

**Effectiveness and Usefulness of the Cationic Antimicrobial Substances Derived
from Natural Products: Bacteriocin from Lactic Acid Bacteria, Protamine, and
 ϵ -Poly-L-Lysine**

Tomomi Hata

Summary

On the way of the wide screening for natural cationic antimicrobial materials, two types of the cationic antimicrobial proteins (1. bacteriocins, 2. protamine and ϵ -poly-L-lysine) were evaluated.

1. Bacteriocins

A lot of lactic acid bacteria (LAB) were screened, and some promising bacteriocins were isolated from various fermented foods. The continuous screening was lead to obtain two bacteriocin-producing LAB strains. One of the strains was determined as *Lactobacillus plantarum* A-1, and an another strain was *Enterococcus faecalis* N1-33 with the conventional method. The bacteriocins produced by two LAB strains were isolated, purified, and structural

analyzed.

As to the strain of *Lactobacillus plantarum* A-1, the novel bacteriocin, which contained a double glycine type leader peptides and two disulfide bonds in the structure, was isolated. This bacteriocin was termed **Plantaricin ASM1 (PASM1)**.

About the strain of *Enterococcus faecalis* N1-33, the hitherto known bacteriocin “**Enterocin MR10A**” was isolated and determined. **Enterocin MR10A** has excellent thermal and pH stability. Furthermore, **Enterocin MR10A** showed a broad antimicrobial spectrum including some food pathogenic bacteria such as *Bacillus cereus* and *Listeria monocytogenes*. Therefore, we tested whether the antibacterial activity of **Enterocin MR10A** against *Bacillus cereus* and *Listeria monocytogenes* is the practical effect or not in the actual food systems by using several food models. For example, the actual antibacterial activity of **Enterocin MR10A** against *Bacillus cereus* was examined by using a custard cream model, and also against *Listeria monocytogenes* was examined by using a fermented cucumber model, respectively. **Enterocin MR10A** was suppressed the superiority in growth of both strains in these food models, and so that **Enterocin MR10A** was suggested to be an actually useful bacteriocin which can be expected to strong growth inhibition against food poisoning bacteria.

Additionally, attempting to eliminate the plasmid from the strain N1-33 using the plasmid-curing agent novobiocin was obtained the mutant strain, which was seemed to

paradoxically overproduction of bacteriocin than the original strain, termed N41-51. The number of originated **Enterocin MR10A** structural gene was detected by real-time PCR. It showed that its level of the strain N41-51 was about two-fold higher than that of original strain based on the level of originated 16S rDNA. Correspondingly, the strain of N41-51 appeared to produce about two fold more bacteriocin compared with the original strain in these culture broth, respectively. It was suggested that the structural gene of **Enterocin MR10A** was coded in the plasmid. It was namely shown that the copy number of the plasmid of the strain N41-51 was increased from that of the wild type strain with the plasmid curing treatment. It could be shown the one meaningful way to increase the amount of bacteriocin production easily and effectively.

2. Protamine and ϵ - poly-L-lysine

About another cationic antimicrobial protein, the usefulness of antifungal effects of both "protamine salmine hydrochloride (PSH)" and " ϵ - poly-L-lysine (ϵ -PL)" were verified.

The antifungal activities of PSH and ϵ -PL were examined in the medium supplemented with 50% (wt/vol) sucrose, as a model of confectioneries, because fungal contamination often occurs in confectioneries with high saccharide content during storage. And it was considered a serious problem in practice. As a result, both PSH and ϵ -PL showed antifungal effects of the level that deserves to practical use in the high saccharides condition. The sugar itself was considered to contribute to the stabilization of the conformation of PSH or ϵ -PL, in addition to the free water

in the medium was reduced (the reduction of water activity) by the presence of saccharides.

To confirm these antifungal activities into actual food systems, the dumpling and the steamed bread were prepared as the model of rice- or wheat-based confectioneries, which have currently problems of contamination of fungus. They substantiated practicable antifungal activities against confectionery pollution fungus in these models. The results demonstrated that both PSH and ϵ -PL are effective not only about antibacterial agents but also antifungal agents in actual food systems with high saccharide contents such as confectioneries.