Duplication of the pepF Gene and Shuffling of DNA Fragments on the Lactose Plasmid of Lactococcus lactis

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The gene corresponding to the lactococcal oligopeptidase PepF1 (formerly PepF1 [V. Monnet, M. Nardi, A. Chopin, M.-C. Chopin, and L.-C. Gripon, J. Biol. Chem. 269:32070-32076, 1994]) is located on the lactose-proteinase plasmid of Lactococcus lactis subsp. cremoris NCDO763. Use of the pepF1 gene as a probe with different strains showed that pepF1 is present on the chromosome of Lactococcus lactis subsp. lactis IL1403, whereas there is a second, homologous gene, pepF2, on the chromosome of strain NCDO763. From hybridization, PCR amplification, and sequencing experiments, we deduced that (i) pepF1 and pepF2 exhibit 80% identity and encode two proteins which are 84% identical and (ii) pepF2 is included in an operon composed of three open reading frames and is transcribed from two promoters. The protein, encoded by the gene located downstream of pepF2, shows significant homology with methyltransferases. Analysis of the sequences flanking pepF1 and pepF2 indicates that only a part of the pepF2 operon is present on the plasmid of strain NCDO763, while the operon is intact on the chromosome of strain IL1403. Traces of several recombination events are visible on the lactose-proteinase plasmid. This suggests that the duplication of pepF occurred by recombination from the chromosome of an L. lactis subsp. lactis strain followed by gene transfer. We discuss the possible functions of PepF and the role of its amplification.

The composition and the functioning of the proteolytic system of lactic acid bacteria, essential for optimal growth in milk, are now well-known, especially for lactococci (36). In this system, a cell envelope-associated protease hydrolyzes casein into oligopeptides which constitute the main source of nitrogen (18). The oligopeptides are then partly taken up by a specific transport system (47) and internally hydrolyzed by peptidases into amino acids, which are used for synthesis of the bacterial proteins. We already know that oligopeptides could be hydrolyzed from their extremities by about 10 different exopeptidases; they are also probably cleaved internally by two recently characterized oligopeptidases, PepO and PepF (29, 33). The two oligopeptidases are metalloenzymes, but they differ in their substrate specificities and are grouped in different peptidase families (32). In a previous paper (33), the sequence of the pepF gene was reported and it was shown that PepF belongs to the M3 peptidase family, which includes the mammalian oligopeptidase 24.15, according to the classification of Rawlings and Barrett (38). This gene is located on a large, 55-kb plasmid which also carries the genes coding for lactose and casein utilization, essential for optimal growth in milk (21).

Three observations suggested the existence of another gene (pepF2), homologous to that carried by the plasmid (now named pepF1) but located on the chromosome of strain NCDO763. First, we copurified with PepF1 another protein displaying the same properties; second, we measured a residual PepF activity in strains cured of the plasmid carrying the pepF1 gene; and third, by hybridization experiments, we identified a DNA fragment having some homology with pepF1, used as a probe. The hybridization experiments also showed that pepF1 was present on the chromosome of strain IL1403.

In the present study, we characterized the two genes coding for PepF and their flanking sequences in Lactococcus lactis subsp. cremoris NCDO763 and L. lactis subsp. lactis IL1403. Our observations suggest that the duplication of pepF occurred by recombination events, traces of which are numerous on the conjugative lactose-proteinase plasmid. We discuss the possible roles of PepF1 and PepF2, which may be an example of the first step of gene divergence in the evolution of the lactococcal genome.

MATERIALS AND METHODS

Purification and N-terminal sequencing of PepF2. PepF1 and PepF2 were copurified as previously described (33). The purified oligopeptidases were separated by electrophoresis under denaturing and reducing conditions using 12.5% acrylamide gels as described by Laemmli (22). The oligopeptidases were blotted on a polyvinylidene difluoride membrane (Problott; Applied Biosystems, San Jose, Calif.) with a Bio-Rad apparatus (1 h, 100 V). The blotting was done in 10 mM CAPS buffer (pH 11) containing 10% methanol and 0.5 mM dithiothreitol (27). The proteins were stained with Ponceau red. The N-terminal sequence was determined directly on the part of the membrane containing PepF2 with an automatic sequencer (model 477A; Applied Biosystems).

Bacterial strains and plasmids, transformation, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. According to its genotype (15), strain NCDO763 is designated an L. lactis subsp. cremoris strain throughout the paper. L. lactis strains were grown at 30°C in M17 medium (46) supplemented with 0.5% lactose or glucose. TIL80 and MG1363 were grown on a minimal medium containing vitamins, salts, nucleic acids, buffer, and glucose as described for the chemically defined medium by Otto et al. (34) and the following amino acids: serine (0.34 g/liter), histidine (0.11 g/liter), methionine (0.12 g/liter), isoleucine (0.20 g/liter), valine (0.33 g/liter), leucine (0.47 g/liter), and glutamic acid (0.20 g/liter) (5). The Escherichia coli strain was grown in Luria-Bertani medium (42) at 37°C. When needed, erythromycin (5 μg/ml for L. lactis subsp. lactis and 150 μg/ml for E. coli) or ampicillin (50 μg/ml for E. coli) was added to the culture medium. L. lactis electroporation was done as previously described (17). E. coli was transformed as described by Sambrook et al. (42).

DNA techniques. Plasmid and chromosomal DNAs were prepared as previously described (25, 42, 44). The classical pulsed-field gel electrophoresis procedure for L. lactis was used (24). Restriction endonucleases, T4 DNA ligase, and T4 polymerase were obtained from Boehringer Mannheim or Eurogentec and used as recommended by the suppliers. After agarose gel electrophoresis, Southern blotting and DNA or RNA hybridization were performed either as described by Amersham International (ECL gene detection system) or by the
DUPPLICATION OF AN OLIGOPEPTIDASE IN LACTOCoccus LACTIS

TABLE 1. Strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Description</th>
<th>Source or reference</th>
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<tbody>
<tr>
<td><strong>L. lactis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL1403</td>
<td>Plasmid free</td>
<td>Chopin et al. (4)</td>
</tr>
<tr>
<td>NCD0765 lac&lt;sup&gt;+&lt;/sup&gt; prt&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Wild-type strain</td>
<td>Davies et al. (6)</td>
</tr>
<tr>
<td>TIL75</td>
<td>NCD0763 cured of the lac-prt plasmid</td>
<td>V. Juillard (17a)</td>
</tr>
<tr>
<td>TIL79</td>
<td>NCD0763 lac&lt;sup&gt;+&lt;/sup&gt; prt&lt;sup&gt;+&lt;/sup&gt; pepF1</td>
<td>Monnet et al. (33)</td>
</tr>
<tr>
<td>TIL79</td>
<td>NCD0763 lac&lt;sup&gt;+&lt;/sup&gt; prt&lt;sup&gt;+&lt;/sup&gt; pepF2</td>
<td>This work</td>
</tr>
<tr>
<td>TIL246</td>
<td>NCD0763 containing pTIL125 (high copy number)</td>
<td>This work</td>
</tr>
<tr>
<td>TIL248</td>
<td>NCD0763 containing pTIL125 (low copy number)</td>
<td>This work</td>
</tr>
<tr>
<td>TIL247</td>
<td>NCD0763 containing pTIL126 (high copy number)</td>
<td>This work</td>
</tr>
<tr>
<td>TIL249</td>
<td>NCD0763 containing pTIL126 (low copy number)</td>
<td>This work</td>
</tr>
<tr>
<td>TIL250</td>
<td>NCD0763 containing pJM2366 (high copy number)</td>
<td>This work</td>
</tr>
<tr>
<td>TIL251</td>
<td>NCD0763 containing pJM2366 (low copy number)</td>
<td>This work</td>
</tr>
<tr>
<td>NCD0712 lac&lt;sup&gt;+&lt;/sup&gt; prt&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Wild-type strain</td>
<td>M. J. Gasson (12)</td>
</tr>
<tr>
<td>MG1363</td>
<td>Plasmid-free derivative of NCD0712</td>
<td>M. J. Gasson (12)</td>
</tr>
<tr>
<td>TIL80</td>
<td>MG1363 pepF1 pepF2</td>
<td>This work</td>
</tr>
<tr>
<td><strong>E. coli</strong> TG1</td>
<td></td>
<td>T. J. Gibson (14)</td>
</tr>
</tbody>
</table>

**Plasmids**

- pBluescript SK<sup>+</sup>
- pIL253
- pJIM2366
- pTIL28
- pTIL34
- pTIL35
- pTIL36
- pTIL37
- pTIL3725
- pTIL126

**Strains**

- **L. lactis**
- **E. coli** TG1

**Construction of plasmids.** A pepF1 probe (33) was prepared from the BstECI-ClaI DNA fragment (positions 416 to 1934) purified from pTIL28. PCR amplifications were done on a Perkin-Elmer DNA thermal cycler 480. The internal pepF2 fragment was amplified by using degenerate oligonucleotides 1 [5'-GAAGC(A/G)TTAACTTGGGA(3')] corresponding to the N-terminal sequence of PepF2(1-904) and 2 [5'-C(G/A)TG 3'], used to amplify the fragments flanking the pepF<sup>+</sup> gene. To clone the entire pepF2 gene and its flanking regions, we used the integrative plasmid pTIL25, which contains a 1.4-kb fragment of pTIL28. This plasmid was digested with NcoI and XhoI and ligated in diluted solution (500 ng per ml), and amplified by PCR (with annealing at 48°C). Oligonucleotides 3 [5'-ATGGCAGCGTTGACACTCCTGCTG 3'] and 4 [5'-GAGGAATTTAGCAGTAC 3'], corresponding to the N-terminal and C-terminal ends of pepF1 and pepF2, oligonucleotides 3 (5'-CATAGAAAACGGGAGGATG 3') and 4 (5'-AGGAGTGACCTTACACGCTAGTAC 3'), used to amplify the fragments flanking pepF1, were deduced from the sequences of S1904 (37) and lac<sup>+</sup> (8), respectively. To test the functionality of the two promoters located upstream of pepF1 and pepF2, oligonucleotides 3 (5'-CATAGAAAACGGGAGGATG 3') and 4 (5'-GAGGAATTTAGCAGTAC 3'), used to amplify the fragments flanking pepF1, were deduced from the sequences of S1904 (37) and lac<sup>+</sup> (8), respectively. Finally, to complete the sequence, two oligonucleotides deduced from the sequence of the amplified fragment (with oligonucleotides 3 and 5) were used. Plasmid pTIL35 contains the luciferase gene, whose expression can be easily measured. Plasmid pTIL25 was cloned upstream of the luciferase gene, whose expression can be easily measured. Plasmid pTIL25 was cloned upstream of the luciferase gene, whose expression can be easily measured.

**DNA sequencing.** The amplified fragments were extracted from 0.7% agarose gels containing 0.1% agarose and 0.1% agarose and 0.1% agarose and 0.1% agarose. To test the functionality of the luciferase gene, its expression can be easily measured. Plasmid pTIL25 was cloned upstream of the luciferase gene, whose expression can be easily measured. Plasmid pTIL25 was cloned upstream of the luciferase gene, whose expression can be easily measured.
RESULTS

Presence of two PepF oligopeptidases in L. lactis subsp. cremoris NCDO763. During the purification of oligopeptidase PepF1, another protein which has the same endopeptidase activity on bradykinin was copurified (33). The N-terminal sequence of this protein, determined up to the 14th residue (Val-Lys-Asn-Arg-Asn-Glu-Ile-Pro-Glu-Ala-Leu-Thr-?-Ile), was identical to 10 of the 13 amino acids of the N-terminal sequence of PepF1 (identical residues underlined) (33), which suggested the existence of two homologous oligopeptidases in strain NCDO763. At the DNA level, hybridization with a pepF1 probe under stringent conditions revealed a single 4-kb and 3.2-kb HindIII band in strains NCDO763 and IL1403, respectively. No band was detected in TIL75 (NCDO763 cured of the 55-kb lactose-proteinase plasmid) or in the plasmid-free MG1363 strain. This confirmed that pepF1 is located on the lactose-protease plasmid of strain NCDO763 and showed that it is located on the chromosome of the plasmid-free IL1403 strain. We have named the plasmid copy of pepF1 pepF1p and the chromosomal copy pepF1c. pepF1p was further mapped by pulsed-field gel electrophoresis to the SmaI and ApaI DNA restriction fragment of strain IL1403. Under less stringent conditions, a 1.6-kb band of low intensity could be visualized in strains NCDO763 and MG1363, but another band in addition to the 3.2-kb fragment could not be detected in strain IL1403 (Fig. 1). This implied the existence of a second gene, homologous but nonidentical to pepF1, located on the chromosome of strain NCDO763 but absent in IL1403. We therefore decided to characterize the second pepF gene, named pepF2.

pepF2 is included in an operon. A 1.1-kb fragment of the pepF2 gene was amplified by PCR with oligonucleotides deduced from the N-terminal sequence of PepF2 and from the active site of PepF1. The nucleotide sequence of the fragment showed 80% identity with the corresponding nucleotide sequence of pepF1 and encoded a peptide 84% identical to the corresponding part of PepF1. From these results, we concluded that a second oligopeptidase gene, pepF2, homologous to pepF1, was indeed present on the chromosome of strain NCDO763. To sequence the entire pepF2 gene and its flanking sequences, we cloned the 1.1-kb fragment in an integrative plasmid to produce pTIL35, which was used to transform L. lactis subsp. cremoris NCDO763; lanes 3, NCDO763 pnt lac; lanes 4, NCDO763 pepF1p; lanes 5, L. lactis subsp. lactis IL1403.

The presence of a potential lactococcal promoter (P1) upstream of ORF2 and of a potential terminator downstream of ORF4 suggested that ORF2, pepF2, and ORF4 are organized in an operon. To test this hypothesis, we measured the mRNA spanning this region with a pepF2 probe and detected a small amount of 3.7-kb transcript, whose size fits well with the predicted size (3.6 kb) of the operon. A second potential ~10 extended promoter sequence (P2) (41) was found upstream of pepF2, which would produce a 2.6-kb transcript (Fig. 4). We could not clearly state if the signal detected at this position corresponded to this transcript because it would comigrate with the abundant 23S RNA, which often produces artifactual bands. Consequently, P1 and P2 were cloned upstream of the luciferase gene in L. lactis and their expression was tested during exponential growth in milk and in M17 medium. At low copy number, the expression of the lux gene under the control of P1 was not detectable, whereas the same gene under the control of P2 is expressed in M17 medium and in milk (Table 2). At high copy number, with P2, the luciferase activity was saturating and the growth rate of strain TIL247 was reduced. This indicated that P2 is much stronger than P1 under the conditions used. However, the activity of the P1 promoter was evidenced in the high-copy-number plasmid (Table 2).

We found no significant homology between the protein encoded by ORF1 and other proteins in the databases. The protein encoded by ORF2 presents a weak overall homology (15% identity, 39% similarity) with the tomato protein vsf-1, which is involved in the regulation of vascular system-specific gene expression (46a).

PepF2 is homologous (80% identity) to PepF1 (33). Both the zinc-binding motif (91% identity between the two PepF proteins for 45 amino acids) and the area showing homology with creatine and arginine kinases found for PepF1 are well-conserved, also for PepF2. No indication of different locations and functions for the two PepF oligopeptidases was found within the sequences.

ORF4 displays a significant homology with several methyltransferases from various origins (plants, mammals, and bacteria). The highest homologies were found with bacterial O-methyltransferases from Synechocystis (31% identity, 55% similarity) (21) and Streptomyces (27% identity, 56% similarity) (16), caffeoyl-coenzyme A 3-O-methyltransferases from plants (26% identity, 52% similarity) (28), and catechol-O-methyltransferases from mammals (27% identity, 55% similarity) (2). ORF4 was also related, though to a lesser extent, to protein 1-isoaspartate-O-methyltransferases (10, 19).

Recombination events around the plasmid pepF1 gene. Our results revealed the existence of two homologous genes, pepF1 and pepF2, in L. lactis. Hybridization experiments showed first that pepF1 is present both on the lactose-proteinase plasmid of strain NCDO763 (pepF1p) and on the chromosome of strain IL1403 (pepF1c) and second that pepF2 is located on the chromosome of strain NCDO763. The high homology observed between these genes suggested that they are evolutionarily related. To evaluate the relationship between them and the
possibility that one was derived from the other, we sequenced larger regions upstream and downstream of the pepF1p and pepF1c genes in strains NCDO763 and IL1403. Two striking observations were made. First, the sequences flanking pepF1c in strain IL1403 are 80% identical to those flanking pepF2 in strain NCDO763. Second, the sequences flanking pepF1c and pepF1p are identical from base 339 of ORF2 to base 113 of ORF4 (Fig. 3B and C; Fig. 5). Beyond these limits, the sequences carried on the plasmid of strain NCDO763 were totally different from those located on the chromosomes of strains IL1403 and NCDO763. ORF2 and ORF4 are therefore truncated and the operon is incomplete on the plasmid of strain NCDO763. Moreover, the extended P2 promoter is present on the plasmid.

A sequence identical to IS904 (37) is present on the plasmid, 1.4 kb upstream of the truncated ORF2. Downstream of the
FIG. 4. Nucleotide and deduced amino acid sequences of ORF1, ORF2, ORF3 (pepF2), and ORF4 from the chromosome of *L. lactis* subsp. *cremoris* NCDO763. The numbers to the right refer to nucleotides (top lines) and amino acid residues (bottom lines). The putative 210 and 235 regions and ribosome binding sites (boldface), stop codons (asterisks), putative terminators (underlined), and the zinc-binding motif of PepF2 (underlined and boldface) are indicated.
We found a sequence almost identical to that found downstream of the terminator of the *L. lactis* *llaII* restriction/modification genes and a sequence coding for a DNA invertase from *L. lactis* (49) (Fig. 3C) homologous to *binR* from *Staphylococcus aureus* (40). A small DNA fragment of 68 bp is inserted between the latter sequence and the truncated ORF2; the junctions between these fragments are shown in Fig. 5A and B.

Downstream of *pepF1p*, the truncated sequence of ORF4 directly abuts a sequence identical to a truncated IS981 (bases 591 to 1, from accession no. M33933) (35) (Fig. 5C). At 34 bp downstream of the IS, there is a sequence, *lin2*, that could code for an invertase, different from that encoded by *lin1* present upstream of *pepF1*. The 133-amino-acid protein encoded by *lin2* seems to be truncated at its C terminus, since the usual length for bacterial invertases is 180 amino acids. The region between *lin2* and *lacR* does not contain a potential ORF and does not show any homology to known sequences. This fragment contains two potential terminators (Fig. 3C).

Northern (RNA) blotting showed that the abundant *pepF1p* transcripts from the plasmid of strain NCDO763 were significantly smaller (3.2 kb) than the transcripts of the operons located on the chromosomes of strains NCDO763 and IL1403 (3.7 kb). The size of the *pepF1p* transcript corresponded to an mRNA initiated at *P2* and ending at the potential terminator found downstream of the truncated *lin2* sequence (Fig. 3C). Since we demonstrated that *P2* was functional on a plasmid, its presence upstream of *pepF1* on the lactose plasmid most probably induced the amplification of expression of *pepF*.

**Function of *pepF* operon.** The amplification of PepF activity by gene duplication in *L. lactis* raises the question of the function of PepF. Preliminary investigations of the growth of strains MG1363 (*pepF2*Δ*pepF1*) and TIL80 (*pepF2*Δ*pepF1*) on different media revealed that the two strains grow at the

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**TABLE 2.** Luciferase expression under the control of promoter sequences P1 and P2 found upstream of *pepF2* at high and low copy numbers in *L. lactis*<sup>a</sup>

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Luciferase activity (kl/10&lt;sup&gt;8&lt;/sup&gt; CFU) in the indicated medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milk</td>
</tr>
<tr>
<td>Low copy number</td>
<td></td>
</tr>
<tr>
<td>None (control)</td>
<td>NS&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P1</td>
<td>NS</td>
</tr>
<tr>
<td>P2</td>
<td>1,550</td>
</tr>
<tr>
<td>High copy number</td>
<td></td>
</tr>
<tr>
<td>None (control)</td>
<td>25</td>
</tr>
<tr>
<td>P1</td>
<td>110</td>
</tr>
<tr>
<td>P2</td>
<td>(34,860)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Luciferase activities were measured during growth in milk or M17 medium when the cell concentration reached 10<sup>8</sup> CFU/ml.

<sup>b</sup> NS, value obtained not significant compared to the background level.

<sup>c</sup> Luciferase activities in these experiments were saturating and thus probably underestimated.

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**FIG. 5.** Junctions corresponding to probable recombination sites on the plasmid of *L. lactis* subsp. *cremoris* NCDO763 upstream (A and B) and downstream (C) of the *pepF1* gene (see Fig. 3). The sequence of the plasmid in the first line is aligned with the *llaII/lin* sequence (accession no. U16027 and Z48180) and the chromosomal *pepF* operon as indicated. The ORFs corresponding to the sequences are indicated below. Identical amino acids (boxed), amino acids corresponding to totally different proteins (italics), and DNA sequences corresponding to the same part of the plasmid (boldface) are shown.

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**FIG. 6.** Putative recombination sites upstream of the *pepF* gene

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**FIG. 6.** Putative recombination sites downstream of the *pepF* gene

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**FIG. 6.** Putative recombination sites downstream of the *pepF* gene
same rate in M17 rich medium. However, different phenotypes were evidenced in minimum medium containing only the seven amino acids essential to the two strains. TIL80, devoid of PepF, grew significantly more slowly (maximum growth rate $\mu_{\text{max}} = 0.12 \, \text{h}^{-1}$) in this medium than its parental strain ($\mu_{\text{max}} = 0.14 \, \text{h}^{-1}$). This suggests that PepF could be involved in protein turnover, since starvation for required nutrients leads to an elevated rate of the degradation of proteins (30).

Similar results obtained with TIL70 (pepF2$^{+}$ pepFlp) and TIL79 (pepF2 pepFlp$^{-}$) compared to NCDO763 seem to indicate that the presence of both genes is beneficial in minimal medium.

**DISCUSSION**

We report the presence of two homologous genes coding for oligopeptidases in *L. lactis* subsp. cremoris NCDO763. One of the genes (pepFlp) is carried on the 55-kb plasmid which also contains the genes necessary for lactose and casein utilization. The other (pepF2) is located on the chromosome.

During a search for pepF genes in the chromosome of several lactococcal strains, we observed that pepF2 is present on the chromosome of *L. lactis* subsp. cremoris NCDO763, while pepF1 (identical to the plasmid copy of the gene in strain NCDO763) is present on the chromosome of *L. lactis* subsp. lactis IL1403. The two chromosomal genes are 98% identical, which corresponds to the divergence usually observed for *L. lactis* subsp. lactis and cremoris genes (16). The almost complete identity between the chromosomal gene of strain IL1403 and the plasmid gene of strain NCDO763 indicates that the two genes diverged more recently than the two subspecies and strongly supports the hypothesis of a recent gene transfer. The fact that pepF is part of a gene cluster in the chromosome of the two subspecies whereas it is restricted to an intact copy of pepF1 on the plasmid suggests that pepF1p is a derivative of pepF1c. The precise mechanism which allowed this event is not known. However, the presence of IS904 and IS981 upstream and downstream, respectively, of pepF1p suggests the involvement of IS-directed mobilization of pepF1 from the chromosome to the plasmid. Since the lactose-proteinase plasmid is conjugative (13), it could have then been transferred to different strains, such as *L. lactis* subsp. cremoris NCDO763. The sequences upstream and downstream of pepF1p possess several fragments identical to previously identified sequences. The direct linkages of these fragments are visible proof of numerous reorganizations that have occurred during the evolution of the lactose-proteinase plasmid.

Two duplicated pepO genes coding for homologous PepO oligopeptidases were recently shown to exist in lactococci (16a). Therefore, both the PepO and the PepF oligopeptidase genes are duplicated in *L. lactis*. The occurrence of such independent duplications of oligopeptidase genes suggests that these events are beneficial to the cell.

The first role attributed to gene duplication is amplification of the level of expression of a gene. This event can be fixed in a population by natural selection if the amplification confers a selective advantage. The best-known examples of amplification concern genes conferring a selective advantage by coding for resistance to antibiotics or drugs. The *tufA* and *nuB* genes of *E. coli* are another example of duplicated genes. The two genes are 98% identical, and their products are functionally indistinguishable. However, the TuA protein appears to be produced at a higher level (11, 31), which suggests that the duplication enables the cell to provide larger amounts of the translation elongation factor EF-Tu when demand is high (1). In the case of pepF, its presence on the lactose plasmid allows a fourfold increase in enzymatic activity in *L. lactis* subsp. cremoris NCDO763, which fits with the above hypothesis. The selective advantage that the strain derives from pepF overexpression remains to be determined.

Once retained on a plasmid, a duplicated gene can be assembled into new pathways to respond to environmental changes and participate in an important manner in the evolution of the genome. In the present case, the enzymatic properties of pepF genes seem to remain unchanged but the expression of the two genes is under the control of different promoters and in different backgrounds (the chromosome for pepF2 and the plasmid for pepFlp). Studies of the regulation of pepF genes are in progress in our laboratory and will provide a better understanding of the function of PepF.

Although PepF is biochemically well characterized, its role in *L. lactis* remains unclear. A mutant devoid of PepF activity (TIL80) is fully viable in M17 rich medium. However, the slower growth of pepF1 or pepF2 mutants than of the parental strains in minimum medium suggests that this oligopeptidase could be involved in protein turnover. This hypothesis is reinforced by the fact that pepF is cotranscribed with a gene coding for a methyltransferase. Most of the methyltransferases showing homology to ORF4 are involved in defense and survival responses and metabolism of altered proteins (43). The latter role is attributed to 1-isoaspartyl methyltransferases, which help the cells to repair or degrade the altered proteins that accumulate in aging cells and limit their viability (10, 24, 26). A similar function may be postulated for ORF4 and the chromosome-encoded PepF. Lastly, a gene coding for a protein showing 30% identity with PepF was detected in the genome of *Mycoplasma genitalium* (9). This genome is one of the smallest known for a self-replicating organism and probably possesses a minimal functional gene set. This suggests that PepF oligopeptidases could play an important role in the cells. Such a fundamental function was already proposed for the homologous oligopeptidase from *Salmonella typhimurium* (OpdA), which is involved in protein turnover during carbon deficiency and in the degradation of signal peptides (48).

The presence of an isolated copy of pepFplp (without the methyltransferase) suggests that the plasmid and chromosome pepF genes have different functions. The linkage of pepFplp with the genes necessary for optimal growth in milk would indicate a role in peptide assimilation. However, experiments testing growth in milk showed that pepF amplification does not confer a significant advantage during exponential growth. Alternatively, the two pepF genes may still have the same function and a sufficient amount of the methyltransferase from the chromosome could be expressed while PepF activities could be limiting in milk cultures.

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**REFERENCES**


