCTGGATTTAAAATCTTTAATTAAAGATTTAGTAAAAACTGAACCTGATAAAGTCATTGTTTATGAAGACCGTTTTTACGAATCATT

      D  S  L  I  N  E  E  N  W  S  L  I  K  A  W  M  L  T  K  I  A  R  G  A  T  S  F  F  N  E  D  L  R
6801 TGATTCACTTATCAATGAAGAAAATTGGTCACTCATTAAAGCTTGGATGCTGACTAAAATTGCACGTGGCGCTACTTCTTTCTTCAATGAAGACCTTAGA

      I  L  G  G  A  Y  G  R  F  L  S  N  V  Q  E  A  R  S  Q  K  H  Q  L  D  L  T  E  S  Y  F  S  Q  V
6901 ATTCTTGGTGGAGCTTACGGACGTTTCCTTTCAAATGTTCAAGAAGCAAGAGTCAAGAAAAACATCAACTTGATTTGACTGAGTCTTATTTTAGCCAAG

      I  G  L  F  Y  G  K  K  Y  F  G  E  A  A  K  A  D  V  K  R  M  V  T  A  M  I  K  V  Y  Q  V  R  L
7001 TGATTGGTTTATTCTATGGTAAAAAATATTTTGGTGAAGCTGCTAAGGCCGATGTCAAACGGATGGTTACTGCGATGATTAAAGTTTACCAAGTGCGTTT

      S  K  N  E  W  L  S  Q  E  T  A  E  K  A  I  E  K  L  D  A  I  T  P  F  I  G  F  P  D  K  L  P  E
7101 GTCTAAAAATGAGTGGCTTAGTCAAGAAACAGCTGAAAAAGCCATTGAAAAATTGGATGCTATTACTCCTTTCATTGGTTTCCCAGATAAATTGCCTGAA

      I  Y  S  R  L  K  T  T  S  G  S  L  Y  E  D  A  L  K  F  D  K  I  L  T  A  R  T  F  E  K  F  S  E  D
7201 ATTTATAGTCGTTTAAAAACAACTTCTGGCTCTCTCTATGAAGATGCCCTTAAATTCGATAAAATTTTGACTGCTCGAACTTTTGAAAAATTCTCAGAAG

      V  D  K  T  S  W  H  M  P  A  H  M  V  N  A  Y  Y  S  P  D  S  N  T  I  V  F  P  A  A  I  L  Q  A
7301 ATGTTGATAAAACAAGCTGGCATATGCCTGCTCATATGGTCAATGCTTATTATAGTCCTGATAGTAATACAATTGTTTTTCCAGCAGCAATTTTGCAAGC

      P  F  Y  S  L  E  Q  S  S  S  Q  N  Y  G  G  I  G  A  V  I  A  H  E  I  S  H  A  F  D  N  N  G  A
7401 TCCTTTTTACTCTCTTGAACAATCTTCATCACAAAATTACGGTGGAATTGGCGCTGTCATTGCTCATGAAATTTCTCATGCTTTTGATAACAACGGTGCC

      Q  F  D  K  E  G  N  L  N  K  W  W  L  D  E  D  Y  E  A  F  E  E  K  Q  K  E  M  I  A  L  F  D  G  V
7501 CAATTCGATAAAGAAGGAAATTTGAACAAATGGTGGTTAGATGAAGATTATGAAGCTTTTGAAGAAAAGCAAAAAGAAATGATTGCACTCTTTGACGGCG

      E  T  E  A  G  P  A  N  G  K  L  I  V  S  E  N  I  A  D  Q  G  G  I  T  A  A  L  T  A  A  K  D  E
7601 TAGAAACTGAAGCTGGTCCAGCAAACGGAAAACTCATTGTGTCAGAAAATATTGCTGACCAAGGAGGAATTACAGCGGCACTGACAGCCGCTAAAGATGA

      K  D  V  D  L  K  A  F  F  S  Q  W  A  K  I  W  R  M  K  A  S  K  E  F  Q  Q  M  L  L  S  M  D  V
7701 GAAAGACGTTGATTTGAAAGCTTTCTTTAGTCAGTGGGCTAAGATTTGGCGCATGAAAGCAAGTAAAGAATTCCAACAAATGCTTTTGTCAATGGATGTT

      H  A  P  A  K  L  R  A  N  I  P  P  T  N  L  E  E  F  Y  E  T  F  D  V  K  E  T  D  K  M  Y  R  A  P
7801 CATGCACCCGCTAAACTTCGTGCCAATATTCCTCCAACAAATCTCGAAGAATTTTACGAAACTTTTGATGTTAAAGAAACAGATAAGATGTATCGTGCAC

      E  N  R  L  K  I  W  *
7901 CAGAAAATCGTCTTAAAATTTGGTAATCATTTAATTTATATTAATGATATATTGAAATTGAGTCAAATAATTTAGAAAAAGCTCCTATAATCACAAAGTT
         ----->    <-----
8001 ATGAGTGCTTTTTCCACTTTTTGATATATTATAAAAAGAGTTATTGCTAGCACGGTTAGGACACCAGAGTATACGCCAATGTCATTAAGTTAAGGAAAAC
     ----->      <----------

8101 AGAAAAACAGGAAGGATCTAATCACCTCCTGTTTTTATTTTATTCTAAGCAAGAAGAACACCCTCTTGATACAATTATTTTATCAATAATAAAACGTCAAA
     ---------->      <----------

8201 GCGTTACGTATTCTGGAGGGATGTCCTATTGCTTAATACTACCAAAGTTCCATCAACACTTCAAGTGTCTCATCAAATAAAGAAAAACTTAGAAGATC
```

FIG. 2—*Continued.*

```
OPPF        ETVGLVGESGSGKSTIGKTIVGLEQMTSGQLIYKGQDVSKKKIRNQLK-Y          90
OPPFSALTY   ETLGVVGESGCGKSTFARAIIGLVKATDGKVAWLGKDLLGMKA-DEWREV          99
SPKEBACSU   ETLGLVGESGCGKSTTGRSIIRLYEATDGEVLFNGENVHGRKSRKKLLEF          88
AMIFSTRPN   ETFSLVGESGSGKTTIGRAIIGLNDTSNGDIIFDGQKINGKKSREQAAEL          85
                  WA
            **  ..*****.**.*  ....*. * ...*... * .....  .* ..
```

```
OPPF        NKDVQMIFQDAFSSLNPRKTIYDIIAEPIRNFEKIDANTENK-RIHELLD          139
OPPFSALTY   RSDIQMIFQDPLASLNPRMTIGEIIAEPLRTYHPKLSRQDVRDRVKAMML          149
SPKEBACSU   NRKMQMIFQDPYASLNPRMTVADIIAEGLDIHKLAKTKKERMQRVHELLE          138
AMIFSTRPN   IRRIQMIFQDPAASLNERATVDYIISEGLYNHRLFKDEEERKEKVQSIIR          135
            . .******. .*** * *. **.* .     . ........
```

```
OPPF        IVGLPKQALEQYPFQFSGGQQQRIGIARAVATNPKLIVADEPVSALDLSV          189
OPPFSALTY   KVGLLPNLINRYPHEFSGGQCQRIGIARALILEPKLIICDDAVSALDVSI          199
SPKEBACSU   TVGLNKEHANRYPHEFSGGQRQRIGIARALAVDPEFIIADEPISALDVSI          188
AMIFSTRPN   EVGLLAEHLTRYPHEFSGGQRQRIGIARALVMQPDFVIADEPISALDVSV          185
                                               WB
            ***  .  ..** .***** ********.  .*..... *...****.*.
```

```
OPPD        AIVGESGSGKSTFATAVMGLHNPNQTQIT-GSILLDDEEVIGKTGDS-MA          87
OPPDSALTY   GIVGESGSGKSQTAFALMGLIA-TNGRIG-GSATFNGREILNLP-ERELN          97
AMIESTRPN   ALVGESGSGKSVLTKTFRGMLE-ENGRIAEGSIDYRGQDLTALSSHKDWE          90
SPKDBACSU   AIVGESGSGKSVTSQAIMKLIPMPPGYFKRGEILFEGKDLVPLS-EKEMQ          89
DCIAD       AIVGESGCGKSVTSQSIMGLLPPYSAKVTDGRILFKNKDLCRLS-DKEMR          86
                WA
            ..*****.***   ....    .  *... ...
```

```
OPPD        SIRGSKVGMIFQNPLTALNPLMKIGQQIKEMLAVHDVYPENQYESRIFQL          137
OPPDSALTY   TLRAEQISMIFQDPMTSLNPYMRVGEQLMEVLMLHKGMSKAEAFEESVRM          147
AMIESTRPN   QIRGAKIATIFQDPMTSLDPIKTIGSQITEVIVKHQGKTAKEAKELAIDY          140
SPKDBACSU   NVRGKEIGMIFQDPMTSLNPTMKVGKQITEVLFKHEKISKEAAKKRAVEL          139
DCIAD       GIRGADISMIFQDPMTALNPTLTVGDQLGEALLRHKKMSKKAARKEVLSM          136
            .*. ... ***.*.*.*.*  .* *.. *.  *. ...
```

```
OPPD        LEQVGIPNPKRVVNQFPHQLSGGMRQRVMIAIAIANDPDLIIADEPTTAL          187
OPPDSALTY   LDAVKMPEARKRMKMYPHEFSGGMRQRVMIAMALLCRPKLLIADEPTTAL          197
AMIESTRPN   MNKVGIPDADRRFNEYPFQYSGGMRQRIVIAIALACRPDVLICDEPTTAL          190
SPKDBACSU   LELVGIPMPEKRVNQFPHEFSGGMRQRVVIAMALAANPKLLIADEPTTAL          189
DCIAD       LSLVGIPDPGERLKQYPHQFSGGMRQRIVIAMALICEPDILIADEPTTAL          186
                                                  WB
            .. * .* .    . .* . *******..**.*.  *...* *******
```

FIG. 3. Part of the alignment of the deduced amino acid sequences of ATP-binding proteins from various oligopeptide transport systems: OppF and OppD from *L. lactis*, OppF and OppD from *S. typhimurium* (SALTY), SpoOKE and SpoOKD from *B. subtilis* (BACSU), AmiF and AmiE from *S. pneumoniae* (STRPN), and DciAD, the dipeptide transport system of *B. subtilis*. Identical (asterisks) and similar (dots) residues are indicated. The two Walker motifs, A (WA) and B (WB), characteristics of many nucleotide-binding proteins, are also indicated.

the entire *opp* operon, nor in the *oppA* disruption mutant VS772 (Fig. 6D). Interestingly, *L. lactis* carrying the *opp* operon on plasmid pVS8 (strain VS230) accumulates the amino acid residues with a much higher rate than the wild-type strain, in which the *opp* operon is located on the chromosome (Fig. 6C).

The uptake rates based on tyrosine and glycine accumulation (the latter value was divided by two, because Leu-enkephalin contains two glycine moieties) were approximately the same, suggesting that these residues are transported across the cytoplasmic membrane in the form of the pentapeptide Leu-enkephalin (Fig. 6). The apparent uptake rates and final accumulation levels of leucine and phenylalanine are somewhat lower than those of tyrosine and glycine. Based on the rates of tyrosine and glycine accumulation, the rates of Leu-enkephalin uptake were 56 and 20 nmol min^{-1} mg of protein^{-1} for the strain carrying *opp* on a plasmid (VS230) and the wild-type strain ML3, respectively. From these results, we conclude that pVS8, indeed, encodes an oligopeptide transport system. Apparently, the expression of the *opp* genes is not tightly controlled, since an increase of the number of copies of the *opp* genes led to an enhanced uptake rate.

Opp is essential for uptake of peptides larger than 3 amino acids and for growth in milk. Leucine is essential for growth of *L. lactis* ML3, MG1363, and MG1614V (42, 52). To establish the role of the oligopeptide transport system and the endopeptidase in the utilization of peptides, growth of wild-type and mutant lactococcal strains was tested in a chemically defined medium in which leucine was replaced by leucine-containing peptides. As is illustrated in Table 2, all strains tested were able to grow on di- and tripeptides, except for the ML3-derived di- and tripeptide transport mutant (21, 44). The fact that the di- and tripeptide transport mutant of ML3 (strain MLDT1) can grow on oligopeptides supports the view that the growth observed on these peptides does not result from extracellular breakdown and subsequent uptake of smaller peptides. The oligopeptide transport mutant *L. lactis* MG1614V was unable to use peptides longer than 3 residues, whereas the parental strain MG1363 grew on all peptides provided. The phenotype of the mutant MG1614V can be restored to wild type by the introduction of the *opp* genes on plasmid pVS8: strain VS230 grows on all Leu-containing peptides. When the chromosomal gene for the substrate-binding protein OppA is interrupted, as is the case in strain VS772, only growth on di- and tripeptides was observed. Growth of the *pepO* integration mutant VS798 was similar to that of MG1363. These results show that the oligopeptide transport system is essential for growth on peptides larger than 3 residues, whereas the endopeptidase PepO does not appear to be essential for oligopeptide utilization. As the strains used so far lacked both the proteinase and the lactose genes, the possible requirement of the *opp* and *pepO* genes for growth in milk could not be examined. Therefore, the lactose-proteinase plasmid pLP712 was introduced in the
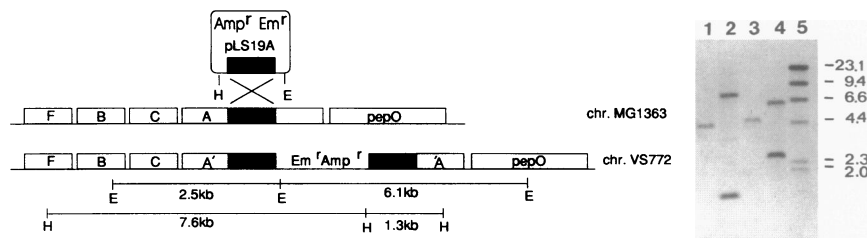

FIG. 4. Schematic representation of Campbell-like integration of plasmid pLS19A into the chromosomal (chr.) *oppA* gene in *L. lactis* MG1363 (left) and Southern hybridization of chromosomal DNA from the parental strain MG1363 and the integrant VS772 digested with *Eco*RI and *Hin*dIII (right). The 1,130-bp *Xba*I-*Eco*RV fragment of pVS8 was used as a probe. The internal fragment of *oppA* present in pLS19A is represented by a shaded bar. The sizes of the expected hybridizing restriction fragments of the integrant, VS772, are indicated. The parental strain MG1363 is expected to give a hybridizing band of 3.8 kb with *Eco*RI and 4.1 kb with *Hin*dIII. Lanes: 1, MG1363 digested with *Hin*dIII; 2, VS772 digested with *Hin*dIII; 3, MG1363 digested with *Eco*RI; 4, VS772 digested with *Eco*RI. Phage lambda DNA digested with *Hin*dIII was used as a marker (lane 5); sizes in kilobases are indicated on the right. E, *Eco*RI; H, *Hin*dIII.
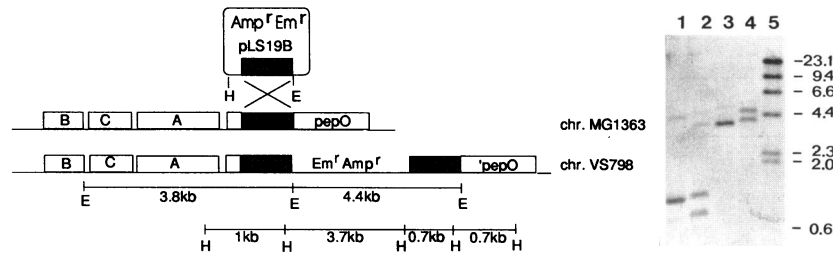
FIG. 5. Schematic representation of the integration of plasmid pLS19B into the chromosome (chr.) of *L. lactis* MG1363 (left) and Southern hybridization of chromosomal DNA from the parental strain MG1363 and the integrant VS798 digested with *Eco*RI and *Hin*dIII (right). The internal fragment of *pepO* present in pLS19B is represented by a shaded bar. The same 800-bp *Pst*I-*Eco*RI fragment of pVS8 was used as a probe. The sizes of the expected hybridizing restriction fragments of the integrant VS798 are shown. The parental strain MG1363 is expected to give hybridizing bands of 3.8 kb with *Eco*RI and 1 and 0.7 kb with *Hin*dIII. Lanes: 1, MG1363 digested with *Hin*dIII; 2, VS798 digested with *Hin*dIII; 3, MG1363 digested with *Eco*RI; 4, VS798 digested with *Eco*RI. Phage lambda DNA digested with *Hin*dIII was used as a marker (lane 5); sizes in kilobases are indicated on the right. E, *Eco*RI; H, *Hin*dIII.

various strains by conjugation, by using *L. lactis* subsp. *lactis* NCDO 712 as a donor (8). Growth in milk of the transconjugants was monitored by a plating assay and by measuring the acid production. The results (Fig. 7) compare well to those obtained in the chemically defined medium. The wild-type strain MG1363, MG1614V carrying multiple copies of *opp* and *pepO* on pVS8 (VS230), and the endopeptidase-deficient mutant VS798 grew well in milk. The *opp*-deficient strain MG1614V did not grow in milk. As is illustrated in Fig. 7, strain VS772 (OppA⁻) did grow to some extent, albeit extremely slowly. Apparently, the *oppA* mutation results in a leaky phenotype. These results indicate that oligopeptide transport is essential for rapid growth of lactococci in milk.

DISCUSSION

We have sequenced over 8.9 kb of chromosomal DNA of *L. lactis* subsp. *lactis* SSL135 and have proven that it encodes a functional oligopeptide transport system (Opp) and an endopeptidase (PepO). Because of their tight genetic organization, the genes *oppDFBCApepO* seem to be located in an operon-like structure. Putative promoters are present in the nucleotide sequences immediately upstream of *oppD* and *oppA*. Using in vitro transcription-translation studies, at least four proteins were shown to be encoded by this chromosomal DNA fragment as present in pVS8 (52).

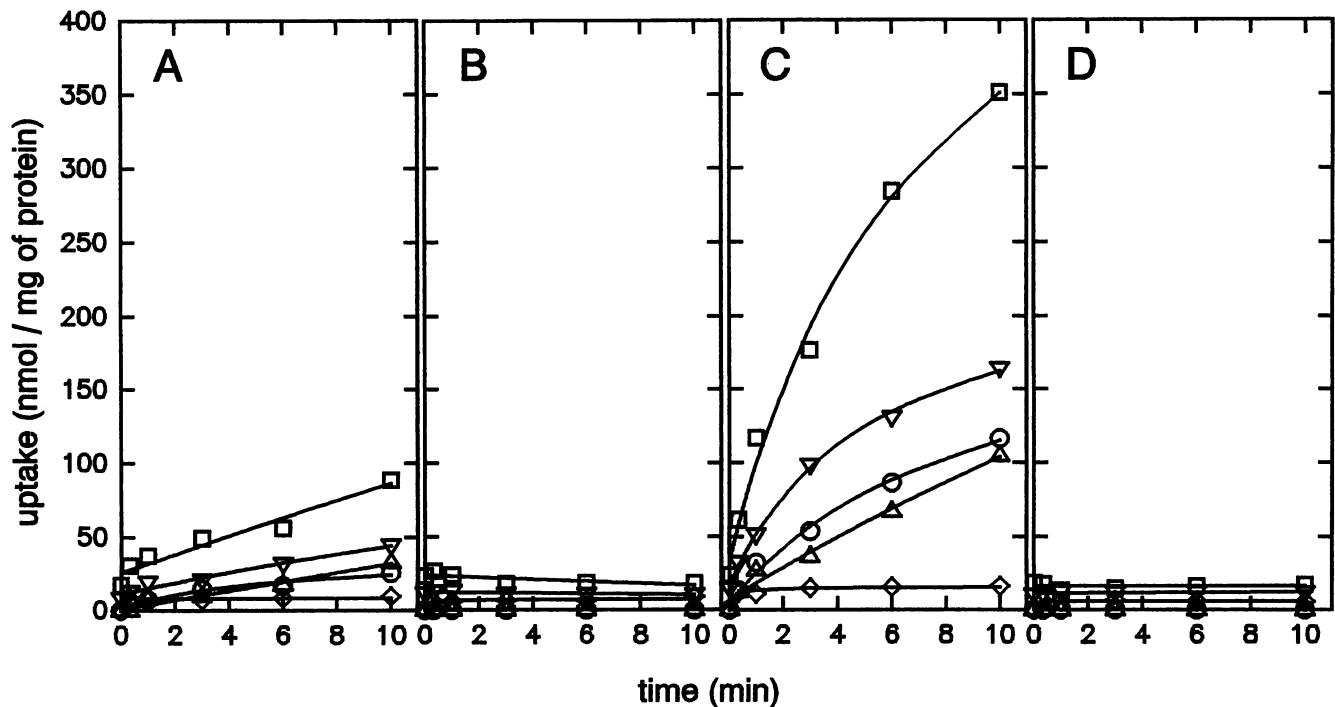The proteins of the oligopeptide transport system of *L. lactis*

FIG. 6. Time course of internal amino acid pools in glycolyzing cells of *L. lactis* ML3 (Opp⁺ PepO⁺) (A), MG1614V (Opp⁻ PepO⁺) (B), VS230 (Opp⁺ PepO⁺) (C), and VS772 (OppA⁻ PepO⁻) (D) upon the addition of 1 mM Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu). Accumulation of tyrosine (▽), glycine (□), phenylalanine (○), leucine (△), and Leu-enkephalin (◇) is shown.

TABLE 2. Growth of *L. lactis* strains in chemically defined medium lacking leucine supplemented with leucine-containing peptides

Peptide	Growth of strain[a]:					
	MG1363 (Opp$^+$ PepO$^+$)	MG1614V (Opp$^-$ PepO$^+$)	VS230 (Opp$^+$ PepO$^+$)	VS772 (OppA$^-$ PepO$^-$)	VS798 (Opp$^+$ PepO$^-$)	MLDT1 (Opp$^+$ Dpp$^-$)
Leu-Gly	+	+	+	+	+	-
Leu-Gly-Gly	+	+	+	+	+	-
Gly-Leu-Gly-Leu	+	-	+	-	+	+
Tyr-Gly-Gly-Phe-Leu	+	-	+	-	+	+
Tyr-Gly-Gly-Phe-Leu-Lys	+	-	+	-	+	ND
Ser-Ile-Gly-Ser-Leu-Ala-Lys	+	-	+	-	+	ND
Val-His-Leu-Thr-Pro-Val-Gly-Lys	+	-	+	-	+	ND

[a] Opp$^+$, functional oligopeptide transport system; Opp$^-$, deficiency in oligopeptide transport; OppA$^-$, deficiency in the substrate-binding protein of the oligopeptide transport system; PepO$^+$ and PepO$^-$, production and lack of production of the endopeptidase PepO, respectively; Dpp$^-$, deficiency in di- and tripeptide transport. +, good growth; -, no growth; ND, not done.

are similar to the corresponding proteins of *S. typhimurium* (14), *B. subtilis* (36, 41), and *S. pneumoniae* (1), although the gene organization in these operons is different. In *B. subtilis*, *S. pneumoniae*, and *S. typhimurium* *oppA* is the first gene of the operon. In *L. lactis*, however, *oppA* is the last of the *opp* genes. A gene encoding an endopeptidase not involved in oligopeptide transport, *pepO*, is transcribed from the same promoter as *oppA*, since the OppA-deficient mutant (strain VS772) also lacks PepO (30a, 31). In the case of the *B. subtilis* dipeptide transport system, the gene for the substrate-binding protein DciAE is the last gene in the operon, which contains an additional gene of unknown function (28). Similarly, the AmiB protein of the *S. pneumoniae* oligopeptide transport system has no counterpart in other known systems (1).

Oligopeptide transport systems are members of a large family of so-called ABC (ATP-binding cassette) transporters or traffic ATPases, which includes uptake and excretion systems of both prokaryotic and eukaryotic origins (2, 13, 18). On the basis of the homology between OppF and OppD of *L. lactis* and other ABC transporter proteins, the *L. lactis* Opp system can be classified as a member of this group. The basic organization of ABC transporters consists of four distinct domains. Two highly hydrophobic integral membrane proteins that span the membrane five to six times mediate substrate translocation across the cytoplasmic membrane. Two highly conserved proteins on the cytoplasmic side of the membrane most likely couple ATP hydrolysis to transport. The bacterial ABC uptake systems also encompass a substrate-binding protein which is located in the periplasm in gram-negative bacteria. The substrate-binding proteins of the gram-positive species (i.e., OppA and DciAE of the *B. subtilis* oligopeptide and dipeptide transport systems, respectively, AmiA of *S. pneu-*

moniae, and OppA of *L. lactis*) all have a signal peptide typical for prokaryotic prolipoproteins. Perego et al. (36) have shown that OppA of *B. subtilis* is bound to the cell, and it is expected that all of these proteins are anchored to the cell membrane by an amino-terminal lipoyl group. In addition to serving as a transporter for the uptake of nutrients (oligopeptides), oligopeptide transport systems have been shown to be involved in the recycling of cell wall peptides in gram-negative bacteria (10) and in sporulation in *B. subtilis* (36, 41).

The present study convincingly shows that the pentapeptide Leu-enkephalin is transported into the cell by the oligopeptide transport system encoded by *oppDFBCA*. Subsequent hydrolysis leads to accumulation of the amino acid residues constituting Leu-enkephalin. No detectable uptake of Leu-enkephalin in the oligopeptide transport-deficient strains MG1614V and VS772 was observed. Therefore, the accumulation of tyrosine, glycine, phenylalanine, and leucine in strain VS230 is the result of Leu-enkephalin uptake and is not due to extracellular breakdown of the oligopeptide and subsequent uptake of the hydrolysis products. Uptake of Leu-enkephalin is increased in a strain carrying multiple copies of *opp* (strain VS230 with pVS8) relative to uptake by the wild-type strain ML3, indicating that the proteins are overproduced. The differences in the apparent uptake rates and the accumulation levels of glycine and tyrosine versus leucine and phenylalanine could be due to the more lipophilic nature of the latter two amino acids. The higher hydrophobicity of leucine and phenylalanine could lead to passive leakage of these amino acids from the cells (6, 38). In addition, if the concentration gradients of the amino acids exceed the driving force imposed by the amino acid carrier mechanism, the residues may also leave the cell by facilitated diffusion (20). Therefore, the estimated uptake rates of Leu-enkephalin based on the concentration of intracellular amino acids will always be underestimates of the actual oligopeptide uptake rate. Nevertheless, the transport assays provide convincing evidence that the transport system encoded by the *oppDFBCA* genes indeed facilitates the uptake of the pentapeptide Leu-enkephalin. All present evidence suggests that the *opp* genes described in this article encode the oligopeptide transport system whose energetics has been described recently (21).

The growth experiments using chemically defined medium supplemented with leucine-containing peptides clearly demonstrate that the oligopeptide transport system is essential for growth of lactococci on peptides longer than 3 residues. It has been suggested that the size restriction for peptide uptake by lactococci is 5 or 6 amino acids (23, 40). In the present study, however, normal growth of *L. lactis* utilizing a leucine-contain-
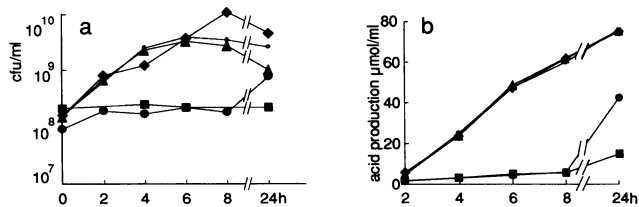

FIG. 7. Growth (a) and acid production (b) of *L. lactis* strains in pasteurized milk. Plasmid pLP712, carrying the genes for lactose utilization and proteinase production, was introduced via conjugation in all strains used in the experiment. ■, MG1614V (Opp$^-$ PepO$^+$); ●, VS772 (OppA$^-$ PepO$^-$); ▲, MG1363 (Opp$^+$ PepO$^+$); ●, VS230 (Opp$^+$ PepO$^+$); ◆, VS798 (Opp$^+$ PepO$^-$).

ing octapeptide was observed. Current studies are directed towards a further biochemical analysis of the substrate specificity and size restriction of the oligopeptide-binding protein. Growth and acid production of the various strains in milk clearly show that the transport of oligopeptides is an essential part of the proteolytic system of lactococci.

The close genetic linkage of the *opp* genes and *pepO* suggests that the gene products are also physiologically linked: it would make sense if oligopeptides taken up by the Opp system are subsequently hydrolyzed by the endopeptidase PepO. However, whereas the *opp* genes are essential, disruption of the endopeptidase gene *pepO* had no apparent effect on the utilization of milk protein or peptides by *L. lactis*. It is possible, of course, that the activity of PepO can be taken over by other peptidases with overlapping specificities (29).

Because of their presumed role in casein degradation, an extracellular location has been envisaged for a number of lactococcal proteolytic enzymes. However, PrtP, the lactococcal proteinase, remains the only proteolytic enzyme whose extracellular location is certain. Recent genetic, biochemical, and immunological data strongly suggest an intracellular location for all of the lactococcal peptidases (4, 46). The present results indicate that the presence of an oligopeptide transport system allows lactococci to utilize oligopeptides containing up to 8 amino acid residues. Therefore, the emerging picture of the lactococcal proteolytic system assumes that extracellular hydrolysis of casein is accomplished by the proteinase PrtP alone and that this hydrolysis produces sufficient ingestible di-, tri-, and oligopeptides for the sustenance of growth of lactococci in milk.

ACKNOWLEDGMENTS

REFERENCES

1. **Alloing, G., M.-C. Trombe, and J. P. Claverys.** 1990. The ami locus of the Gram-positive bacterium *Streptococcus pneumoniae* is similar to binding protein-dependent transport operons of Gram-negative bacteria. Mol. Microbiol. **4:**633–644.
2. **Ames, G. F.-L.** 1992. Bacterial periplasmic permeases as model systems for the superfamily of traffic ATPases, including the multidrug resistance protein and the cystic fibrosis transmembrane conductance regulator. Int. Rev. Cytol. **137A:**1–35.
3. **Anderson, D. G., and L. L. McKay.** 1983. Simple and rapid method for isolating large plasmid DNA from lactic streptococci. Appl. Environ. Microbiol. **46:**549–552.
4. **Baankreis, R.** 1992. Ph.D. thesis. University of Amsterdam, Amsterdam.
5. **Chapot-Chartier, M.-P., M. Nardi, M.-C. Chopin, A. Chopin, and J.-C. Gripon.** 1993. Cloning and sequencing of *pepC*, a cysteine aminopeptidase gene from *Lactococcus lactis* subsp. *cremoris* AM2. Appl. Environ. Microbiol. **59:**330–333.
6. **Driessen, A. J. M., K. J. Hellingwerf, and W. N. Konings.** 1987. Mechanism of energy coupling to entry and exit of neutral and branched chain amino acids in membrane vesicles of *Streptococcus cremoris*. J. Biol. Chem. **262:**12438–12443.
7. **Feirtag, J. M., J. M. Petzel, E. Pasalodos, K. A. Baldwin, and L. L. McKay.** 1991. Thermosensitive plasmid replication, temperature-sensitive host, and chromosomal plasmid integration conferred by *Lactococcus lactis* subsp. *cremoris* lactose plasmids in *Lactococcus lactis*. Appl. Environ. Microbiol. **57:**539–548.
8. **Gasson, M. J.** 1983. Plasmid complements of *Streptococcus lactis* NCDO 712 and other lactic streptococci after protoplast-induced curing. J. Bacteriol. **154:**1–9.
9. **Gasson, M. J., and F. L. Davies.** 1980. Conjugal transfer of the drug resistance plasmid pAMβ1 in the lactic streptococci. FEMS Microbiol. Lett. **7:**51–53.
10. **Goodell, E. W., and C. F. Higgins.** 1987. Uptake of cell wall peptides by *Salmonella typhimurium* and *Escherichia coli*. J. Bacteriol. **169:**3861–3865.
11. **Hagting, A., K. Leenhouts, B. Poolman, and W. N. Konings.** Submitted for publication.
12. **Higgins, C. F.** 1992. ABC transporters: from microorganisms to man. Annu. Rev. Cell Biol. **8:**67–113.
13. **Higgins, C. F., I. D. Hiles, G. P. C. Salmond, D. R. Gill, J. A. Downie, I. J. Evans, I. B. Holland, L. Gray, S. D. Buckel, A. W. Bell, and M. A. Hermodson.** 1986. A family of related ATP-binding subunits coupled to many distinct biological processes in bacteria. Nature (London) **323:**448–450.
14. **Hiles, I. D., M. P. Gallagher, D. J. Jamieson, and C. F. Higgins.** 1987. Molecular characterization of the oligopeptide permease of *Salmonella typhimurium*. J. Mol. Biol. **195:**125–142.
15. **Holo, H., and I. F. Nes.** 1989. High frequency transformation by electroporation of *Lactococcus lactis* subsp. *cremoris* grown with glysin in osmotically stabilized media. Appl. Environ. Microbiol. **55:**3119–3123.
16. **Horng, J. S., K. M. Polzin, and L. L. McKay.** 1991. Replication and temperature-sensitive maintenance functions of lactose plasmid pSK11L from *Lactococcus lactis* subsp. *cremoris*. J. Bacteriol. **173:**7573–7581.
17. **Hugenholtz, J., D. van Sinderen, J. Kok, and W. N. Konings.** 1987. Cell wall-associated proteases of *Streptococcus cremoris* Wg2. Appl. Environ. Microbiol. **35:**853–859.
18. **Hyde, S. C., P. Emsley, M. J. Hartshorn, M. M. Mimmick, U. Gileadi, S. R. Pearce, M. P. Gallagher, D. R. Gill, R. E. Hubbard, and C. F. Higgins.** 1990. Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. Nature (London) **346:**362–365.
19. **Kok, J.** 1990. Genetics of proteolytic system of lactic acid bacteria. FEMS Microbiol. Rev. **87:**15–42.
20. **Konings, W. N., B. Poolman, and A. J. M. Driessen.** 1989. Bioenergetics and solute transport in lactococci. Crit. Rev. Microbiol. **16:**419–476.
21. **Kunji, E. R. S., E. J. Smid, R. Plapp, B. Poolman, and W. N. Konings.** 1993. Di-tripeptides and oligopeptides are taken up via distinct transport mechanisms in *Lactococcus lactis*. J. Bacteriol. **175:**2052–2059.
22. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157:**105–132.
23. **Law, B. A.** 1978. Peptide utilization by group N streptococci. J. Gen. Microbiol. **105:**113–118.
24. **Law, B. A., and J. Kolstadt.** 1983. Proteolytic systems in lactic acid bacteria. Antonie Leeuwenhoek **49:**225–245.
25. **Leenhouts, K. J., J. Kok, and G. Venema.** 1990. Stability of integrated plasmids in the chromosome of *Lactococcus lactis*. Appl. Environ. Microbiol. **56:**2726–2735.
26. **Ludwig, W., E. Seewalt, R. Klipper-Bältz, K. H. Schleiffer, L. Magrum, C. R. Woese, G. E. Fox, and E. Stackebrandt.** 1985. The phylogenetic position of *Streptococcus* and *Enterococcus*. J. Gen. Microbiol. **131:**543–551.
27. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Manthiopoulos, C., J. P. Mueiler, F. J. Slack, C. G. Murphy, S. Patankar, G. Bukusoglu, and A. L. Sonenshein.** 1991. A *Bacillus subtilis* dipeptide transport system expressed early during sporulation. Mol. Microbiol. **5:**1903–1913.
29. **Mayo, B., J. Kok, W. Bockelmann, A. Haandrikman, K. J. Leenhouts, and G. Venema.** 1993. Effect of X-prolyl dipeptidyl aminopeptidase deficiency on *Lactococcus lactis*. Appl. Environ. Microbiol. **59:**2049–2055.
30. **Mayo, B., J. Kok, K. Venema, W. Bockelmann, M. Teuber, H. Reinke, and G. Venema.** 1991. Molecular cloning and sequence analysis of the X-prolyl dipeptidyl aminopeptidase gene from *Lactococcus lactis* subsp. *cremoris*. Appl. Environ. Microbiol. **57:**38–44.
30a.**Mierau, I.** Personal communication.
31. **Mierau, I., P. S. T. Tan, A. J. Haandrikman, J. Kok, K. Leenhouts, W. N. Konings, and G. Venema.** 1993. Cloning and sequencing of the gene for a lactococcal endopeptidase, an enzyme with se-

quence similarity to mammalian enkephalinase. J. Bacteriol. **175:** 2087–2096.

32. **Monnet, V., W. Bockelmann, J.-C. Gripon, and M. Teuber.** 1989. Comparison of cell wall bound proteinases from *Lactococcus lactis* subsp. *cremoris* AC1 and *Lactococcus lactis* subsp. *lactis* NCDO763. II. Specificity towards bovine β casein. Appl. Microbiol. Biotechnol. **31:**112–118.

33. **Monnet, V., D. Le Bars, and J.-C. Gripon.** 1986. Specificity of a cell wall proteinase from *Streptococcus lactis* NCDO763 towards bovine β casein. FEMS Microbiol. Lett. **36:**127–131.

34. **Nardi, M., M.-C. Chopin, A. Chopin, M.-M. Cals, and J.-C. Gripon.** 1991. Cloning and DNA sequence analysis of an X-prolyl dipeptidyl aminopeptidase gene from *Lactococcus lactis* subsp. *lactis* NCDO 763. Appl. Environ. Microbiol. **57:**45–50.

35. **Pearson, W., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.

36. **Perego, M., C. F. Higgins, S. R. Pearce, M. P. Gallaher, and J. A. Hoch.** 1991. The oligopeptide transport system of *Bacillus subtilis* plays a role in the initiation of sporulation. Mol. Microbiol. **5:**173–185.

37. **Petzel, J. P., and L. L. McKay.** 1992. Molecular characterization of the integration of the lactose plasmid from *Lactococcus lactis* subsp. *cremoris* SK11 into the chromosome of *L. lactis* subsp. *lactis*. Appl. Environ. Microbiol. **58:**125–131.

38. **Poolman, B., and W. N. Konings.** 1988. Relation of growth of *Streptococcus lactis* and *Streptococcus cremoris* to amino acid transport. J. Bacteriol. **170:**700–707.

39. **Poolman, B., E. J. Smid, H. Veldkamp, and W. N. Konings.** 1987. Bioenergetic consequences of lactose starvation for continuously cultured *Streptococcus cremoris*. J. Bacteriol. **169:**1460–1468.

40. **Rice, G. H., F. H. C. Stewart, A. J. Hillier, and G. R. Jago.** 1978. The uptake of amino acids and peptides by *Streptococcus lactis*. J. Dairy Res. **45:**93–107.

41. **Rudner, D. Z., J. R. LeDeaux, K. Ireton, and A. D. Grossman.** 1991. The *spoOK* locus of *Bacillus subtilis* is homologous to the oligopeptide permease locus and is required for sporulation and competence. J. Bacteriol. **173:**1388–1398.

42. **Smid, E.** 1991. Ph.D. thesis. University of Groningen, Groningen, The Netherlands.

43. **Smid, E., A. J. M. Driessen, and W. N. Konings.** 1989. Mechanism and energetics of dipeptide transport in membrane vesicles of *Lactococcus lactis*. J. Bacteriol. **171:**292–298.

44. **Smid, E., R. Plapp, and W. N. Konings.** 1989. Peptide uptake is essential for growth of *Lactococcus lactis* on the milk protein casein. J. Bacteriol. **171:**6135–6140.

45. **Strøman, P.** 1992. Sequence of a gene (*lap*) encoding a 95.3 kDa aminopeptidase from *Lactococcus lactis* ssp. *cremoris* Wg2. Gene **113:**107–112.

46. **Tan, P. S. T., M.-P. Chapot-Chartier, K. M. Pos, M. Rousseau, C.-Y. Boquien, J.-C. Gripon, and W. N. Konings.** 1992. Localiza-tion of peptidases in lactococci. Appl. Environ. Microbiol. **58:**285–290.

47. **Tan, P. S. T., I. J. van Alen-Boerrigter, B. Poolman, R. J. Siezen, W. M. de Vos, and W. N. Konings.** 1992. Characterization of the *Lactococcus lactis pepN* gene encoding an aminopeptidase homologous to mammalian aminopeptidase N. FEBS Lett. **306:**9–16.

48. **Tapuhi, Y., D. E. Schmidt, W. Lindner, and B. L. Karger.** 1981. Dansylation of amino acids for high-performance liquid chromatography analysis. Anal. Biochem. **115:**123–129.

49. **Terzaghi, B. E., and W. E. Sandine.** 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. **29:**807–813.

50. **Tinoco, I., P. N. Borer, B. Dengler, and M. D. Levine.** 1973. Improved estimation of secondary structure in ribonucleic acids. Nature (London) New Biol. **246:**40–41.

51. **Tynkkynen, S., and A. von Wright.** 1988. Characterization of a cloned chromosomal fragment affecting the proteinase activity of *Streptococcus lactis* ssp. *lactis*. Biochimie **70:**531–534.

52. **Tynkkynen, S., A. von Wright, and E.-L. Syväoja.** 1989. Peptide utilization encoded by *Lactococcus lactis* subsp. *lactis* SSL135 chromosomal DNA. Appl. Environ. Microbiol. **55:**2690–2695.

53. **van Alen-Boerrigter, I. J., R. Baankreis, and W. M. de Vos.** 1991. Characterization and overexpression of the *Lactococcus lactis pepN* gene and localization of its product, aminopeptidase N. Appl. Environ. Microbiol. **57:**2555–2561.

54. **Van der Guchte, M., J. Kok, and G. Venema.** 1992. Gene expression in *Lactococcus lactis*. FEMS Microbiol. Rev. **88:**73–92.

55. **van der Vossen, J. M. B. M., D. van der Lelie, and G. Venema.** 1987. Isolation and characterization of *Streptococcus cremoris* Wg2-specific promoters. Appl. Environ. Microbiol. **53:**2452–2457.

56. **Visser, S., C. J. Slangen, F. A. Exterkate, and G. J. de Veer.** 1988. Action of a cell wall proteinase (PI) from *Streptococcus cremoris* HP on bovine β casein. Appl. Microbiol. Biotechnol. **29:**61–66.

57. **Von Heijne, G.** 1989. The structure of signal peptides from bacterial lipoproteins. Protein Eng. **2:**531–534.

58. **Von Wright, A., S. Tynkkynen, and M. Suominen.** 1987. Cloning of a *Streptococcus lactis* subsp. *lactis* chromosomal fragment associated with the ability to grow in milk. Appl. Environ. Microbiol. **53:**1584–1588.

59. **Walker, J. E., M. Saraste, M. J. Runswick, and N. J. Gay.** 1982. Distantly related sequences in the α and β subunits of ATP synthetase, myosin, kinases and other ATP requiring enzymes and a common nucleotide binding fold. EMBO J. **1:**945–951.

60. **Wiedmeier, W. T., S. P. Porterfield, and C. E. Hendrich.** 1982. Quantitation of Dns-amino acids from body tissues and fluids using high-performance liquid chromatography. J. Chromatogr. **231:**410–417.

61. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.