

# Inhibition of Spoilage and Pathogenic Bacteria by Lacticin JW3, a Bacteriocin Produced by *Lactococcus lactis* JW3 Isolated from Commercial Swiss Cheese Products

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## Abstract

Strain JW3 was isolated from commercial Swiss cheese products and identified as a bacteriocin producer. *Lactococcus lactis* JW3 showed a broad spectrum of activity against most of the non-pathogenic and pathogenic microorganisms tested by the modified deferred method. Lacticin JW3 also showed a relatively broad spectrum of activity against non-pathogenic and pathogenic microorganisms as assessed using the spot-on-lawn method. It demonstrated a typical bactericidal mode of inhibition against *Leuconostoc mesenteroides* KCCM 11324.

## Introduction

Many lactic acid bacteria produce antimicrobial substances such as organic acids, hydrogen peroxide, diacetyl, carbon dioxide, and bacteriocins.<sup>2)</sup> Bacteriocins are defined as bactericidal proteins, which typically have a narrow spectrum of activity targeted toward a species related to the producer culture.<sup>4)</sup> Recently, bacteriocins have aroused great interest in the context of food preservation, and the possibility of genetically manipulating the genes which encode bacteriocins is considered as one of the major reasons for undertaking bacteriocin research. In this study we report on the inhibition of spoilage and pathogenic bacteria by lacticin JW3, a Swiss cheese bacteriocin produced by *Lactococcus lactis* JW3.

## Materials and Methods

### Bacterial strains and media

Producer strain *Lactococcus lactis* JW3 was isolated from commercial Swiss cheese products and *Leuconostoc mesenteroides* KCCM 11324 was used as the indicator strain. The strains used as for the determination of the antimicrobial spectrum of activity were obtained from different culture collections and indicator strains were grown in appropriate media as indicated in Tables 1 and 2.

### Detection of antimicrobial activity

*L. lactis* JW3 was examined for antimicrobial activity against indicator organisms on MRS agar plates using the modified deferred method.<sup>1)</sup>

### Lacticin JW3 assay

Lacticin JW3 assay was performed by the spot-on-lawn method.

### Production of lacticin JW3

Lacticin JW3 production was performed in a 5L jar fermenter (3.0-liter working volume; Korea Fermenter Co., Inchon, Korea) in a fermentation medium of MRS broth.

### Preparation of cell-free supernatant

Culture broth from the jar fermenter was centrifuged at  $8,000 \times g$  for 20 min at 4°C and the supernatant was filter-sterilized by passing 0.22  $\mu$ m cellulose acetate.

### Partial purification of lacticin JW3

Partially purified lacticin JW3 was obtained as previous study.<sup>3)</sup>

### Antimicrobial spectrum of activity

The modified deferred and spot-on-lawn methods were used to assess the antimicrobial activity of

the cell-free supernatant and the partially purified preparation of lacticin JW3 against several Gram-positive and -negative strains, which included food spoilage and pathogenic organisms.

#### Mode of inhibition

Cells from the log-phase of *L. mesenteroides* KCCM 11324 were suspended in sterile 100 mM phosphate buffer (pH 7.0). The test was conducted out at 32°C by adding 800 AU/mL of partially purified lacticin JW3. At specific times, the viable cells (CFU/mL) were determined on MRS agar plates by the standard plate counting method.

## Results and Discussion

### Partial purification of lacticin JW3

*L. lactis* JW3 was cultured in a jar fermenter at 32°C for 9 hr and the culture broth centrifuged. Partially purified preparations of lacticin JW3 were made by ammonium sulfate precipitation.

### Antimicrobial spectrum of activity

*L. lactis* JW3 showed a broad spectrum of activity against all of the non-pathogenic and pathogenic bacteria tested by the modified deferred method. Lacticin JW3 also showed a relatively broad spectrum of activity against *Bacillus cereus*, *B. pumilis*, *B. subtilis* ATCC 6633, *Clostridium perfringens* ATCC 3624, *Enterococcus faecalis* ATCC 19433, *Lactobacillus delbrueckii* ATCC 4797, *Leuconostoc mesenteroides* KCCM 11324, *Listeria innocua*, *L. monocytogenes* ATCC 15313, *L. monocytogenes*, *Micrococcus flavus* ATCC 10240, *Pediococcus acidilactici* KCTC 1626, *Propionibacterium acnes* P1, *P. acnes* P2, *P. acnes* P3, *P. acnes* P4, *P. acnes* P5, *Rhodococcus equi*, *Staphylococcus aureus* ATCC 6538, *Streptococcus bovis* ATCC 9809, *Escherichia coli* KCCM 32396, *Pseudomonas cepacia* SBB9611, and *Sphingomonas paucimobilis* BNJ 9964.

### Mode of inhibition

Lacticin JW3 showed a bactericidal mode of action (Fig. 1). However, the intrinsic nature of this inhibition has not been identified and requires further investigation.

## References

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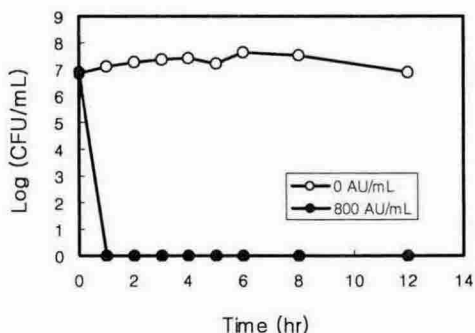


Fig. 1. Mode of action of lacticin JW3 against *L. mesenteroides* KCCM 11324 in phosphate buffer.

**Table 1. Antimicrobial spectrum of activity of *L. lactis* JW3 by the modified deferred method**

Organisms	Culture medium	Incubation temp.	Inhibition zone diameter (mm)	
			Nisin producer (ATCC 40140)	<i>L. lactis</i> JW3
<b>Gram positive bacteria</b>				
<i>Bacillus cereus</i>	NB	30°C	30	>30
<i>Bacillus pumilis</i>	NB	30°C	ND <sup>1)</sup>	ND
<i>Bacillus subtilis</i> ATCC 6633	TSB	37°C	>30	20
<i>Clostridium perfringens</i> ATCC 3624	TSB	37°C	10	11
<i>Enterococcus faecalis</i> ATCC 19433	TSB	37°C	15	15
<i>Lactobacillus delbrueckii</i> ATCC 4797	MRS	37°C	30	28
<i>Leuconostoc mesenteroides</i> KCCM 11324	MRS	25°C	24	21.5
<i>Listeria innocua</i>	TSB	37°C	23	16
<i>Listeria monocytogenes</i> ATCC 15313	TSB	37°C	21.5	20
<i>Listeria monocytogenes</i>	TSB	37°C	25	20
<i>Micrococcus flavus</i> ATCC 10240	NB	37°C	ND	ND
<i>Pediococcus acidilactici</i> KCTC 1626	MRS	37°C	25	23
<i>Propionibacterium acidipropionici</i> P200910	NLB	32°C	14	15
<i>Propionibacterium acnes</i> P1	NLB	32°C	14	15
<i>Propionibacterium acnes</i> P2	NLB	32°C	15.5	14.5
<i>Propionibacterium acnes</i> P3	NLB	32°C	18	16.5
<i>Propionibacterium acnes</i> P4	NLB	32°C	ND	ND
<i>Propionibacterium acnes</i> P5	NLB	32°C	ND	ND
<i>Rhodococcus equi</i>	TSB	37°C	17	17
<i>Staphylococcus aureus</i> ATCC 6538	TSB	37°C	23	26
<i>Staphylococcus aureus</i> ATCC 25923	TSB	37°C	27	25
<i>Streptococcus bovis</i> ATCC 9809	TSB	37°C	27	21
<b>Gram negative bacteria</b>				
<i>Chryseomonas luteola</i> SBA 9634	NB	30°C	ND	ND
<i>Escherichia coli</i> ATCC 8739	TSB	37°C	>30	>30
<i>Escherichia coli</i> ATCC 25922	TSB	37°C	24	16
<i>Escherichia coli</i> O157:H7	TSB	37°C	20	16
<i>Escherichia coli</i> KCCM 32396	LB	37°C	ND	ND
<i>Escherichia coli</i> JM109	LB	37°C	ND	ND
<i>Pseudomonas aeruginosa</i> ATCC 15422	TSB	37°C	20	20
<i>Pseudomonas syringae</i> ATCC 12885	TSB	37°C	16	15
<i>Pseudomonas cepacia</i> SBB 9611	NB	30°C	ND	ND
<i>Pseudomonas putida</i>	NB	30°C	ND	ND
<i>Salmonella enteritidis</i>	TSB	37°C	21	18
<i>Salmonella london</i> E	TSB	37°C	18	18
<i>Salmonella paratyphi</i>	TSB	37°C	>30	25
<i>Salmonella typhi</i>	TSB	37°C	27	20
<i>Salmonella typhimurium</i>	TSB	37°C	24	20
<i>Shigella flexneri</i>	TSB	37°C	21	23
<i>Shigella sonnei</i>	TSB	37°C	25	20
<i>Sphingomonas paucimobilis</i> BNJ 9964	NB	30°C	ND	ND
<i>Vibrio cholerae</i> O139	TSB	37°C	17	17
<i>Vibrio parahaemolyticus</i> ATCC 17802	TSB	37°C	21	16
<i>Vibrio parahaemolyticus</i>	TSB	37°C	17	16
<i>Vibrio vulnificus</i>	TSB	37°C	>30	>30
<i>Yersinia enterocollicus</i> ATCC 27729	TSB	37°C	18	18
<i>Xanthomonas maltophilia</i> SBC 9611	NB	30°C	ND	ND

<sup>1)</sup>Not detected

**Table 2. Antimicrobial spectrum of activity of partially purified lacticin JW3 by spot-on-lawn method**

Organisms	Culture medium <sup>2)</sup>	Incubation temp.	Bacteriocins	
			Nisin producer (ATCC 40140)	<i>L. lactis</i> JW3
<b>Gram positive bacteria</b>				
<i>Bacillus cereus</i>	NB	30°C	+	+
<i>Bacillus pumilis</i>	NB	30°C	+	+
<i>Bacillus subtilis</i> ATCC 6633	TSB	37°C	+	+
<i>Clostridium perfringens</i> ATCC 3624 <sup>1)</sup>	TSB	37°C	+	+
<i>Enterococcus faecalis</i> ATCC 19433	TSB	37°C	+	+
<i>Lactobacillus delbrueckii</i> ATCC 4797	MRS	37°C	+	+
<i>Leuconostoc mesenteroides</i> KCCM 11324	MRS	25°C	+	+
<i>Listeria innocua</i>	TSB	37°C	+/- <sup>3)</sup>	+
<i>Listeria monocytogenes</i> ATCC 15313	TSB	37°C	+/-	+
<i>Listeria monocytogenes</i>	TSB	37°C	+	+
<i>Micrococcus flavus</i> ATCC 10240	NB	37°C	+	+
<i>Pediococcus acidilactici</i> KCTC 1626	MRS	37°C	+	+
<i>Propionibacterium acidipropionici</i> P200910	NLB	32°C	+	+
<i>Propionibacterium acnes</i> P1	NLB	32°C	+	+
<i>Propionibacterium acnes</i> P2	NLB	32°C	+	+
<i>Propionibacterium acnes</i> P3	NLB	32°C	+	+
<i>Propionibacterium acnes</i> P4	NLB	32°C	+	+
<i>Propionibacterium acnes</i> P5	NLB	32°C	+	+
<i>Rhodococcus equi</i>	TSB	37°C	+	+
<i>Staphylococcus aureus</i> ATCC 6538	TSB	37°C	+	+
<i>Staphylococcus aureus</i> ATCC 25923	TSB	37°C	+/-	+/-
<i>Streptococcus bovis</i> ATCC 9809	TSB	37°C	+	+
<b>Gram negative bacteria</b>				
<i>Chryseomonas luteola</i> SBA 9634	NB	30°C	-	-
<i>Escherichia coli</i> ATCC 8739	TSB	37°C	-	-
<i>Escherichia coli</i> ATCC 25922	TSB	37°C	-	-
<i>Escherichia coli</i> O157:H7	TSB	37°C	-	-
<i>Escherichia coli</i> KCCM 32396	LB	37°C	+	+
<i>Escherichia coli</i> JM109	LB	37°C	-	-
<i>Pseudomonas aeruginosa</i> ATCC 15422	TSB	37°C	-	-
<i>Pseudomonas syringae</i> ATCC 12885	TSB	37°C	-	-
<i>Pseudomonas cepacia</i> SBB 9611	NB	30°C	+	+
<i>Pseudomonas putida</i>	NB	30°C	ND <sup>4)</sup>	-
<i>Salmonella enteritidis</i>	TSB	37°C	-	-
<i>Salmonella london</i> E	TSB	37°C	-	-
<i>Salmonella paratyphi</i>	TSB	37°C	-	-
<i>Salmonella typhi</i>	TSB	37°C	-	-
<i>Salmonella typhimurium</i>	TSB	37°C	-	-
<i>Shigella flexneri</i>	TSB	37°C	-	-
<i>Shigella sonnei</i>	TSB	37°C	-	-
<i>Sphingomonas paucimobilis</i> BNJ 9964	NB	30°C	+	+
<i>Vibrio cholerae</i> O139	TSB	37°C	-	-
<i>Vibrio parahaemolyticus</i> ATCC 17802	TSB	37°C	-	-
<i>Vibrio parahaemolyticus</i>	TSB	37°C	-	-
<i>Vibrio vulnificus</i>	TSB	37°C	-	-
<i>Yersinia enterocolitica</i> ATCC 27729	TSB	37°C	-	-
<i>Xanthomonas maltophilia</i> SBC 9611	NB	30°C	-	-

<sup>1)</sup>Incubated in anaerobic Gaspak jar

<sup>2)</sup>NB, Nutrient broth; LB, Luria broth; TSB, Tryptic soy broth; MRS, Lactobacilli MRS broth; PDB, Potato dextrose broth; NLB, Sodium lactate broth

<sup>3)</sup>Not clear

<sup>4)</sup>Not detected