Understanding the regulation mechanisms of two novel Bacteriocins

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Background

Bacteriocins have been widely studied for their antimicrobial characteristics, especially against harmful pathogens. In the bacterial community, many Gram positive (Gm+) and Gram negative (Gm–) bacteria are known to produce bacteriocins. Generally, the genes cluster, transcriptional regulation and mode of action is related to the class of bacteriocins. Considering the extraordinary variety of antimicrobial compounds (AMP) such as bacteriocins, their production are influenced by biotic and abiotic factors.
This study aimed to understand the regulation of the production of two new bacteriocins. Test strains did not show antimicrobial activity in vitro, suggesting a potential downregulation of these novel bacteriocins with a unique operon organization.. However, there are notable similarities between the two operons and other bacteriocins already known (under the same class) reinforces the interest for future application in food bioperservation.

Materials and Methods

In silico bacteriocin detection

In silico genome analysis of the whole genome of the strains under investigation was studied using three online programs which includes Bagel 4 [5] antiSMASH 5.0 [2] and RiPPMiner [1] dedicated to this purpose.

Bacteriocin operon evaluation

Coding sequences were identified using the NCBI database and blastx and tblastn software. Putative bacteriocin genes within the respective genomes were annotated using the CLC Main Workbench software.

Assay for antimicrobial activity

Well-diffusion method and colony overlay assay were used as described respectively by [3][4] .

Construction of plasmids

The integration vector were based on the pNZ8048 *Lc. lactis* NICE system plasmid and cloned in *Lactococcus lactis* Strain NZ9000. Nucleic acid manipulations and general cloning procedures were carried out according to standard protocols, as described by Sambrook and Russel [6]. Electro transformation of *E. coli* MC1061 and *Lactococcus lactis* expression Strain NZ9000 was achieved following [7].

Results & Discussion

Both strains under study doesn't show constitutive bacteriocin production. Multiple growing conditions were tested including different media, pH, temperature, co-culture with pathogen or probiotic strains. However, the presence of these operons have been confirmed. The results of the alignment of the genome area containing the Bacteriocin loci with and without the mobile elements detected was summarized in Figure 1. There are several ways in which the activity of mobile element can positively and negatively impact a genome; for example, gene inactivation, modulate gene expression or induce illegitimate recombination.

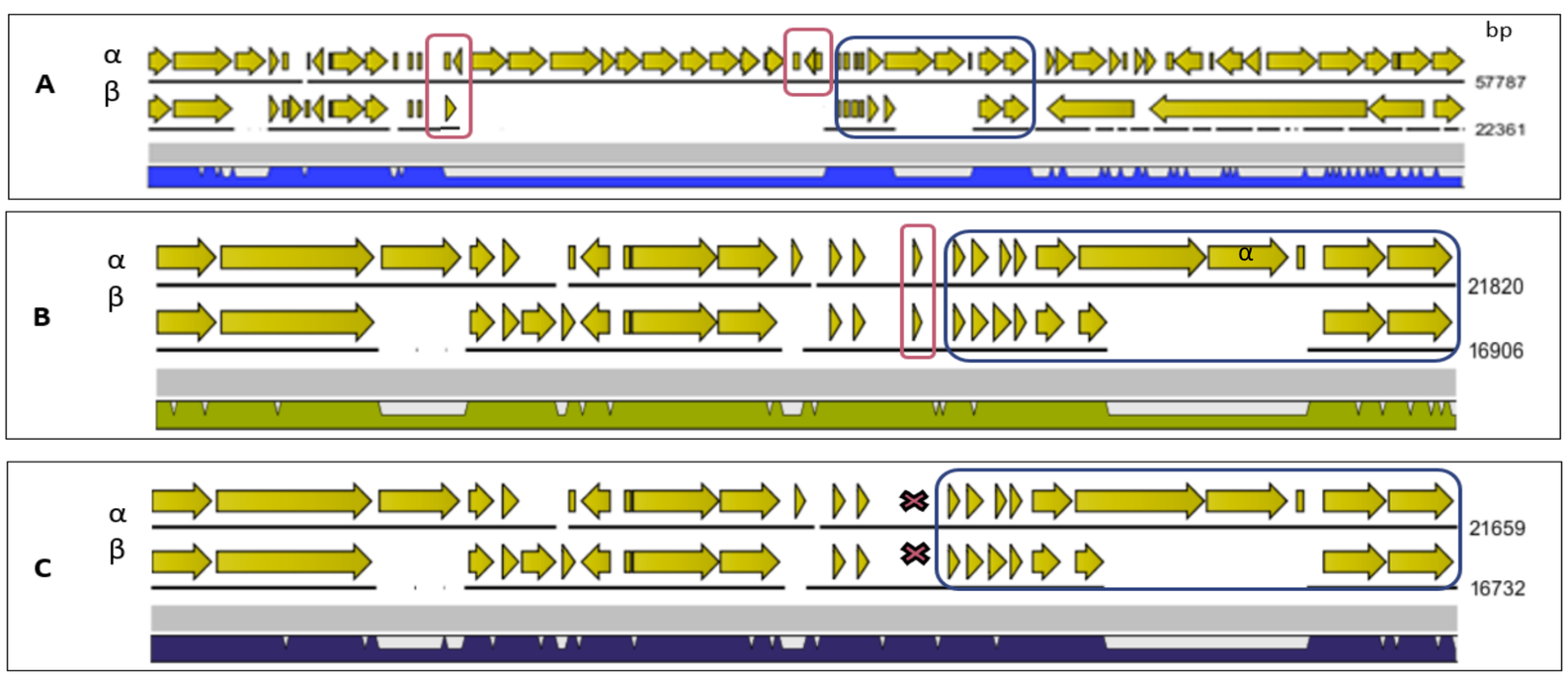
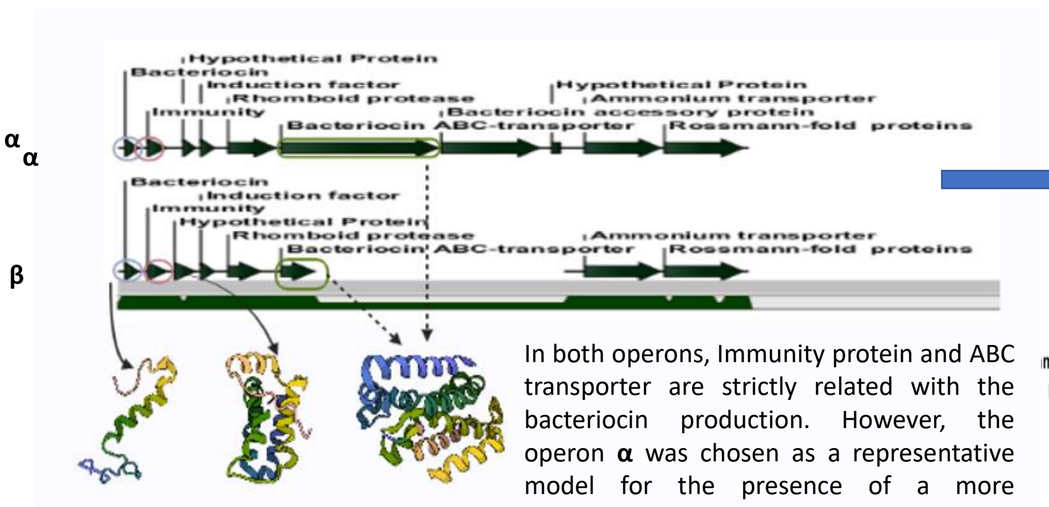
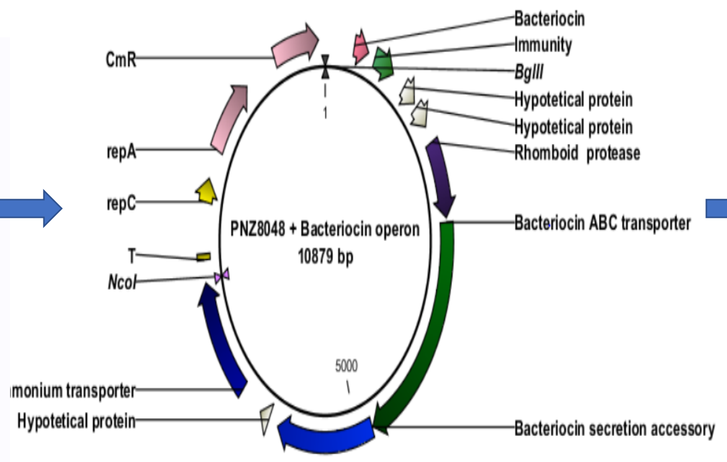


Figure 1 : Alignment *of genomic sequence including the bacteriocin operon from Strain 1 and 2.*

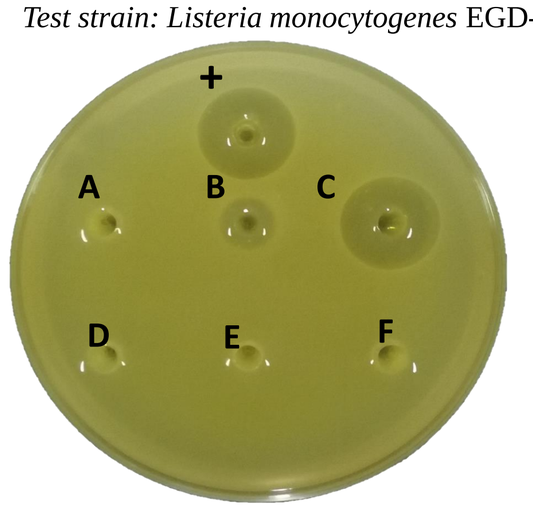
Strain 1	Strain 2	Genes Similarity (%)
BACTERIOCIN	BACTERIOCIN	97
Immunity	Immunity	98
Hypothetical	Hypothetical	100
IF Induction factor (potential)	IF Induction factor (potential)	
Rhomboid family	Rhomboid family	78
ABC Transporter	ABC Transporter	72
Accessory Bacteriocin protein	-	-
Hypothetical	-	-
Ammonium transporter	Ammonium transporter	90
NAD(P)-binding domain-containing protein	NAD(P)-binding domain-containing protein	76



In both operons, Immunity protein and ABC transporter are strictly related with the bacteriocin production. However, the operon **α** was chosen as a representative model for the presence of a more representative ABC transporter and a bacteriocin accessory protein.



Test strain: Listeria monocytogenes EGD-e



Well-diffusion : Spot of 50 µL supernatant cell free pH adjusted 6,5.
A: *Lactococcus lactis* PN9000
B: *Lactococcus lactis* PN9000-PNZ8048
C: *Lactococcus lactis* PN9000-PNZ8048+Bacteriocin operon
D-E-F : Same order above + Proteasi K (2 mg/ml)
Positive control: Plantarocin 423

Conclusion

- ✓ **Bacteriocin mining and in silico genome analysis showed that the two strains under study were carriers of different operons responsible for producing two novel bacteriocins.**
- ✓ **Mobile elements upstream to the bacteriocin loci can downregulate the bacteriocin production in the wild strain.**
- ✓ **Successful cloning of the complete α operon confirmed that the operons' functionality can transcribe and secrete the bacteriocin of interest.**

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