Lactococcus lactis subsp. *lactis* 431, a nisin producing strain isolated from kimchi

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Introduction

Antimicrobial peptides, including bacteriocins, have already almost one century of history. First reports on bacteriocins, produced by *E. coli*, called colicins were described in 1925, and in 1928, the first report on nisin was published [1]. Initially proposed as antimicrobials with application in the food industry, in the last decade bacteriocins have won scientific and pharmaceutical attention as potentially therapeutical antimicrobials [2]. Bacteriocins are described as via ribosomal produced antimicrobial peptides, normally not subjected to postproduction modifications, with activity against closely related to the producer species [3]. An iconic example for bacteriocins is nisin, produced by different strains of *Lactococcus* spp.

To our best of knowledge this is the first report on strains of *Lactococcus lactis* isolated from kimchi, producer of nisin.

***Purpose*: The goal of this study was to explore bacteriocinogenic properties for LAB isolated from kimchi.**

Results: Selected isolate 431 was identified as *Lactococcus lactis* subsp. *lactis* based on biochemical, physiological, and biomolecular 16S rRNA gene sequencing. The bacterial effect of bacteriocin produced by strain 431 on *Listeria* spp. and *Staphylococcus* spp., has been shown for actively growing and stationary cells. Similar growth and bacteriocin production were observed when *Lactococcus lactis* subsp. *lactis* 431 was cultured in MRS at 30ºC or 37ºC. The presence of nisin operon with some point mutations on the genomic DNA was recorded based on the performed PCR reactions targeting different genes associated with nisin expression. Good growth for strain 431 was recorded in MRS broth with pH 5.0 to 9.0 and in absence of oxbile or concentration below 0.8%. *Lactococcus lactis* subsp. *lactis* 431 was shown that can be considered as safe for human and animals application based on negative reaction for production of biogenic amines, lipolytic, proteolytic and γ-hemolytic activity and susceptibility to different antibiotics and growth not affected by different commercial drugs.

***Significance*: Nisin producing *Lactococcus lactis* subsp. *lactis* 431 shows to have potential beneficial properties: production of nisin, be safe and suitable to GIT environment.**

Material and methods

Methods: Different LAB were isolated from kimchi, obtained from the region of Pohang, Korea and identified based on physiological, biochemical, and molecular methods. The promising isolate (*Lactococcus lactis* subsp. *lactis* 431) was evaluated for production of bacteriocin, including stability in presence of enzymes, chemicals, pH and temperatures. Adherence properties for the expressed bacteriocins by *Lactococcus lactis* subsp. *lactis* 431 were evaluated at presence of selected chemicals, pH and temperatures. The presence of bacteriocin genes in strain 431 was investigated and analysed. Growth of *Lactococcus lactis* subsp. *lactis* 431 in different pH values and presence of ox-bile was explored. Safety properties for *Lactococcus lactis* subsp. *lactis* 431 were evaluated.

References

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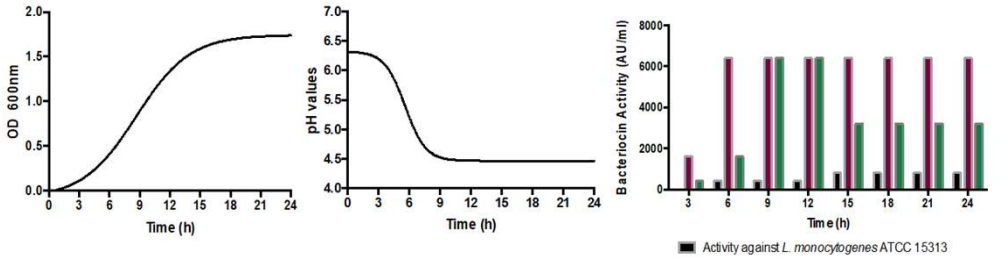
Dynamic of bacterial growth (evaluated as changes in OD at 600 nm), acidification (evaluated as changes in pH) for *Lactococcus lactis* subsp. *lactis* 431 cultured in MRS at 37ºC and production of bacteriocins evaluated versus *L. monocytogenes* ATCC15313, *S. simulans* KACC13241 and *S. carnosus* subsp. *carnosus* KACC13250.



Analysis of the data obtained from the sequencing of amplicons generated from positive PCR results targeting genes parts of nisin operon in *Lactococcus lactis* subsp. *lactis* 431

Lactococcus lactis subsp. lactis 431					
Primer	DNA level		Protein level		function
	Identity	Accession #	Identity	Accession #	
nisA_F	99% antibiotic nisin (spaN)gene, complete cds 99% nisZ gene, complete cds 99% NisB gene, complete cds	J04057.1 AF420259.1 FJ972141.1	95% NisB 95% lantibiotic dehydratase	ACQ65769.1 WP_014570406.1	structure
nisA_R	99% antibiotic nisin (spaN)gene, complete cds 99% nisZ gene, complete cds 99% NisB gene, complete cds	J04057.1 AF420259.1 FJ972141.1	100% NisB 100% lantibiotic dehydratase	ACQ65769.1 WP_014570406.1	structure
nisB_F	99% nisin A (nisA) and nisB, nisC, nisT, and nisI genes, complete cds	L16226.1	98% NisB 98% lantibiotic dehydratase	ACQ65769.1 WP_014570406.1	maturation
nisB_R	99% nisZ gene, complete cds 98% NisB gene, complete cds	AF420259.1 FJ972141.1	95% NisB 95% lantibiotic dehydratase	ACQ65769.1 WP_014570406.1	maturation
nisI_F	94% nisI gene, complete cds 93% nis genes	AY380742.1 Z18947.1	66% Nisin immunity protein	AIS03234.1	immunity
nisI_R	96% nisI gene, complete cds 95% nis genes	AY380742.1 Z18947.1	92% Nisin immunity protein	AAQ89591.1	immunity
nisRK_F	99% nisR and nisK genes in nisin Z operon 99% nisI, nisP, nisR and nisK genes	Z22813.1 X76884.1	99% histidine kinase NisK	KST83030.1	regulator
nisRK_R	99% nisR and nisK genes in nisin Z operon 99% nisI, nisP, nisR and nisK genes	Z22813.1 X76884.1	99% histidine kinase NisK	KST83030.1	regulator
nisF_F	94% NisF , NisE, NisG, and SacR genes, complete cds	U17255.1	57% ABC transporter ATP-binding protein	WP_058220517.1	immunity
nisF_R	99% NisF , NisE, NisG, and SacR genes, complete cds	U17255.1	99% ABC transporter ATP-binding protein	WP_058220517.1	immunity
nisFEG_F	97% NisF , NisE, NisG, and SacR genes, complete cds	U17255.1	92% ABC transporter ATP-binding protein	WP_058220517.1	immunity
nisFEG_R	99% NisF , NisE, NisG, and SacR genes, complete cds	U17255.1	98% lantibiotic ABC transporter permease 96% transporter	WP_017864241.1 WP_191670030.1	immunity

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