Characterization of bacteriocin produced *Lactobacillus bulgaricus* acting on bovine mastitis pathogens

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Abstract

The antimicrobial substance produced by *Lactobacillus bulgaricus* was inactivated by protease, which confirmed it as a bacteriocin and referred to 'bulgaricin HJ'. The bulgaricin HJ showed the inhibitory activity against mastitis pathogens, gram-positive and gram-negative bacteria. The optimal conditions for the production of bulgaricin HJ were at the temperature of 30℃ and 10 h after cultivation of *L. bulgaricus*.

Staph. aureus and *Strep. agalactiae*, common bovine mastitis pathogens, were treated with bulgaricin HJ by the agar well diffusion method and showed antimirobial activities to the bovine mastitis pathogens. The activity of the bulgaricin HJ was maintained at pH 6-7 and 100℃ for 60 min against the mastitis pathogens. The bulgaricin HJ was determined as class Ⅳ bacteriocin by various enzyme treatments. Colony forming units analysis with indicator strains by the treatments of bulgaricin HJ indicates that the mode of bacteriocin action was bactericidal rather than bacteriostatic.

Introduction

Bovine mastitis can be defined as the inflammation of udder resulting from infection or trauma, which has been generally thought as a critical disease in cattle. Mastitis results in reduced quantity and quality of milk and products from milk. These losses are primarily due to less milk production, increased veterinarian costs, increased cow mortality and discarded milk [3]. Therefore, the bovine mastitis is one of major problems in dairy farming. Currently, the cows with bovine mastitis have been treated using antibiotics [1]. However, antibiotics have been detected in the raw milk of cows with bovine mastitis by the antibiotics residue-test. From this reason, bacteriocins have

been emerged as an alternative to the antibiotics to treat the cows infected with bovine mastitis pathogens. In this study, the bacteriocin producing LAB strain killing mastitis pathogens was screened, and the characteristics and the optimal production conditions of the bacteriocin were evaluated.

Materials and Method

Lactobacillus bulgaricus screened from kimchi was cultured at 30℃ for one day either in MRS broth (Difco Lab., Detroit, U.S.A.) or on the equivalent solid medium prepared by adding 1.5% agar. The indicator strains, *Staph. aureus* and *Strep. agalactiae*, were incubated at 37℃. *Staph. aureus* was grown in TSA (Trypticase Soy Agar) and *Strep. agalactiae* was incubated in TSA with 5% defibrinated sheep blood. The bacteriocin produced by *L. bulgaricus* was detected by the agar well diffusion method and its activity was measured by a serial dilution method. The bacteriocin activity was defined as the reciprocal of the highest dilution showing definite inhibition of the indicator lawn and was expressed as activity units (AU) per milliliter.

Results and Discussions

The antimicrobial spectrum of bulgaricin HJ was broad and effective not only against LAB including genus *Lactobacillus, Pediococcus* and *Leuconstoc* but also against gram-negative bacteria such as *Acetobacter* and *Pseudomonas*. Especially, bulgaricin HJ showed inhibitory activity for mastitis pathogens which are *Staph. aureus* and *Strep. agalactiae*.

The cell growths at 30 and 37℃ were almost same, while the cell density at 25℃ was higher than those at 30 and 37℃ (Fig. 1A). Figs. 1B and C show the activity of the bacteriocin, bulgaricin HJ, during the cultivation of *L. bulgaricus* at different temperatures. The bacteriocin prodution at 30℃ showed highest concentration compared to other temperature. Bulgaricin HJ was more effective to *Strep. agalactiae* rather than *Staph. aureus* as shown in Figs. 1B and C. The bacteriocin is effective to the strains of the same or closely related to the bacteriocin producing bacterium. Therefore, *Strep. agalactiae* was more sensitive than *Staph. aureus* with same concentration of bulgaricin HJ.

Proteolysis enzymes caused complete loss of the bulgaricin HJ activity. This indicates that bulgaricin HJ was the proteionous substances. The loss of an antimicrobial activity of bulgaricin HJ by α-amylase, β-amylase, glucoamylse indicated that carbohydrate composition on the bacteriocin is related to the antimicrobial activity of the bulgaricin HJ. Therefore, bulgaricin HJ was determined as a class IV bacteriocin according to reports [2]. Hundred % of bulgaricin HJ activity was maintained with pH 2~7 for 30 min heat treatment at 100℃. However, the activity decreased to 50% after 60 min of heat treatment. The activity decreased rapidly at pH 10-11 with the heat treatment. This result indicates that bulgaricin HJ was stable in acidic conditions (pH 2~7) with heat treatment (Fig. 2). The colony forming units (CFU) of *Strep. agalactiae* and *Staph. aureus* with bulgaricin HJ were measured to determine whether the bacteriocin has a bactericidal or bacteriostatic mode as shown in Fig. 3. Five hours of incubation with indicator strains and bulgaricin HJ resulted in the decrease of live cell number. However, the optical density increased for 3 h then kept constant in both case of indicator strains as shown in Fig. 3. These results indicate that bulgaricin HJ acts in bactericidal mode, not bacteriostatic mode [3].

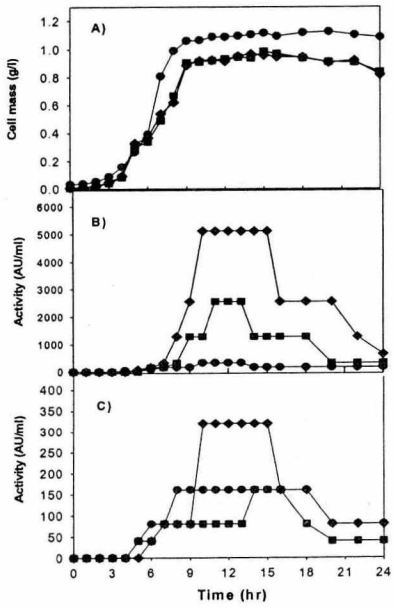

Fig. 1. Time course on the cell mass and the production of bulgaricin HJ against *Strep. agalactiae* and *Staph. aureus*. The cell density (A) and the change of bulgaricin HJ activity were monitored every hour against *Strep. agalactiae* (B) and *Staph. aureus* (C). Symbols: ●, 25℃ ◆, 30℃ ■, 37℃.

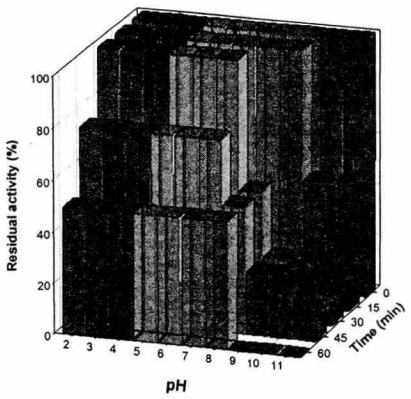

Fig. 2. Effects of pH and heat treatment at 100℃ on the bactericidal activity of bulgaricin HJ against *Strep. agalactiae* and *Staph. aureus*.

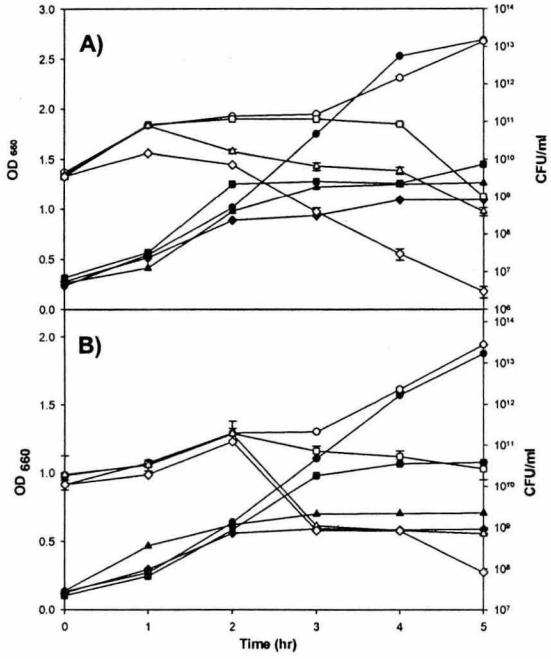

Fig. 3. The activities CFU of indicator strains by the treatment of on bulgaricin HJ on *Strep. agalactiae* and *Staph. aureus* as indicator strains for the determination of mode of action.

A) The effects of bulgaricin HJ on *Strep. agalactiae*; B) The effects of bulgaricin HJ on *Staph. aureus*.

Symbols: ●, Optical density of control; ■, Optical density of culture treated by 5120AU/ml(A) or 320AU/ml(B) of bulgaricin HJ; ▲, Optical density of culture treated by 10240AU/ml(A) or 640AU/ml(B) of bulgaricin HJ; ◆, Optical density of culture treated by 15360AU/ml(A) or 960AU/ml(B) of bulgaricin HJ; ○, CFU of control; □, CFU of culture treated by 5120AU/ml(A) or 320AU/ml(B) of bulgaricin HJ; △, CFU of culture treated by 10240AU/ml(A) or 640AU/ml(B) of bulgaricin HJ; ◇, CFU of culture treated by 15360AU/ml(A) or 960AU/ml(B) of bulgaricin HJ.

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