CHARACTERIZATION, PRODUCTION AND APPLICATION OF A NOVEL LANTIBIOTIC FROM BACILUS AMYLOLIQUEFACIENS

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Introduction. The class I bacteriocin, the so-called lantibiotic, are heat stable post-translationally modified peptides that contain the thioether amino acids lanthionine and methyllanthionine. These are capable to inhibit the growth of gram-positive bacteria, including Listeria monocytogeneses, Staphylococcus aureus or Bacillus cereus, the causative agents of food-borne diseases or nosocomial infections. Lantibiotics, such as nisin have been widely studied and used commercially as food preservative. However, limitations of use due to its low solubility at pH above 6, its inactivation in certain type of food and the emergence of nisin-resistant strains point out the need for the identification and characterization of novel bacteriocin or bacteriocin-like compounds to overcome these problems.
Here, we report on the biochemical and genetic characterization of a novel lantibiotic, named amylolysin from Bacillus amyloliquefaciens GA1, the development of an efficient production and purification process, and application as food-preservative on poultry meat**.**

Methods. Amylolysin gene cluster was characterized from a shot-gun sequencing of the producer strain genome. Amylolysin was produced in 10 h process in a 80-liters bioreactor in rich medium and purified from the culture supernatant by extraction on Amberlite XAD-16 resin and two steps of reverse-phase chromatography. Pure amylolysin was subsequently analyzed by MALDI-TOF and LC-MS analysis. MIC was determined by a standard microdilution method. Dissruption of Amy ORF was performed by a method derived from (1). Interaction with the peptidoglycan synthesis was demonstrated in vitro by direct interaction ^{14}C labeled Lipid II by thin layer chromatography and in vivo using the LiaRS based reporter gene system (2). The membrane pore forming mode of action was evidenced by electric potential measurements and cell leaking experiments as described in (3). Antilisterial activity in chicken meat was evaluated by analyzing *L. monocytogense* growth in meat during 21-days of storage at 4°C.

Results. In silico analysis revealed that the amylolysin biosynthetic genes span over 9.5 kb and contain seven ORFs clustered into four operons. Highly conserved characteristic structural motifs were highlighted, notably the one involved in the interaction with the peptidoglycan precursor Lipid II. The presence of two separate AmyM and AmyT genes suggest that amylolysin belongs to the type B lantibiotic. Disruption of the Amy locus in B. amyloliquefaciens GA1 confirmed that the identified locus is responsible for amylolysin synthesis. Beside this, the biological mode of action of amylolysin was investigated. It was evidenced either, in vitro, by means of 14C labeled lipid II and in vivo, using the LiaRS based reporter gene, that amylolysin interact directly with Lipid II. In addition, transmembrane electric potential measurements and cell leaking experiments demonstrate clearly the ormembrane pore-forming mode of action of amylolysin. Amylolysin was found effective on different Gram-positive bacteria including MRSA strains.
The capacity of amylolysin to inhibit proliferation of different isolates of L. monocytogenes in poultry meat was demonstrated for different amylolysin concentrations. By contrast to nisin, amylolysin had a bacteriostatic effect on L. monocytogenese in our experimental condition. Moreover, the stability of amylolisin during incubation with meat protease points to its low sensitivity to meat endogenous proteolitic content by contrast to nisin.

Conclusions. This study is a first report an the genetic, structural and mode of action characterization of a type B lantibiotic in B. amyloliquefaciens that could be used as food preservative.

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References.

1. Fickers P, Le Dall MT, Gaillardin C, Thonart P, Nicaud JM (2003) J. *Microbiol Meth.* 55:727-737
2. Burkard M, Stein T (2008) *J. Microbiol. Meth*. 75:70-74
3.Fickers P, Guez JS, Damblon C, Leclere V, Bechet M, Jacques P, Joris B (2009) App. Env. Microbiol. 75:4636-4640