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Production and Partial Characterization of 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, which has bactericidal activity against *Leuconostoc mesenteroides* KCCM 11324. Strain JW3 was identified tentatively as *Lactococcus lactis* by the API test. The activity of lacticin JW3, named tentatively as the bacteriocin produced by *Lactococcus lactis* JW3, was detected during the mid-log growth phase, and reached a maximum during the early stationary phase, and decreased after the late stationary phase. Its antimicrobial activity on sensitive indicator cells was completely disappeared by protease Ⅳ. The inhibitory activities of lacticin JW3 were detected during treatments of up to 121℃ for 15 min. Lacticin JW3 was very stable over a pH range of 2.0 to 9.0 The apparent molecular mass of lacticin JW3 was estimated to be in the region of 3-3.5 kDa, which was determined by the direct detection of bactericidal activity after SDS-PAGE.

Introduction

Lactic acid bacteria are common microflora in various fermented foods such as dairy products and processed vegetables, and play an essential role in food fermentation processes. Many lactic acid bacteria produce antimicrobial substances such as organic acids, hydrogen peroxide, diacetyl, carbon dioxide, and bacteriocins.[2)]

Bacteriocins are defined as bactericidal proteins, which typically have a narrow spectrum of activity targeted toward a species related to the producer culture.[6)] 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 production and partial characterization of lacticin JW3, a Swiss cheese bacteriocin produced by *Lactococcus lactis* JW3.

Materials and Methods

Bacterial strains

Producer strain *Lactococcus lactis* JW3 was isolated from commercial Swiss cheese products by general spreading method and incubating at 32℃.

Detection of antimicrobial activity

L. lactis JW3 was examined for antimicrobial activity against indicator organisms on MRS agar plates using the modified deferred method.[1)]

Lacticin JW3 assay

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

Identification of lacticin JW3 producer

Bacteriocin-producing strain JW3 was identified by Gram staining, morphology by SEM (scanning electron microscopy), a catalase test, and biochemical carbohydrates fermentation patterns using a API 50 CHL kit (BioMerieux, France).[4)]

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×g for 20 min at 4℃ and the supernatant was filter-sterilized by passing 0.22 m cellulose acetate.

Partial purification of lacticin JW3

Partially purified lacticin JW3 was obtained as previous study.[5)]

Effects of enzymes, heat, and pH

For enzyme stability, partially purified lacticin JW3 was treated at 30℃ for 1 hr with various enzymes at a final concentration of 1 mg/mL. To determine the effect of heat on bacteriocin activity, aliquots (500 L) of partially purified lacticin JW3 were incubated at various temperatures (40, 50, 60, 70, 80, 90, and 100℃) for 30 min or 121℃ for 15 min.[5)] pH stability was estimated in the partially purified lacticin JW3 after 4 hr of storage at 4℃ in the following buffers: 50 mM citrate buffer at pH 3-6; 50 mM phosphate buffer at pH 7.0; 50 mM Tris-HCl buffer at pH 8-9.[5)] The residual bacteriocin activity was determined by the spot-on-lawn method. Results are presented means of tests performed in duplicate.

Molecular Weight Determination by SDS-PAGE

To estimate the molecular weight of partially purified lacticin JW3, sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed on 18% discontinuous gel.[3)]

Results and Discussion

Screening of bacteriocinogenic lactic acid bacteria

To obtain bacteriocinogenic lactic acid bacteria, 17 lactic acid bacteria were successfully isolated from the various commercial Swiss cheese products using MRS agar media. These all isolates were tested for their antimicrobial activity against ten indicator strains, using the spot-on-lawn method. The antagonistic activity of an isolate (strain JW3) among them was sensitive to proteolytic enzymes, which indicated that its activity was due to bacteriocin.

Identification of lacticin JW3 producer isolated from commercial Swiss cheese products

Information on carbohydrate utilization patterns of strain JW3 coincided with those of *Lactococcus lactis* subsp. *lactis*.(data not shown) This isolate was Gram-positive, nonmotile and catalase-negative, and the cells were of the coccus type. On the basis of these results this strain was identified as *Lacococcus lactis*, and the isolate was tentatively named as *Lactococcus lactis* JW3. Lacticin JW3 is proposed as the tentative name of the bacteriocin produced by *Lactococcus lactis* JW3.

Production of Lacticin JW3

L. lactis JW3 produced extracellular inhibitory activity against *L. mesenteroides* KCCM 11324, in a MRS medium of pH 6.0 and incubated at 32℃. Production of lacticin JW3 seems to follow the typical kinetics of primary metabolite synthesis. Lacticin JW3 activity reached a maximum (2,400 AU/mL) after incubation for 9 hr, the early stationary phase, it was dropped after the late stationary phase.

Partial purification of lacticin JW3

L. lactis JW3 was cultured in a jar fermenter at 32℃ for 9 hr and the culture broth centrifuged. Partially purified preparations of lacticin JW3 were made by ammonium sulfate precipitation. The activity of the partially purified lacticin JW3 was 38,400 AU/mL.

Effects of various enzymes, heat, and pH

As is shown in Table 1, treatment with protease IV caused a complete loss of bacteriocin activity. No modification of activity was observed when lacticin JW3 was treated with the other enzymes tested (protease I, protease IX, protease XIII, protease XIV, α-chymotrypsin, β-chymotrypsin, trypsin, trypsin III, and pepsin). When the partially purified bacteriocin was treated with α-amylase, the bacteriocin activity was decreased, but not lost completely. This result confirm the proteinaceous nature of the antimicrobial substance and suggest that carbohydrate moiety is essential for the

bacteriocin activity.

Lacticin JW3 proved to be relatively heat stable (Table 1); partially purified lacticin JW3 was stable to a heat treatment of 70℃ for 30 min, and inhibitory activity was detected during treatments up to 121℃ for 15 min.

Finally, partially purified bacteriocin was pH stable in the range 2.0 to 9.0 as is shown in Table 1. This aspect of its stability is of particular importance to the food industry.

Molecular weight of lacticin JW3

As is shown in Fig. 1, the bactericidal activity of lacticin JW3 is associated with a band having an apparent molecular mass of about 3-3.5 kDa. Thus, the apparent molecular mass of lacticin JW3 was determined to be about 3-3.5 kDa by the direct detection of bactericidal activity after SDS-PAGE.

References

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Table 1. Effect of heat, various pH and enzyme on partially purified lacticin JW3

Treatment (Heat)	Residual activity (AU/mL)	Treatment (pH)	Residual activity (AU/mL)	Treatment (Enzyme)	Residual activity (AU/mL)
Control	25,600	Control	25,600	Control	51,200
40℃[1)]	25,600	2	25,600	Protease	51,200
50℃[1)]	25,600	3	25,600	Protease Ⅳ	0
60℃[1)]	25,600	4	25,600	Protease Ⅸ	51,200
70℃[1)]	25,600	5	25,600	Protease XIII	51,200
80℃[1)]	12,800	6	25,600	Protease XIV	51,200
90℃[1)]	12,800	7	25,600	α-Chymotrypsin	51,200
100℃[1)]	6,400	8	25,600	β-Chymotrypsin	51,200
120℃[2)]	400	9	25,600	Trypsin	51,200
				Trypsin Ⅲ	51,200
				Proteinase K	51,200
				Pepsin	51,200
				α-Amylase	6,400

[1)]Heat treatment for 30 min

[2)]Autoclave for 15 min

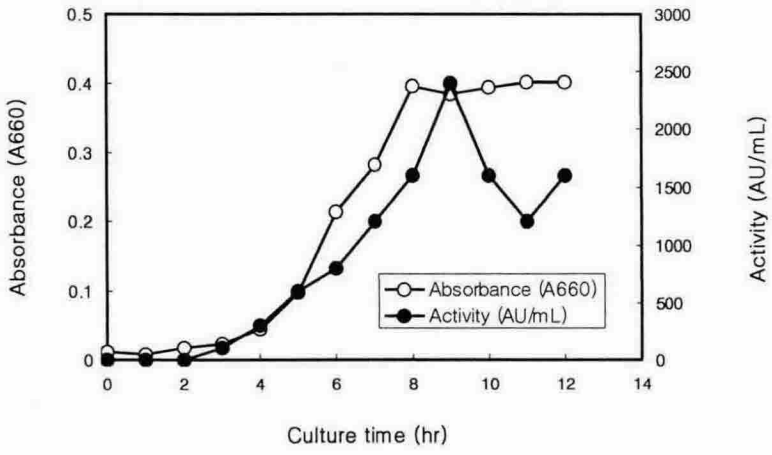

Fig. 1. Production of lacticin JW3 in jar fermenter.

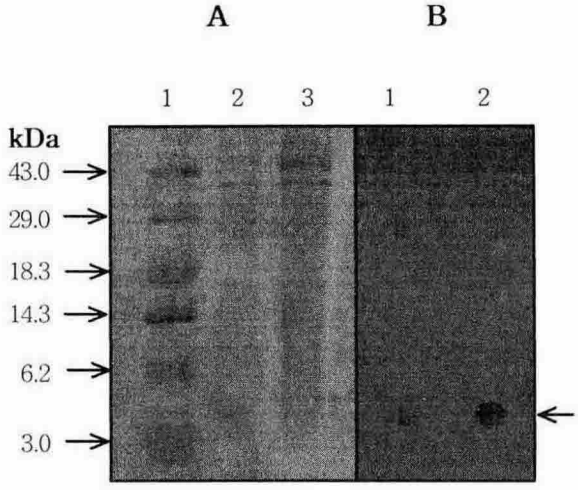

Fig. 3. SDS-PAGE of partially purified lacticin JW3 for determination of molecular weight.

(A) SDS-PAGE of partially purified lacticin JW3 after coomassie brilliant blue stained. Lane 1: size marker (43, 29, 18.3, 14.3, 6.2, 3 kDa); Lane2 : nisin; Lane 3: partially purified lacticin JW3.

(B) The gel overlayed with indicator strain, *L. mesenteroides* KCCM 11324. Lane1 : nisin; Lane 2: partially purified lacticin JW3.