Study on the biosynthetic mechanism and the mode of action of a circular bacteriocin, leucocyclicin Q

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（環状バクテリオシン，ロイコサイクリシン Q の生合成機構と作用機構に関する研究）

区　　分　：甲

論　文　内　容　の　要　旨

Leucocyclicin Q (LcyQ), a circular bacteriocin produced by *Leuconostoc mesenteroides* TK41401, is active against a unique range of Gram-positive bacteria. LcyQ shares a remarkable sequence identity with lactocyclicin Q (LycQ), a circular bacteriocin from *Lactococcus* sp. strain QU 12. Although circular bacteriocins constitute one of the most attractive groups of inhibitors described to date, their biosynthetic mechanism is still little known. Therefore, the aim of this study is to clarify the biosynthetic mechanisms and the mode of action of LcyQ.

Sequence analysis and database search of the regions flanking the LcyQ structural gene *lcyQ* revealed four open reading frames (*lcyR*, *lcyB*, *lcyC*, and *lcyD*) related to bacteriocin biosynthesis. LcyR shares 43% identity to a TetR family transcriptional regulator, LcyB shows 32% identity to an acyltransferase, and LcyC showes 37% identity to an ATP-binding protein. LcyD shares some similarity to the DUF95 superfamily proteins, often found in the biosynthetic gene clusters of circular bacteriocins. The *lcyD* knockout mutant accumulates active mature LcyQ inside the cells. Heterologous expression of *lcyC* and *lcyD* demonstrated that they confer robust immunity (self-protection) against LcyQ. Peptide release/binding assay revealed that the immunity is attributed to the release of LcyQ to the cell exterior. Thus, the DUF95 superfamily protein, LcyD, has a dual function in the biosynthesis of LcyQ, as an immunity-associated transporter and as a secretion-aiding agent. Accumulation of mature LcyQ inside the cells in the *lcyD* knockout mutant further implied that cyclization of LcyQ occurs within the cell.

The immunity mechanism was further verified by the FITC-LcyQ interaction assay. The results indicated that the main immunity mechanism of LcyQ is conferred by the transporter systems employing immunity protein LcyC and LcyD. Furthermore, carbohydrate fermentation assay showed that immunity proteins LcyC and LcyD might interact with maltose ABC transporters or some proteins that can inhibit the function of maltose ABC transporters.

The mode of action of LcyQ was characterized both *in vitro* and *in vivo*. Calcein leakage and translocation assay verified that LcyQ can bind cell membrane rapidly and significantly, make pores and induce the release of cell solutes such as ATP to the outside, leading the cell death. Calcein leakage assay also showed that the different mode of action between LcyQ and LycQ. The further prediction of second structures indicated that their helix 1 and helix 2 are important for the different modes of action of LcyQ and LycQ. In addition, linear LcyQ-C-His did not show any antimicrobial activity, which indicated that the circular structure is important in the mode of action of LcyQ.

The main immunity mechanism, the important role of a DUF95 superfamily protein in the biosynthesis, and the mode of action of LcyQ were clarified in this thesis. These will provide a platform for the further understanding the bioengineering of circular bacteriocins, which might have exciting applications in future.