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-Abstract Sample-

1P-138 **Elucidation of the biosynthetic mechanism of the circular bacteriocin, enterocin NKR-5-3B**

○ Rodney Perez[1], Tomoko Inoue[1], Kohei Himeno[1], Naoki Ishibashi[1], Takeshi Zendo[1], Jiro Nakayama[1], Kenji Sonomoto[1,2]
([1]Fac. Agric., Kyushu Univ., [2]Bio-Arch., Kyushu Univ.)
XXXXXXX@gmail.com

Recently a class of bacteriocins characterized by its circular structure have attracted a great attention among researchers due to its better structural stability, higher thermal stress resistance, and superior proteolytic stability compared to other bacteriocins. The sturdiness of this group of bacteriocins is attributed by its circular structure where the N- and C- termini are covalently bounded. Understanding the detailed mechanism by which these bacteriocins are synthesized will materialize its full application potential as biological delivery agents and as scaffolds in drug design. One of the bacteriocins produced by *Enterococcus faecium* NKR-5-3, enterocin NKR-5-3B (Ent53B), is a novel circular bacteriocin. The putative structural gene and 9 putative *orf*s were identified after sequencing a region of 8 kb in the producer strain' s genome. To know the functions of these putative Orfs, plasmids encoding different combinations of these genes were constructed using pMG36c vector and cloned into *E. faecalis* JH2-2 and subsequently evaluated for Ent53B production and self-immunity through direct colony overlay assay and spot-on-lawn assay respectively. The production of Ent53B was only observed from the mutant expressing *ent53B-orf1234*, suggesting that its production and secretion require a cooperative function of these Orfs. Moreover, self-immunity assay of these mutants revealed that strain NKR-5-3 has two self-immunity systems. Transformants expressing *orf4* and/or combination of *orf1* and *orf3* were immune to the inhibitory action of Ent53B.

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