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NOTE

Antibacterial Activities of *Lactobacillus crispatus* ATCC 33820 and *Lactobacillus gasseri* ATCC 33323

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***Lactobacillus crispatus* ATCC 33820 and *L. gasseri* ATCC 33323 were grown in MRS broth (pH 6.5) at 37°C for 24 h and the antibacterial activities of cell free culture supernatants were determined by the agar well diffusion method. The culture supernatants were inhibitory to *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pediococcus acidilacticii*, and *Lactobacillus helveticus*. The supernatants did not show any lysozyme activity. Addition of catalase did not affect the antibacterial activities of the supernatants. The antibacterial substances were heat stable (100°C for 60 min) and sensitive to proteases.**

Key words: lactobacilli, *Lactobacillus crispatus*, *Lactobacillus gasseri*, bacteriocin

Bacteriocins are antimicrobial polypeptides that are usually inhibitory to strains closely related to the bacteriocin producing bacteria. These antimicrobial compounds are thought to provide a selective advantage to the producer strains over other strains (6). Lactic acid bacteria (LAB) have been studied extensively for the production of antimicrobial compounds such as bacteriocins for their potential use as biopreservatives in foods (2, 7, 11). Bacteriocins from LAB have advantages as food preservatives because LAB are common microflora in various fermented foods and they have been used for centuries in the preparation and preservation of foods (2). Moreover, the new generation of foods that demand minimal processing would benefit from the development of antimicrobial agents from LAB (8, 13). However, the ineffectiveness of bacteriocins from LAB against Gram-negative bacteria, and many Gram-positive bacteria associated with food spoilage and food-borne illness have limited the application of bacteriocins in food preservation. This suggests a need for broad host range antimicrobials for use in food preservation.

Several species of the genus *Lactobacillus* produce bacteriocins (4, 5, 7). However, little is known about the antimicrobial activities of *L. crispatus* and *L. gasseri*. *L. crispatus* and *L. gasseri* are thermophilic homofermentatative lactobacilli and have been isolated from the intestinal tracts and other organs of human beings and animals (3). The present study examines the antimicrobial spectrum of cell free culture supernatants from *L. crispatus* and *L. gasseri*.

Lactobacillus crispatus 33820 and *L. gasseri* 33323 were obtained from the American Type Culture Collection (Rockville, MD). The indicator strains came from the stock culture collections of the Departments of Microbiology at University of Wisconsin-La Crosse and Oregon State University. The LAB were propagated in MRS broth (Difco Laboratories, Detroit, MI) and non-LAB cultures were cultivated in brain heart infusion (BHI) broth (Difco) at 37°C. Agar media were prepared by adding 1.5% (wt/vol) Bacto agar (Difco) to the above broth media. Soft agar for overlay was prepared by adding 0.75% (wt/vol) Bacto agar.

The culture supernatants of *L. crispatus* and *L. gasseri* were prepared by growing the cultures in MRS broth for 24 h at 37°C and removing the cells by centrifugation. The supernatant was adjusted to pH 6.5 with 1N NaOH, sterilized by filtering through a 0.45 μm-pore-size cellulose acetate filter membrane and stored at -80°C until used (9).

The cell free culture supernatants from *L. crispatus* and *L. gasseri* were examined for their antimicrobial activity by the agar well diffusion method as described by Schillinger and Lucke (9). Prepoured MRS or BHI agar plates were overlayed with 10 ml of soft MRS or BHI agar inoculated with 0.3 ml of 1:10 dilution of an overnight culture of the indicator organism. After allowing the media to

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Table 1. Antibacterial spectrum of the culture supernatants from *L. crispatus* ATCC 33820 and *L. gasseri* ATCC 33323

Organisms	Source[a]	Inhibition	
		L. crispatus	*L. gasseri*
Gram positive bacteria			
Bacillus subtilis	UWL	+	+
Enterococcus faecalis	UWL	+	+
Lactobacillus helveticus	OSU	+	-
Micrococcus flavus	OSU	+	+
Pediococcus acidilacticii	OSU	+	+
Staphylococcus aureus	UWL	+	+
Gram negative bacteria			
Escherichia coli	UWL	+	+
Klebsiella pneumoniae	UWL	+	+
Pseudomonas aeruginosa	UWL	+	+

[a]UWL-University of Wisconsin-La Crosse, OSU-Oregon State University.

harden at room temperature for 15 min, wells of 5 mm diameter were made with a sterile cork borer and 100 µl of the cell-free supernatant was placed into each well. *L. helveticus* plates were incubated in an anaerobic jar and other indicator cultures were incubated under aerobic conditions for 24 h at 37°C and were subsequently examined for zones of inhibition.

The cell-free culture supernatants from *L. crispatus* and *L. gasseri* were inhibitory to both Gram positive and Gram negative bacteria (Table 1). The culture supernatant from *L. gasseri* was not inhibitory to *L. helveticus*. However, the culture supernatant from *L. crispatus* was inhibitory to all six Gram positive and three Gram negative isolates tested. The inhibition of bacteria was not due to acid or low pH, because the supernatants were neutralized to pH 6.5 before testing against the organisms. The results indicate that the antimicrobial substances produced by *L. crispatus* and *L. gasseri* have a wider host range than those produced by other lactobacilli.

The effect of proteases on the antimicrobial activity of the culture supernatants was determined by adding filter sterilized α-chymotrypsin, ficin, pepsin, proteinase-k, or trypsin to obtain a final protein concentration of 1 mg/ml. The mixture was incubated for 1 h at 37 °C and the residual antimicrobial activity was determined by the agar well diffusion assay. *L. helveticus* was used as an indicator to test the supernatant from *L. crispatus* and *Klebsiella pneumoneae* was used to test the supernatant from *L. gasseri*. All proteases were from Sigma Chemical Co., St. Louis, MO, USA (10). The culture supernatants, when treated with proteases, lost their ability to inhibit the growth of indicator strains (Fig. 1).

To examine if hydrogen peroxide was involved in the antimicrobial activity of the culture supernatants, filter sterilized catalase (Sigma) was added to the supernatants to obtain a final concentration of 5 mg/ml and agar well

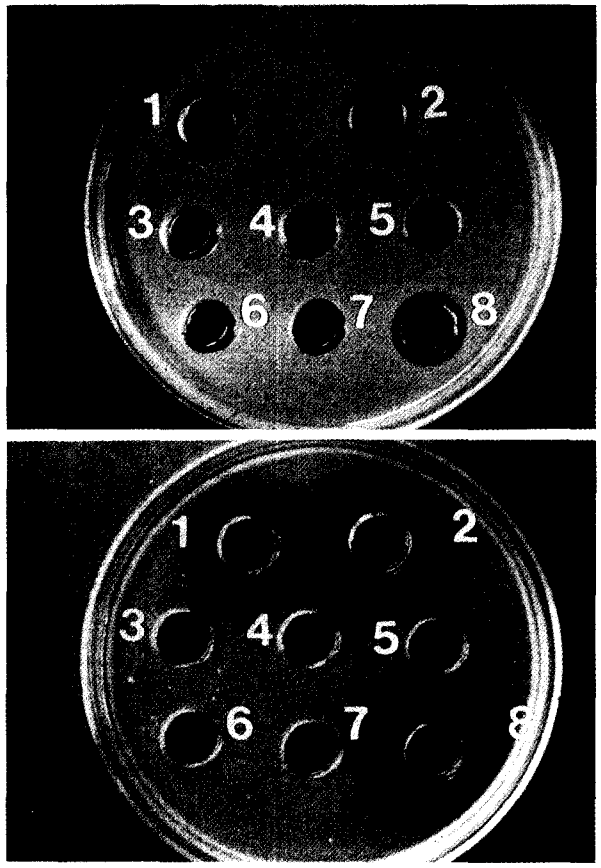

Fig. 1. Effect of catalase, proteases and heat treatment on the antimicrobial activity of the culture filtrates from *L. crispatus* ATCC 33820 (A) and *L. gasseri* ATCC 33323 (B). Indicator cultures: (A) *L. helveticus*, (B) *K. pneumoniae*. Symbols: 1. Culture supernatant (CS) only; 2. CS+catalase; 3. CS+α-chymotrypsin; 4. CS+ficin; 5. CS+pepsin; 6. CS+proteinase-K; 7. CS+trypsin; and 8. Culture supernatant heated to 100°C for 60 min.

diffusion assay was performed. Addition of catalase to the supernatants did not affect the antimicrobial activity indicating that the antimicrobial activity was not due to hydrogen peroxide.

The lysozyme activity in cell-free culture supernatants was determined as described by Cornett *et al.* (1). Lyophilized cells of *Micrococcus lysodeikticus* (Sigma) were used as the substrate for the lysozyme assay. The activity was determined by monitoring the optical density at 450 nm at 25°C for up to 3 h. However, the lysozyme activity was not detected in the supernatants.

The heat stability of the antimicrobial compounds present in the culture supernatants was determined by heating the supernatants for 10, 20, 30, and 60 min at 100°C and analyzing the residual activity by agar well diffusion assay (9, 12). The culture supernatants heated to 100°C for 1 h retained the antimicrobial activity (Fig. 1).

The heat stability of the antimicrobial compounds present in the culture supernatants and their susceptibility to proteases suggests that the antimicrobial compounds are per-

haps bacteriocins or bacteriocin like compounds. Antimicrobial compounds produced by the *Lactobacillus* are generally known to have a narrow spectrum of activity and are mainly active against closely related strains (2, 4, 5). However, given the broad spectrum of inhibition and thermal stability of the antimicrobial substances produced by *L. crispatus* and *L. gasseri,* there is a potential for their use as probiotics and as biopreservatives in foods.

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