Studies on biosynthetic and action mechanisms of a novel bacteriocin, lactococcin Z

ゴソン, モスバー, アブデルカレム, ダバ

Name：Ghoson Mosbah Abdelkarem Daba

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(新奇乳酸菌バクテリオシン、ラクトコッシン Z の生合成機構および作用機構に関する研究)

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Thesis Summary

Bacteriocins produced by lactic acid bacteria (LAB) show variety in their structure, biosynthetic mechanism, antimicrobial spectra, and action mechanisms. One of the most interesting groups of bacteriocins is the *Lactococcus*-specific bacteriocins, as they target only *L. lactis* strains. Such bacteriocins may have limited applications in the field of microbial control, but studying them can participate effectively in designing highly selective antimicrobial peptides. Furthermore, knowing their action mechanisms may contribute in understanding of the competition among closely related species in ecological environment.

Lactococcin Z is a novel bacteriocin produced by *Lactococcus lactis* QU 7 isolated from lettuce grown in Fukuoka, Japan. Its molecular mass is 5041.35 Da, and it has a narrow antimicrobial activity only against *L. lactis* strains (a *Lactococcus*-specific activity). The gene encoding the lactococcin Z prepeptide was identified. However, the gene cluster responsible for the lactococcin Z biosynthesis has not yet been identified, and the killing mechanism exerted by lactococcin Z has not been characterized. The aim of this study was to elucidate the biosynthetic and action mechanisms of lactococcin Z.

The lactococcin Z gene cluster (approximate 5.1 kb) was identified, which consists of the previously identified structural gene (*lczA*) and genes for immunity (*lczB*), and transport (*lczC* and *lczD*). Heterologous expression verified that LczB is the immunity protein responsible for conferring self-immunity to the lactococcin Z producer strain. Moreover, an ABC transporter, LczC, has been proved to be responsible for secretion of lactococcin Z with support of LczD, a transport accessory protein. It was confirmed that biosynthesis of lactococcin Z is conducted by the proteins encoded by *lczABCD*, as proposed in Figure 1.

LczB was further studied in comparison with immunity proteins of two other *Lactococcus*-specific bacteriocins namely LciA and LaqC, the immunity proteins of lactococcins A and Q, respectively. LczB exhibited no cross-immunity neither to lactococcin A nor to lactococcin Q, which suggested that lactococcin Z employs a receptor other than those targeted by lactococcins A and Q. Therefore, the receptor targeted by lactococcin Z was investigated through generating some lactococcin Z resistant mutants from the sensitive strain *L. lactis* IL1403. All tested resistant mutants showed difficulty in growing on mannose or glucose as sole sugar sources. Furthermore, mutations were detected in *ptnC* and *ptnD* encoding respective IIC and IID subunits in the Man-PTS. These results indicated that the receptor employed by lactococcin Z is the Man-PTS subunits IIC and IID, which is the

same receptor targeted by lactococcin A; however, lactococcin Z utilizes different position(s) of this receptor other than those recognized by lactococcin A.

The action mechanism of lactococcin Z was investigated *in vivo* and *in vitro*. Lactococcin Z in 10 x MIC barely induced ATP efflux from the two tested sensitive strains, *L. lactis* IL1403 and *L. lactis* ATCC 19435^{T}, and it failed to dissipate their membrane potential. Moreover, lactococcin Z in 5 x MIC exhibited a bactericidal activity against both tested indicator strains. Furthermore, no leakage was induced from large unilammellar vesicles (LUVs) after treatment with lactococcin Z. These results indicated that lactococcin Z is a non-pore former, and its mode of action differs completely from those exerted by other known *Lactococcus*-specific bacteriocins. It is highly suggested that lactococcin Z interacts with the Man-PTS in a manner that causes growth inhibition of the sensitive cells.

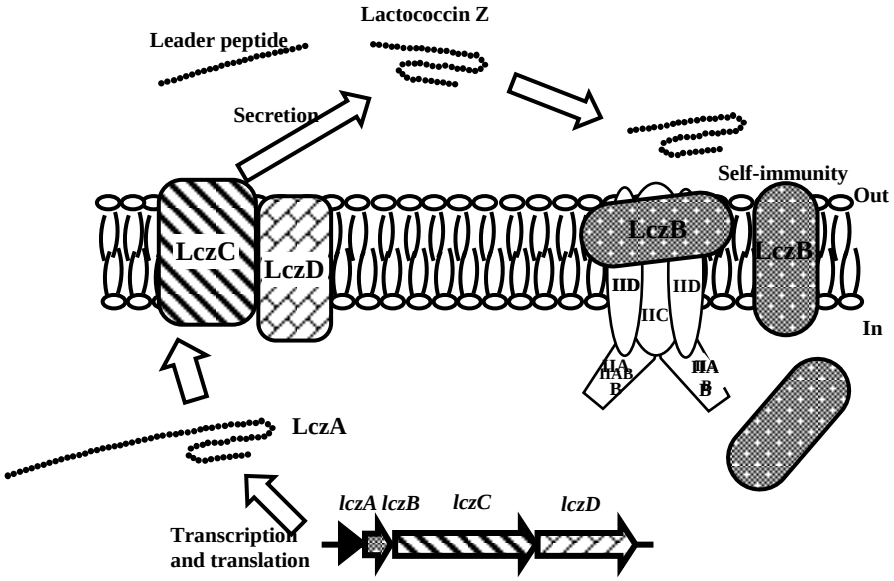

Figure 1 Proposed model for lactococcin Z biosynthesis. Lactococcin Z is produced by expressing *lczABCD*. The structural gene (*lczA*) encoding the lactococcin Z precursor is transcribed and translated. Then, the resulting peptide is secreted by the ABC transporter (LczC) through support by the transport accessory protein (LczD), coincidentally with cleavage of the leader peptide. The immunity protein (LczB) confers self-immunity to the lactococcin Z producer cells probably by protecting the receptor Man-PTS subunits IIC and IID.

Our data elucidated the biosynthesis (secretion and immunity) of the novel *Lactococcus*-specific bacteriocin, lactococcin Z. Moreover, Man-PTS components IIC and IID were identified as a receptor targeted by lactococcin Z. Finally, it was verified that lactococcin Z exhibited different immunity and action mechanisms from other known *Lactococcus*-specific bacteriocins. This is the first time to report a non-pore forming, bactericidal *Lactococcus*-specific bacteriocin.