Note

Nucleotide sequence of the gene required for maturation of 763 proteinase of *Lactococcus lactis* subsp. *cremoris* NCDO 763

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In many kinds of lactic acid bacteria, cell wall-bound proteinases are responsible for the initial degradation of caseins and are important for sufficient growth of the bacteria in milk. The proteinase genes (*prtP*) of *Lactococcus lactis* strains were investigated intensively and it was found that they are mostly located on plasmids different in size but closely related to each other (1-3). Similar proteinase genes and their products are also found in *Lactobacillus* species (4-7). It has been shown that a maturation gene (*prtM*) located immediately upstream of and in the opposite direction to *prtP* is required for the processing of the precursor of *PrtP* into an active enzyme (8,9).

During the course of a study to characterize 763 proteinase (*PrtP*) (1), we subcloned a DNA fragment derived from the proteinase plasmid pLP 763 (1) encompassing *Hind*III to *Pst*I (designated as pHD2) (Fig. 1.b).

As shown in Fig. 1, the transformant of *L. lactis* YIT 2081 with pHD2 expressed as much proteinase activity as that with the parental plasmid did, whereas the transformant with a plasmid lacking the fragment of *Hind* III and *Nru* I from pHD2 (designated as pHDN) did not (Fig. 1.c). This indicates the importance of ORF1 for the expression of enzymatically active 763 *PrtP* by the bacteria.

In order to further analyze the relationship between the two genes, we sequenced the flanking region of ORF1. As shown in Fig.1, ORF1 was found to comprise 900 nts. There was a non-coding region consisting of 319 nts between ORF1 and the 763 *prtP* gene, and the promoter and SD sequences were found to be in the opposite direction of 763 *prtP*. Comparison of the nucleotide sequence of ORF1 with those of the *prtM* genes from *L. lactis* strains Wg 2 (8) and SK11 (9) revealed

Fig. 1. Relationship between the DNA fragments subcloned from pLP 763 plasmid and proteinase phenotypes. (a) A DNA fragment encompassing *Bgl* II and the end of 763*prtP*, a positive control, (b) a fragment encompassing *Hind* III to *Pst* I in pHD2, and (c) a fragment encompassing *Nru* I to *Pst* I in pHDN. Arrows indicate the positions and transcriptional directions of the two genes. The phenotypes of the strains carrying these plasmids on glucose-citrate milk agar (1) are shown at the right.

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differences of only one nucleotide with Wg 2, and 2 nucleotides with SK11. These differences, respectively, resulted in only one amino acid substitution at the 46th position from the N-terminus of the deduced protein sequence. From these results, we conclude that ORF1

is the gene that encodes PrtM for pLP 763. The similarities of the nucleotide sequences and the same gene organization of *prtM* and *prtP* from the proteinase plasmids of three strains suggest that they were derived from a common ancestral prototype.

Fig. 2. Nucleotide and deduced amino acid sequences of the gene required for the maturation of 763 proteinase. Sequencing was performed as described previously (1). The different nucleotides and amino acid residues of the maturation genes of Wg2 (8) and SK11 (9) compared to that of 763 are shown above and below the sequences, respectively.

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Lactococcus lactis subsp. *cremoris* NCDO 763 の生産する 763プロテアーゼの成熟に必要な遺伝子の塩基配列

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要 約

Lactococcus lactis subsp. *cremoris* NCDO 763 株のプラスミド pPL 763 において, 細胞壁結合型プロテアーゼ遺伝子上流にそのプロテアーゼ活性の発現に必要な遺伝子 (*prtM*) が検出された。その塩基配列解析の結果, *Lactococcus lactis* Wg 2 および SK11 で報告されている *prtM* と 900 塩基中それぞれ 1 および 2 塩基を除き相同であった。

Key words : *Lactococcus lactis* subsp. *cremoris* NCDO 763, cell wall-bound proteinase, maturation gene (*prtM*)