INFLUENCES OF pH DURING GROWTH ON REMOVAL OF CHOLESTEROL FROM MRS BROTH BY *LACTOBACILLUS CASEI* AND *LACTOBACILLUS ACIDOPHILUS*

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Story in Brief

Two strains of *Lactobacillus acidophilus*, L1 and ATCC 43121, and two strains of *Lactobacillus casei*, E5 and N19, were evaluated for the ability to remove cholesterol from MRS broth during growth at a constant pH of 6.0 and during growth in broth without pH control. Samples grown without pH control dropped to pH levels of 4.2-4.5 depending on the strain and the species. There was very little difference in the amount of cholesterol removed from broth with or without pH control for either strain of *L. acidophilus*. Both strains of *L. casei* removed more cholesterol from the broth during growth without pH control compared to the cells grown with pH control. The total amount of cholesterol removed from the broth for both species was similar for cells grown without pH control.

(Key Words: *Lactobacillus acidophilus*, *Lactobacillus casei*, Cholesterol.)

Introduction

High serum cholesterol levels are associated with the development of coronary heart disease, the leading cause of death in the United States (Kannel et al., 1979; Pekkanen et al., 1990). Studies have indicated that lactobacilli remove cholesterol from laboratory media and have the potential to reduce serum cholesterol levels in humans (Buck and Gilliland, 1994; Danielson et al, 1989; Gilliland et al., 1985). A study by Noh and Gilliland (1995) revealed that *L. acidophilus* incorporates some of the cholesterol removed from the broth into the cellular membrane thus assimilating the cholesterol.

Some researchers have speculated that removal of cholesterol from laboratory media is due to the disruption of the cholesterol micelles and precipitation of the cholesterol with the deconjugated bile acids (Klaver and Van der Meer, 1993). Both *L. casei* and *L. acidophilus* deconjugate bile acids during growth by producing the enzyme bile salt hydrolase. Cholic acid, a deconjugated bile acid, is less soluble as the pH of the media decreases. The cholic acid is especially insoluble at pH levels less than 5 due to its pK of 5-6. As the pH drops due to acid production during bacterial growth, the cholic acid precipitates from the broth and may cause the cholesterol to precipitate also.

Buck and Gilliland (1994) isolated several strains of *L. casei* from human subjects that removed amounts of cholesterol from laboratory broth comparable to isolated strains of *L. acidophilus*. The objective of this study was to determine if the *L. acidophilus* and the *L. casei* removed cholesterol from laboratory media in a similar manner and if there was a difference in the amount of cholesterol removed from the broth when the pH remained constant.

Materials and Methods

The two strains of *L. acidophilus*, L1 and ATCC 43121, and two strains of *L. casei*, E5 and N19, were from our laboratory stock culture collection. They were propagated in MRS broth at 37°C. Cultures were subculture at least three times immediately before they were used experimentally.

To evaluate cholesterol removal from laboratory media, 8 ml of a freshly prepared MRS broth culture of the appropriate strain was added to 800 ml of sterile MRS broth supplemented with .2% sodium thioglycholate, .3% sodium taurocholate, and 10% cholesterol micelles prepared according to Razin et al. (1980). Prior to inoculation, a 10 ml sample of the broth was removed to serve as an uninoculated control in the cholesterol assay. The inoculated broth was divided into two equal portions. Cells in one portion of the broth were grown under static conditions and without pH control. The other portion of cells was grown with agitation, sparged with nitrogen gas at a constant pH of 6.0. The temperature of the samples was maintained at 37°C in a water bath. Samples were taken aseptically at 0, 16, 18, 20, and 22 hours.

The cells were removed from the samples by centrifugation and the pH and the amount of cholesterol removed from the broth were determined (Buck and Gilliland, 1984). The amount of cholesterol removed from the broth was determined by subtracting the amount of cholesterol in the sample from the amount of cholesterol in the uninoculated control.

Results and Discussion

The pH of the samples grown without pH control was 6.5 at the beginning of the experiment. The pH dropped to 4.2 after 18 and 16 hours of growth for the *L. casei* N19 and E5, respectively. The pH of the broth remained at 4.2 for the remainder of the experiment. The pH of the *L. acidophilus* samples grown without pH control dropped to 4.2 at 22 hours and 4.5 at 22 hours for strains 43121 and L1, respectively. The pH of the samples grown with pH control was initially 6.5 but was allowed to drop to 6.0 due to acid produced by the lactobacilli. Thereafter it was maintained at pH 6.0 for the remainder of the experiment for both strains of *L. casei* and *L. acidophilus*.

Cholesterol removal from the MRS broth was variable among the cultures. The amount of cholesterol removed from the MRS broth by *L. acidophilus* L1 during growth with and without pH control was not significantly different at any of the sampling times of 16, 18, 20, and 22 hours (Figure 1). For *L. acidophilus* 43121, there was significantly less cholesterol removed from the broth maintained at pH 6.0 compared to the broth with no pH control at sampling times of 16, 18, and 20 hours (Figure 2). However, by the final sampling time of 22 hours, the difference was not significant.

Noh and Gilliland (1995) reported that *L. acidophilus* incorporates cholesterol into the cellular membrane, thus removing it from the broth. These results suggest that some of the removal of cholesterol from MRS broth was due to assimilation of the cholesterol by the *L. acidophilus*.

The removal of cholesterol from the broth by *L. casei* was very different than the *L. acidophilus*. Generally, the cells grown with pH control removed very little cholesterol from the broth while cells growth without pH control removed cholesterol from the broth in amounts comparable to the *L. acidophilus*. Strain N19 grown without pH control removed 46.76 µg of cholesterol from

the broth by the 22 hour sampling period but the cells grown with pH control had removed only 13.63 µg of cholesterol/ml from the broth (Figure 3). Similarly, strain E5 grown without pH control removed 62.24 µg of cholesterol/ml after 22 hours of growth while the cells grown with pH control removed only 8.96 µg/ml (Figure 4). At all sampling times for both strains, there was significantly less cholesterol removed from the broth maintained at a constant pH compared to the cultures grown without pH control.

These results suggest that the removal of cholesterol from the MRS broth by the *L. casei* is related to the lower pH created in the culture grown without pH control. It likely is due to disruption of the cholesterol micelles as a result of deconjugation of the sodium taurocholate and precipitation of the free cholic acid at the lower pH. *L. casei* deconjugates taurocholic acid to cholic acid (Buck, 1992). The cholesterol thus may be removed from the broth during growth of *L. casei* by coprecipitation with the cholic acid.

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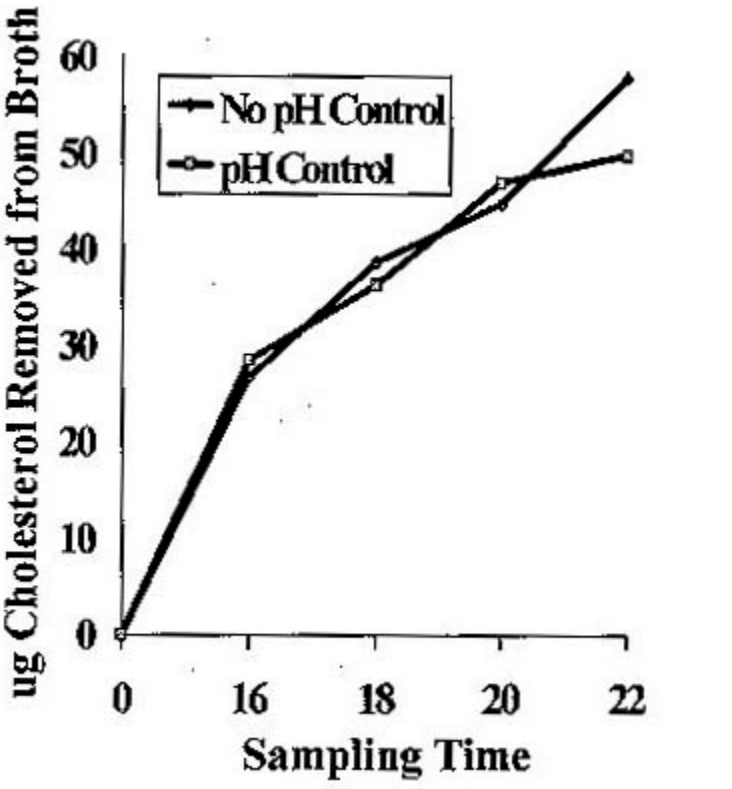

Figure 1. Removal of cholesterol from MRS broth by *Lactobacillus acidophilus* L1 grown with and without pH control.

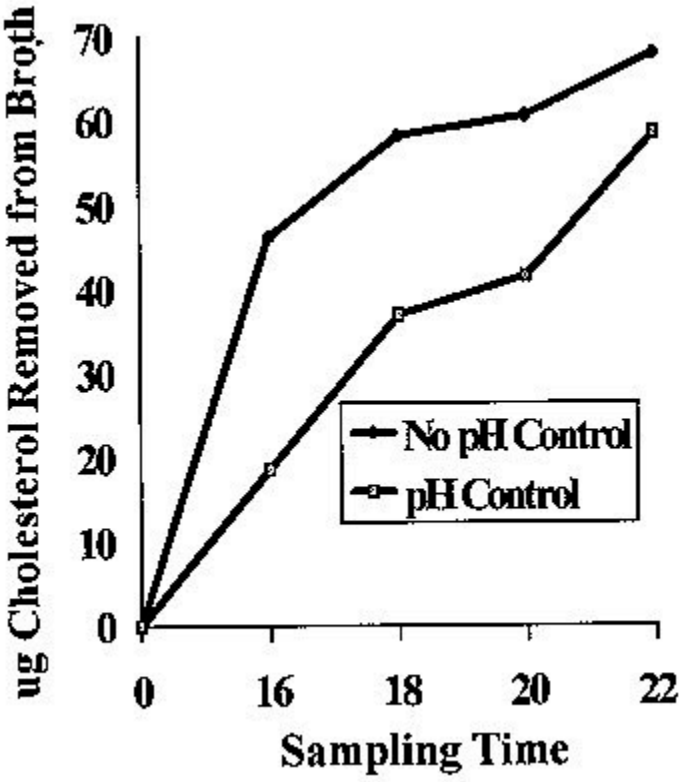

Figure 2. Removal of cholesterol from MRS broth by *Lactobacillus acidophilus* ATCC 43121 grown with and without pH control.

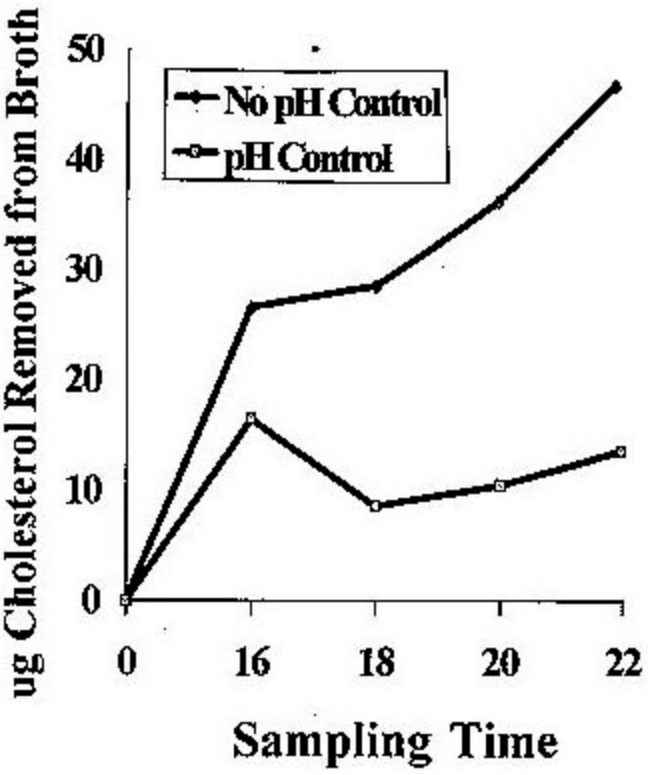

Figure 3. Removal of cholesterol from MRS broth by *Lactobacillus casei* N19 grown with and without pH control.

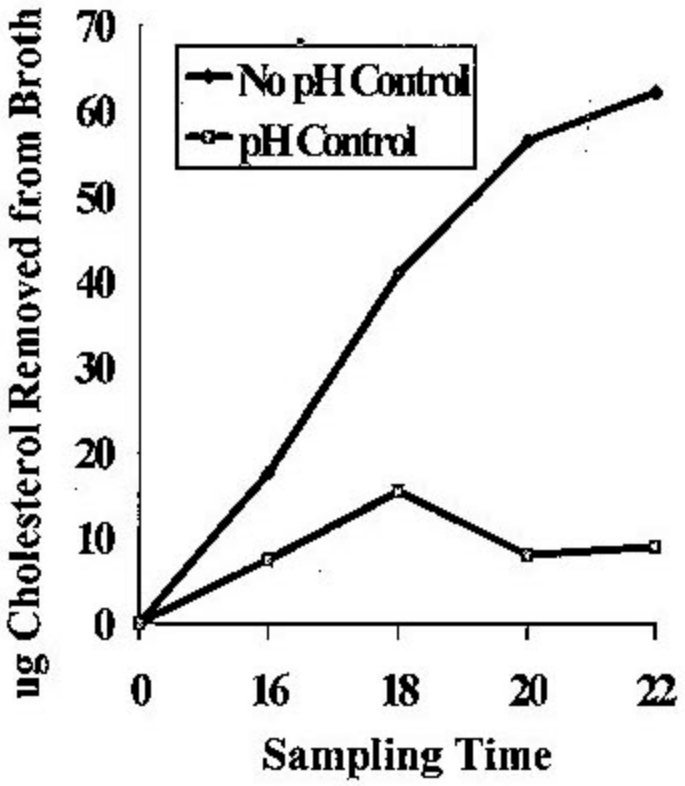

Figure 4. Removal of cholesterol from MRS broth by *Lactobacillus casei* E5 grown with and without pH control.

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