CHOLESTEROL UPTAKE BY CULTURE OF *LACTOBACILLUS ACIDOPHILUS* USED FOR NONFERMENTED ACIDOPHILUS MILK

S.E. Gilliland[1] and D.K. Walker[2]

Story in Brief

Twelve cultures of *Lactobacillus acidophilus* of human origin were compared for several characteristics considered desirable with regard to their use as a dietary adjunct for the possible production of a hypocholesterolemic effect in humans. There were significant variations among the cultures with regard to the ability to grow in the presence of bile and to assimilate cholesterol during growth. The most bile resistant cultures did not necessarily assimilate the greatest amount of cholesterol, suggesting that bile resistance is not directly related to the ability to assimilate cholesterol. However, there were significant differences in bile resistance among the cultures most active in assimilating cholesterol. Thus it is important to consider both characteristics when selecting cultures of the organism for use as dietary adjunct for these purposes. Another important characteristic of cultures of lactobacilli to be used as dietary adjunct is the ability to compete well with other cultures of lactobacilli or lactic acid bacteria. The cultures most active in assimilating cholesterol varied in this regard. Our results indicate that a strain of *L. acidophilus* can be selected which has all three of these desirable characteristics. However, the most desirable one among the twelve cultures of human origin tested in this study may not be sufficiently active with regard to cholesterol assimilation to produce a hypocholesterolemic effect.

(Key Words: *Lactobacillus acidophilus*, Cholesterol, Bile-tolerance, Bacteriocin.)

Introduction

Several studies have indicated a hypercholesterolemic activity produced by *Lactobacillus acidophilus*. We have found that variations exist among

---

[1]Regents Professor [2]Graduate Assistant

strains of *L. acidophilus* isolated from the intestinal tract of pigs with regard to the ability to assimilate cholesterol. Furthermore a strain which actively assimilated cholesterol was beneficial in helping exert beneficial influence on serum cholesterol levels in the pigs (Gilliland et al., 1985). The purpose of the present study was to compare strains of *L. acidophilus* of human origin, including some which are commercially available and used in preparing commercial acidophilus milk, for several characteristics desired for an organism to be used to exert a hypocholesterolemic effect.

Since it is necessary that the organism be able to grow in the intestinal tract it is important that it be bile resistance. While the degree of bile resistance required for maximum growth of the organism in the intestinal tract is not known, it is important to select the most bile resistant strain having the other desired characteristics for use as a dietary adjunct. It is also desirable to select a strain which has optimum ability for assimilating cholesterol during growth. Our earlier study indicated there is not a direct relationship between the ability to grow well in the presence of bile and the ability to assimilate cholesterol, thus both of these characteristics should be considered.

Some strains of *L. acidophilus* produce bacteriocins (antibiotic like materials which are only active against closely related strains or species of bacteria) which provides them with an advantage in being able to compete and grow in the presence of other lactic acid bacteria. This would be especially important in enabling a culture used as a dietary adjunct to be able to compete with other lactobacilli or lactic acid bacteria occurring naturally in the small intestines.

Materials and Methods

Thirteen strains of *L. acidophilus* were included in the study. Twelve of the cultures were originally of human origin. The thirteenth culture was *L. acidophilus* ATCC 43121 which was isolated from the intestines of a pig in our previous studies (Gilliland et al., 1985) and has been deposited with the American Type Culture Collection (ATCC). Because of host specificity exhibited among strains of *L. acidophilus* it is not one, however, being considered for use as a human dietary adjunct.

To determine if the cultures produced materials (bacteriocins) which inhibited other lactobacilli, each culture was grown in MRS broth at 37°C for 24 hours. The resulting cultures were centrifuged to remove the cells, the spent broth was adjusted to pH 6 and passed through a sterile .45 μm filter to remove any residual bacterial cells. The spent broth (10 μl volume) was spotted onto the surface of MRS agar seeded with the desired culture of

lactobacilli. Plates were incubated at 37°C for 24 h. Clear zones where the spent broth had been placed on the surface of the agar indicated inhibition of growth of the test culture.

To compare the ability of the cultures to assimilate cholesterol during growth they were inoculated into MRS broth supplemented with .2% sodium thioglycollate, .3% oxgall (dried bile) and 10% plueropnuemonia-like organism (PPLO) serum. The inoculated media were incubated the desired time (14 or 16 h) at 37°C. Following incubation the cultures were chilled and centrifuged to remove the bacterial cells. The spent broth along with uninoculated control broth was assayed for cholesterol using a colorimetric method (Rudel and Morris, 1973). Differences in the amount of cholesterol in the control broth and the spent broth for each culture were taken as the amounts of cholesterol assimilated by the cultures during the growth period.

Results and Discussion

All of the cultures being compared in this study were confirmed to be *L. acidophilus*.

There was significant variation in the rapidity of growth of the cultures in MRS broth containing .3% oxgall (Table 1). The most rapid growing

Table 1. Comparison of cultures of *L. acidophilus* for ability to grow in presence of bile and to assimilate cholesterol.

Culture	Growth in broth + 0.3% oxgall[1]	Cholesterol assimilated[2]
L. acidophilus ATCC 43121	2.93^{a}	95.7^{ab}
L. acidophilus 107	3.72^{b}	67.1^{c}
L. acidophilus NCFM	4.48^{c}	103.9^{b}
L. acidophilus 606	4.65^{cd}	64.6^{c}
L. acidophilus TKNC3	4.80^{cde}	92.4^{ab}
L. acidophilus 1	4.90^{cdef}	61.3^{c}
L. acidophilus ATCC 4962	5.27^{efg}	102.9^{a}
L. acidophilus TKNC2	5.32^{efgh}	27.5^{d}
L. acidophilus NCFM-L	5.35^{fghi}	90.7^{ab}
L. acidophilus TKNC1	5.73^{ghi}	97.5^{ab}
L. acidophilus 223	$>6.70^{j}$	102.1^{a}
L. acidophilus NCFM-F	$>7.20^{k}$	77.5^{bc}
L. acidophilus ATCC 4356	$>7.40^{k}$	96.3^{ab}

[1]Expressed as hours for growth to increase turbidity by .3 units; each value is an average from 3 trials.

[2]Amount assimilated (μ/ml) during 16 h of growth; each value is an average from 4 trials.

a,b,c,d,e,f,g,h,i,j,kValues in same column followed by different superscript letters differ significantly (P<.05).

culture was *L. acidophilus* ATCC 43121 which was originally isolated from pig intestines. The range in times required for the growth of the other to cause an increase in absorbance of .3 units was from 3.7 hrs for *L. acidophilus* 107 to greater than 7.4 hrs for *L. acidophilus* ATCC 4356. In Table 1 the cultures are arranged in order of decreasing ability to grow in the presence of bile with the most active culture being listed first.

The μg of cholesterol assimilated by the cultures during 16 h of growth also varied significantly among the cultures (Table 1). It is obvious from these data that the cultures exhibiting the greatest ability to grow in the presence of bile did not necessarily assimilate the greatest amount of cholesterol. Cultures ATCC 43121, NCFM, TKNC3, ATCC 4962, NCFM-L, TKNC1, 223, and ATCC 4356 assimilated significantly more cholesterol than did cultures 107, 606, 1, and TKNC2. *L. acidophilus* 107, which was the fastest growing of the cultures of human origin in the presence of bile assimilated significantly less cholesterol than many other cultures. In fact, it assimilated significantly less cholesterol than did the one which grew slowest in the presence of bile (*L. acidophilus* ATCC 4356). It is possible that the group of cultures assimilating the highest levels of cholesterol in these experiments had reached maximum levels of growth and cholesterol assimilation. Thus it may have been possible to detect differences among them if a shorter incubation time had been used.

In order to determine if there were differences among the eight cultures which assimilated the highest amount of cholesterol during the 16 h incubation period, additional experiments were conducted in which the cultures were incubated only 14 h (Table 2). Results from these experiments

Table 2. Comparison of selected cultures of *L. acidophilus* for ability to assimilate cholesterol during a shortened growth period and to produce inhibitory action toward other lactobacilli.

Culture	Cholesterol assimilated[1]	Inhibition of other lactobacilli
L. acidophilus ATCC 43121	55.4[a]	-[2]
L. acidophilus NCFM-L	35.1[b]	Yes
L. acidophilus ATCC 4962	31.0[b]	No
L. acidophilus NCFM	30.6[b]	Yes
L. acidophilus 223	18.6[c]	Yes
L. acidophilus ATCC 4356	12.9[c]	Yes
L. acidophilus TKNC1	10.9[c]	Yes
L. acidophilus TKNC3	10.9[c]	No

[1]Amount (μg/ml) assimilated during 14 hours of growth; each value is an average from 3 trials.
[2]Not listed.
[a,b,c]Values with different superscript letters differ significantly ($P<.05$).

revealed significant differences among the eight cultures. *L. acidophilus* ATCC 43121 assimilated significantly more cholesterol than did the other seven cultures. This is most likely due to the fact that it grew more rapidly than did the others. Of the seven cultures of human origin, *L. acidophilus* NCFM-L, ATCC 4962 and NCFM assimilated significantly more cholesterol than did the remaining 4 cultures.

The seven cultures of *L. acidophilus* of human origin which assimilated the greatest amount of cholesterol during the 16 h incubation period were also compared for the ability to produce substances that inhibited other lactobacilli (Table 2). Of the seven cultures NCFM-L, NCFM, 223, ATCC 4356, and TKNC1 produced inhibitory materials whereas ATCC 4962 and TKNC3 did not.

Because of host specificity among cultures of *L. acidophilus*, the most active culture included in this comparison (ATCC 43121) would not be considered for use as a dietary adjunct for humans. Of the cultures of human origin included in the studies, cultures NCFM-L, ATCC 4962, and NCFM were significantly more active in assimilating cholesterol than were the others. While these three were not the most bile resistant culture included, culture NCFM did exhibit a fair degree of bile resistance. The most bile resistant culture of human origin (107) was rather ineffective in assimilating cholesterol. Thus if the purpose of the dietary adjunct is to provide benefits in helping control serum cholesterol levels it would not be a culture of choice. Of the three which most actively assimilated cholesterol NCFM-L and ATCC 4962 grew significantly slower in the presence of bile than did NCFM. Additionally ATCC 4962 did not produce bacteriocin activity. Thus of these three cultures *L. acidophilus* NCFM would appear to be the culture of choice. However, the fact that *L. acidophilus* ATCC 43121 (of swine origin) was much more active with regard to both bile tolerance and cholesterol assimilation suggests the possibility that better cultures of human origin might occur. Thus, it seems reasonable that additional cultures of human origin should be screened in order to select one having even better characteristics for use as a dietary adjunct produce hypocholesterolemic activity in humans.

Literature Cited

Gilliland, S. E. et al. 1985. Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 49:377.

Rudel, L. L. and M. D. Morris. 1973. Determination of cholesterol using o-phthalaldehyde. J. Lipid Res. 14:364.