S4-1

Enhancement of Bacteriocin Production by Its Sensitive Strain

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Introduction

Bacteriocin are bactericidal proteins that inhibit species closely related to the producer culture. Many bacteriocins have been identified and characterized for the industrially important lactic acid bacteria or *Bacillus* sp.. They include lactacin, nisin, lactocin, helveticin, sakacin, plantaricin, subtilin, bacilysin, botrycidin and others. To date, all characterized bacteriocins of bacteriocin producers have been produced in pure culture. Several reports have shown that production of bacteriocins is regulated via cell density dependent quorum-sensing mechanism. In these case, the bacteriocins also act as autoinducers or via quorum-sensing mechanism mediated by peptide pheromone or autoinducer. While pH, temperature, growth phase, media condition, and other microoganisms have been proposed to play an important role in the regulation of bacteriocin production. In fact, LAB are known to be more or less capable of producing bacteriocins depending on environmental conditions. But little or nothing is known about how these factors interact with the regulatory systems controling bacteriocin production.

We isolated bacteriocin producing some lactic acid bacteria and *Bacillus* sp. from Korea fermented foods, Kimchi and Doeonjang(soybean paste) and characterized the bacteriocins. During this work, we found out that some bacteriocin production was enhanced by presence of its sensitive strain. In this study, we reported the effect of bacteriocin sensitive strain on bacteriocin production and identified the responsible agent that influence the bacteriocin production.

Results

Table 1. Enhancement of bacteriocin GJ7 by *Leu. citreum* GJ7 in the presence of thermally inactivated lactic acid bacteria

Co-cultivation	Antimicrobial activity
Leu. citreum GJ7	+
Leu citreum GJ7 + *Lb Plantarum* KFRI 464	++++
Leu. citreum GJ7 + *Lb. delbruekii* KFRI 347	+++
Leu citreum GJ7 + *Lb Acidophilus* KFRI 150	+
Leu citreum GJ7 + *Leu mesenteroides* KCTC 1628	+++
Leu citreum GJ7 + *Lb plantarum* KFRI 236	+
Leu. citreum GJ7 + *Leu Mesenteroides* KFRI 218	+

Table 2 Stability of the inducing factor of bacteriocin GJ7

Treatment	Antimicrobial activity
Heat treatment	
bacteriocin GJ7(control)	+
bacteriocin GJ7 + IF	++
bacteriocin GJ7 + IF/4 ℃, 3 h	++
bacteriocin GJ7 + IF/37 ℃, 3 h	++
bacteriocin GJ7 + IF/50 ℃, 3 h	++
bacteriocin GJ7 + IF/70 ℃, 3 h	++
bacteriocin GJ7 + IF/100 ℃, 30 min	+++
bacteriocin GJ7 + IF/121 ℃, 15 min	+++
Proteolytic enzymes	
bacteriocin GJ7(control)	+
bacteriocin GJ7 + IF/AEBSF(100 mM)	++
bacteriocin GJ7 + IF/proteinase K(2mg/ml) and then AEBSF(100 mM)	+
bacteriocin GJ7 + IF/trypsin(2mg/ml) and then AEBSF(100 mM)	+
bacteriocin GJ7 + IF/α-chymotrypsin(2mg/ml) and then AEBSF(100 mM)	+

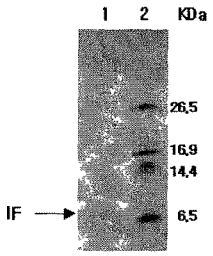

Figure 1. Tricine-SDS-PAGE of purified IF from *Lb. plantarum* KFRI 464

Table 3. Antimicrobial activity of BSCX1 against other bacteria

Indicator strains	Activity[1)]
Bacillus subtilis ATCC6633 gram(+)	+
Curtobacterium sp. cf3 gram(+)	+
Listeria monocytogenes KCTC3569 gram(+)	+
Salmonella typhimurium ATCC19430 gram(-)	+
Escherichia coli ATCC25922 gram(-)	+
Leuconostoc mesenteroides KCTC1628 gram(+)	+
Micrococcus luters ATCC9341 gram(+)	-
Staphylococcus aureus ATCC29213 gram(+)	+
Streptococcus mutans ATCC25175 gram(+)	-
Streptococcus faecalis ATCC29212 gram(+)	+
Pseudomonas aeruginosa ATCC27853 gram(-)	+
Pseudomonas aeruginosa ATCC9027 gram(-)	-

Table 4. Production of bactenocin BSCX1 by pure or mixed culture

Culture condition[1)]	Activity[2)]
producer alone(*B. subtilis* cx1)	+
B subtilis cx1+*B. subtilis* ATCC6633 0.01%	++
B subtilis cx1+*B subtilis* ATCC6633 0.1%	+++
B subtilis cx1+*B subtilis* ATCC6633 1%	++

Table 5. Effect of temperature on inducing activity of inducing factor from *B. subtilis* ATCC6633

Culture condition[1)]		Activity[2)]
control	producer alone (*B subtilis* cx1)	+
	B subtilis cx1+IF	++
IF/heat treated	4℃	++
	30℃	++
	37℃	++
	50℃	+
	70℃	+
	100℃	+
	121℃	+

Table 6. Effect of EDTA concentration on inducing activity of inducing factor from *B. subtilis* ATCC6633

Culture condition	Activity[1)]
producer alone(*B. subtilis* cx1)	+
B. subtilis cx1+IF treated with 0 mM EDTA	++
B subtilis cx1+IF treated with 0.5 mM EDTA	+++
B subtilis cx1+IF treated with 1 mM EDTA	++
B. subtilis cx1+IF treated with 2 mM EDTA	+

Discussion

This study provides evidence that the production of bacteriocin in lactic acid bacteria can be enhanced by the presence of bacteriocin sensitive strains, and identified the responsible agent that influence the bacteriocin production.

Although several repots have documented bacteriocin production by other microorganism, none previously have identified the responsible agent from sensitive cells to enhance the bacteriocin production. We believe that this study is first report to identify the responsible agent from other microorganism that influence the bacteriocin production. Now we are working an cloning of the inducing factor was carried out by using the determined N-terminus probe. The extract mechanism by which enhancement of bacteriocin production occurs should be clear by determination and characterization of the inducing factor whole molecule by further investigation.

References

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