Production of Bacteriocins by Strains of *Lactobacillus acidophilus* from Different Animal Origins

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ABSTRACT

Twenty seven strains of *Lactobacillus acidophilus* among 92 isolated from fecal contents of humans, pigs, calves, chickens, rodents and turkeys demonstrated inhibitory activity attributed to bacteriocin(s). The bacteriocin(s) were heat stable and nondialyzable proteinous compounds and exhibited narrow inhibitory spectra of activity. Neither hydrogen peroxide nor low pH were responsible for inhibitory action. All of the producer strains were resistant to their own bacteriocin or bacteriocin(s) produced by other strains. The bacteriocins from several strains from different host species were purified for further characterization. The bacteriocin(s) all exhibited similar characteristics.

(Key words: *Lactobacillus acidophilus,* Bacteriocin, Purification)

I. INTRODUCTION

Antagonistic effects produced by lactobacilli toward other organisms may play an imoportant role in maintaining a proper microbial balance in the intestinal tract and preserving certain foods. Some bacteria produce bacteriocins which are protein or protein related complexes that inhibit other bacteria that are usually closely related. A number of lactobacilli including strains of *Lactobacillus acidophilus* produce bacteriocins. Most bacteriocins are heat stable and sensitive to certain proteolytic enzymes.

The objectives of this study were: 1) to assay bacteriocin activity by various strains of *L. acidophilus* isolated from intestinal source of different origins including humans, pigs, calves, chickens, and roduents; 2) to purify the bacteriocin(s) and investigate their properties to determine whether or not there were differences in those produced by various strains of *L. acidophilus.*

Ⅱ. MATERIALS AND METHODS

Cells of *L. acidophilus* were removed by centrifugation from broth cultures which had been grown at 98.6°F for 18 h. The supernatant fluids (spent broth) were collected, adjusted to pH 6. 5 and filtered through sterile 0.45 μm acrodise filters into sterile serew cap test tubes. The resulting cell free spent broths were stored at refrigeration temperature (33.8°F).

Ten microliter portions of spent broths were aseptically placed onto the surface of an agar medium seeded with a culture sensitive to bacteriocins. The petri dishes were incubated upright for 24 h at 98.6°F. The presence of inhibitory material in the spent broth samples was indicated by clear zones on the agar.

The inhibitory samples of spent broth or components there of were heated at 250°F for 15 min, cooled to room temperature and assayed for inhibitory activity by the agar plate assay to determine if the inhibitor(s) were heat resistant. To ascertain whether or not the inhibitory activity was due to hydrogen perodxide or protein. The spent broths were treated with the following enzymes: A) catalase, B) trypsin, and C) pepsin.

To purify the bacteriocins, the proper amount of ammonium sulfate was added slowly to spent broth from cultures that produced bacteriocin to make the solution 50% saturated with ammonium sulfate. The preipitated fraction was recovered by centrifugation and dissolved in 0.05 M Tris-HCl buffer (pH 8.0), with or without 0.1% sodium dodecyl sulfate. Further purfication was accomplished by dialysis, gel chromatography and ion exchange chromatography.

Polyacrylamide gel electrophoresis (PAGE) was used to check the number of peptides or proteins in the inhibitory material purified by the above procedures.

Ⅲ. RESULTS AND DISCUSSION

A total of 92 strains of *L. acidophilus* isolated from fecal contents of humans, pigs, calves, chickens, rodents and turkeys were screened for the production of inhibitory activity (Table 1). Among these strains 17 out of 28 from pigs, 4 out of 22 from calves, 3 out of 19 from humans and 3 out 7 from turkeys exhibited inhibitory activity against indicator cultures (*L. acidophilus* La-1, NCFM-F and *L. delbrueckii* subsp. *lactis* 4797) sensitive to bacteriocins. None of 6 from chickens or 10 from rodents produced detectable inhibitory actions.

The sensitivity of each strain to inhibitory action also was tested against all of the strains that produced inhibitory activity against indicator cultures. Among strains that did not produce inhibitor(s) against the indicator cultures, the growth of 5 from pigs, 2 from calves, 10 from humans, and 1 each from chicken, rodent, and turkey were inhibited by some of the inhibitor(s) produced by the 27 inhibitor producing strains of *L. acidophilus*. This shows variations in the inhibitory spectra of the inhibitor(s) produced by these strains. None of those that produced in-

Table 1. Inhibitory activity of *L. acidophilus* toward *L. delbrueckii* subsp *lactis* 4797

Origin	Strains tested	
	Total	Producer strains
Pig	28	17
Calf	22	4
Human	19	3
Turkey	7	3
Rodent	10	0
Chicken	6	0

hibitory activity were sensitive to their own inhibitor or inhibitor(s) produced by other strains.

The inhibitory action of the spent broth was destroyed by treatment with either trypsin or pepsin. Treatment with catalase had no effect on the inhibition. Heating the inhibitory broths for 15 min at 250°F also had no effect on the inhibitory activity. These results demonstreated that the inhibitory substance(s) produced by 27 strains of *L. acidophilus* were heat stable proteinous compounds. Neither hydrogen peroxide nor acid were responsible for inhibitory activity against indicator cultures. Additonal tests revealed that the inhibitory action was restricted to closely related species. The inhibitory activity of spent broth was non-dialyzable. These results show the characteristics of the inhibitor(s) to be consistent to the definition of bacteriocins.

Based on consistency in production of inhibitory activity, and identity characteristics throughout the study, some inhibitory strains were selected for further study. Bacteriocins produced by 8 of the strains were stable through purification steps, and subjected to further purification. The 8 strains included of 1 from human origin, 2 strains from calves and 5 strains from pigs.

Gel chromatography of the ammonium sulfate precipitate fraction of spent broth from *L. acidophilus* GP4A (of pig origin) in the presence of 0.1% SDS resulted in the elution of a single peak of inhibitory activity corresponding to a single absorbance peak (Fig. 1). In addition, larger portion of material absorbing light at 280 nm was fractionated as a second peak in which no inhibitory activity was detected. The elution profiles of inhibitory activity of 4 other strains of *L. acidophilus* from pigs, 2 strains from calves, and 1 strain from human origin were similar to that of *L. acidophilus* GP4A.

The inhibitory fraction from *L. acidophilus* GP4A obtained by gel filtration was subjected to anion exchange column chromatography. Two absorbance peaks at 280 nm were deteced. First peak was eluted befor the sodium chloride gradient was applied. The inhibitory actvity was eluted with second absorbance peak at A 280 nm which corresponded to 0.4 M sodium chloride (Fig. 2). The elution profiles of the 7 other strains of *L. acidophilus* on ion exchange chromatography exhibited similar elution patterns.

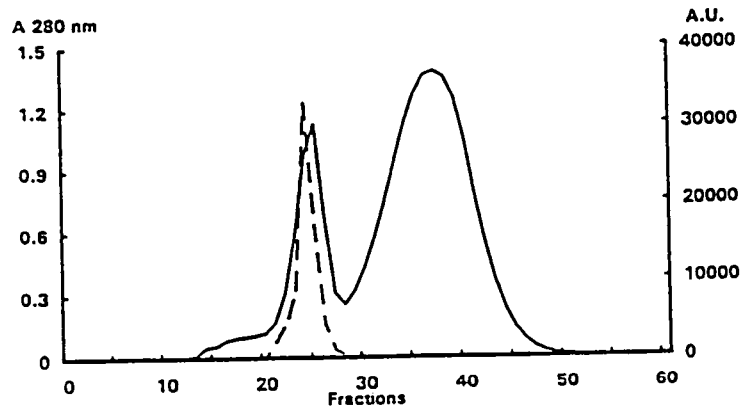

Fig. 1. Elution profile of ammonium sulfate precipitate of inhibitory activity produced by *L. acidophilus* GP4A on Sephadex G-200. Each 5 ml fraction was monitored at 280 nm (−) and was assayed for inhibitory activity(---).

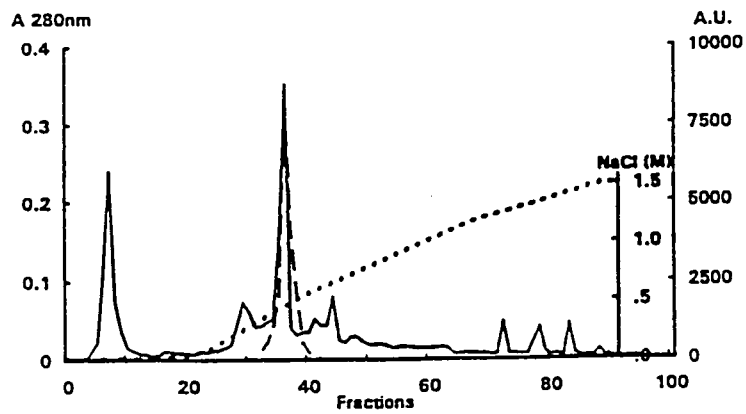

Fig. 2. Chromatography of inhibitory activity obtained from Sephadex G-200 produced by *L. acidophilus* GP4A on DE 52 anion exchange. The activity was eluted with 0 to 1.5 M NaCl(···). Each 4 ml fraction was monitored at 280 nm (−) and was assayed for inhibitory activity(---).

The active fractions from the second peak of ion exchange columns were separated by electrophoresis. A 72 KDa band appeared in the active fractions from all 8 strains of *L. acidophilus*. Attempts to show inhibition zone on the indicator lawn of agar by the bands from gels were not successful, possibly because of lack of sufficient inhibitory substances to produce inhibitory zone on the agar. Because of the presence of only one band in common, it appears that this band may have been responsible for the bacteriocin activity of all 8 strains.

Great diversity in characteristies and properties of bacteriocins produced by lactobacilli have been reported. In this study, however, the results indicated that the bacteriocins produced by

several strains of *L. acidophilus* isolated from different origins exhibited similar characteristics.

Further studies should include amino acid sequence analyses and genetic determination of bacteriocin production and host immunity to determine whether there are differences in bacteriocins produced by various strains of *L. acidophilus*.