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Fengycin or plipastatin? A confusing question in Bacilli

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Abstract

According to most of the related literature published since their discovery in 1986, fengycin and plipastatin are very related molecules. These are lipodecapeptides encoded by operons of five synthetase genes. The most important difference between these two molecules lies in the peptide moiety at the position of the D-tyrosine, which is encoded by the second gene *fenB* of fengycin operon and by the fourth gene *ppsD* of plipastatin operon. Here, we aimed to differentiate between fengycin and plipastatin molecules. We designed degenerate primers using the consensus sequence of the epimerization domain responsible for the transformation of L-tyrosine to D-tyrosine from *Bacillus subtilis* 168, *Bacillus amyloliquefaciens* FZB42, and *Bacillus atrophaeus* 1942. These degenerate primers were then used to amplify fragments from *B. amyloliquefaciens* S499, *B. subtilis* ATCC 21332, and *Bacillus cereus*. Alignment of the sequences of the amplified fragments with the sequences from the mentioned strains deposited in GenBank database showed high similarity with 64 *B. subtilis* strains, 24 *B. amyloliquefaciens* strains, seven *B. atrophaeus* strains, one *B. cereus* strain, one *Bacillus sonorensis* strain, two *Bacillus methylotrophicus* strains, and 45 *Bacillus velezensis* strains. The results confirmed that these *Bacillus* strains *harbor* the tyrosine epimerization domain located on the fourth gene of their fengycin or plipastatin operons, which indicated that these strains synthesize plipastatin rather than fengycin.

Key words: fengycin, plipastatin, Bacilli, epimerization domain, NRPS

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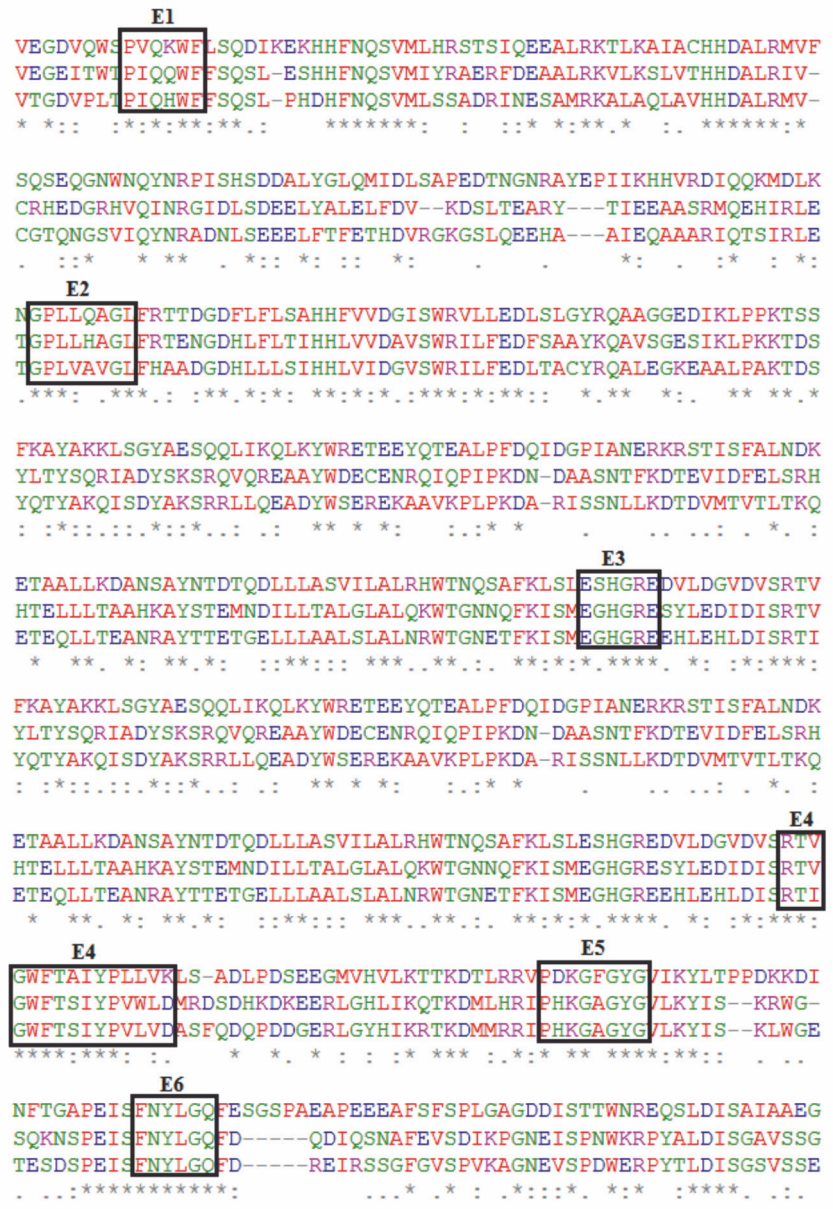

Fig. S1. Clustal multiple-sequence alignment of the sequenced epimerization domain protein from *Bacillus cereus*, *Bacillus subtilis* ATCC 21332, and *Bacillus amyloliquefaciens* S499 amplified by *Ep* and *fenB* degenerate primers; the epimerization domain of six consensus sequences is shown in black boxes

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Fig. S2. The sequences of epimerization domain (partial *ppsD* gene, 1670 bp) and partial *ppsB* (1446 bp) of *Bacillus subtilis* ATCC 21332 amplified by *Ep* and *fenB* degenerate primers; *Ep* fwd and rev primers and *fenB* fwd and rev primers are shown in blue

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Fig. S3. The sequences of epimerization domain (partial *ppsD* gene, 1651 bp) and partial *ppsB* (1447 bp) of *Bacillus cereus* amplified by *Ep* and *fenB* degenerate primers; *Ep* fwd and rev primers and *fenB* fwd and rev primers are shown in blue

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Fig. S4. The sequences of epimerization domain (partial *ppsD* gene, 1641 bp) and partial *ppsB* (1449 bp) of *Bacillus amyloliquefaciens* S499 amplified by *Ep* and *fenB* degenerate primers; *Ep* fwd and rev primers and *fenB* fwd and rev primers are shown in blue