Identification of binding sites of Lactobacillus plantarum enolase involved in the interaction with human plasminogen

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The enolase EnoA1 of Lactobacillus plantarum, previously shown to be cell surface-expressed and involved in binding with the fibronectin, is here shown to interact with human plasminogen (Plg). By sequence allignment of EnoA1 with Streptococcus pneumoniae and Bifidobacterium lactis enolases, we identified putative BS1 and BS2 Plg-binding sites. A structure prediction of EnoA1 generated with the Rosetta server showed lysine residues in position 255 (BS2), and 422 (BS1) exposed on protein surface. Furthermore, a lysine residue in position 259 was as well identified as a surface-exposed aminoacid. The enoA1 gene was site directed-mutagenized to generate four mutated proteins, which were expressed in Escherichia coli and purified along with the wild type EnoA1 protein. The mutated proteins carried the aminoacid substitutions K255A, K259A, K422A, and a double substitution K259A/K422A. The functional role of these lysine residues was assessed by evaluation of specific Plg-binding activity of the mutated proteins. While the binding activity of the mutated proteins was drastically reduced, their enzymatic activity was reduced only to about 50% of the wild type protein. Our results show that L. plantarum EnoA1 exhibits the Plg-BS1 characterized by only one lysine residue, and the Plg-BS2 extending up to the lysine residue in position 259, therefore consisting of 12-aa residues instead of the 9-aa residues described in S. pneumoniae. Furthermore, a test performed on whole cells of L. plantarum, demonstrated that after inducing the conversion of the cell-bound plasminogen to plasmin, this is released into the medium, contrary to the mechanism present in most pathogens, where the plasmin remains cell-surface-bound.
