Studies on a new antimicrobial mechanism of lacticin Q

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論　文　内　容　の　要　旨

Bacteriocins are ribosomally synthesized bacterial antimicrobial peptides or proteins. Bacteriocins produced by gram-positive bacteria are of particular interest due to the industrial use of some strains that belong to lactic acid bacteria (LAB). Nisin A is the most extensively studied LAB bacteriocin, and applied as a food preservative so far. However, the low stability at neutral and alkaline pH conditions limits the range of usage of nisin A. This circumstance gives rise to the need for novel bacteriocins superior to nisin A. A novel LAB bacteriocin, lacticin Q has a wide activity spectrum against gram-positive bacteria. In addition, lacticin Q is highly stable under a wide range of pH and heat treatment. It is expected to be utilized in various areas such as a safe food preservative as nisin A.

The antimicrobial mechanism model of lacticin Q was designated as the huge toroidal pore (HTPs) model. However, the intensity of the activity of lacticin Q against gram-positive bacteria is varied by species, and in some cases, even by strains. Although this selective antimicrobial activity phenomenon is also observed in most of bacteriocins, the reasons still remain unknown. For the practical use of lacticin Q, the mechanism of the selective antimicrobial activity is necessary to be clarified. Since bacteriocins do not always show antimicrobial activity on a single mechanism, new antimicrobial mechanisms would involve in the selective antimicrobial activity other than the HTPs formation. Therefore, the selective antimicrobial activity and a new antimicrobial mechanism were investigated in this thesis.

First, some possible influence factors were investigated for the selective antimicrobial activity of lacticin Q *in vitro* and *in vivo*. *In vitro*, significant relationship was not observed between membrane lipid compositions and the pore formation by lacticin Q. *In vivo*, no direct relationship was also identified between the pore formation and survival ability of indicator strains suffered by lacticin Q. These results implied that the selective antimicrobial activity of lacticin Q depends not only on the membrane lipid compositions, but also on characteristics of the indicator strains against pore formation induced by lacticin Q.

Recently, considerable interest has focused on the accumulation of reactive oxygen species (ROSs), which play an important role in bactericidal antibiotic-induced bacterial cell death. So, it was investigated whether the accumulation of deleterious hydroxyl radicals (a kind of ROSs) is induced by lacticin Q as a contributing factor to cell death. When the minimum bactericidal concentrations (MBCs) of lacticin Q were added to the indicator cultures, hydroxyl radical accumulations were detected in all the indicator strains tested. The rescue of indicator strains by radical scavengers, and accumulation of hydrogen peroxide in the indicator cultures further suggested that lacticin Q exerts activity involving accumulation of hydroxyl radical by the Fenton reaction. The different patterns of accumulation of hydroxyl radical by the minimum inhibitory concentrations (MICs) of lacticin Q suggested that hydroxyl radical accumulation depends on the potentials of indicator strains to scavenge the deleterious hydroxyl radicals. Consequently, these results

suggest that the final antimicrobial mechanism of lacticin Q is the accumulation of hydroxyl radicals, which varies by strains, resulting in the selective antimicrobial activity.

It was further investigated whether other LAB bacteriocins share the antimicrobial activity mechanism involving hydroxyl radical accumulation as lacticin Q. Nisin A, enterocin W and leucocyclicin Q were also found to accumulate hydroxyl radical even though they have different structures and patterns of pore formation activity on bacterial membrane.

In this thesis, a new antimicrobial activity mechanism of lacticin Q involving the accumulation of hydroxyl radicals was identified, which is shared with at least 3 kinds of other LAB bacteriocins. These findings would provide a helpful reference for other LAB bacteriocins and have significant implications for the practical use of lacticin Q and other LAB bacteriocins.