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Comparison of Probiotic Characteristics in *Lactobacillus acidophilus Strains*

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Introduction

Lactobacillus acidophilus strains have the ability to assimilate cholesterol during growth in the presence of bile under anaerobic conditions. A culture of *L. acidophilus* possessing all these characteristics should be advantageous over one that is not capable of establishing the intestinal tract and carrying out cholesterol assimilation [(1)]. Kim and Gilliland (1997) reported that the cells of *L. acidophilus* ATCC 43121 grown in MRS media with cholesterol, were not stained by Gram staining and were more resistant to disruption by lysozyme than those that are grown in normal MRS broth. However, some reports suggest that cholesterol assimilation may be due to the precipitation of cholesterol with deconjugated bile salts [(2)]. Deconjugation of bile acids may play an important role in host serum cholesterol levels since deconjugated bile acids do not function as well as conjugated bile acids in the solublization and absorption of lipids. Bile salt hydrolase (BSH) catalyzes the hydrolysis of conjugated bile acids to produce free bile acids and amino acids [(3,4)]. Thus, the hypocholesteremic effect of *L. acidophilus* strains should be associated with the production of BSH. The purpose of this study was to compare the ability of cholesterol assimilation, acid and bile tolerances, deconjugation of glycocholate and taurocholate, and CLA production of different strains of *L. acidophilus*.

Materials & Methods

L. acidophilus strains were obtained from Dairy Microbiology Laboratory in Oklahoma State University. *B. longum* BBL and S9 were obtained from Culture System Inc. (Mishawaka, IN). The strains of *L. acidophilus* were grown for 18 hrs at 37°C in MRS broth (Difco, Detroit, MI, USA) and bifidobacteria were incubated in Blood liver broth [(5)] at 37°C for 24 h.

The amount of cholesterol in the cell free spent broth was determined by Rudel and Morris [(6)]. Acid tolerance was expressed as the log difference of viable cells between treatment and initial levels. The cells were inoculated at *ca* 10^6 CFU/ml in MRS broth adjusted to pH 2.5 with 1*N* HCl for 1 h. The viable cell count was determined after 0 and 1 h of incubation at 37°C. Bile tolerance was determined by inoculating

(10^6 CFU/ml) MRS broth containing 0.3% oxgall (Difco) inoculated with a resuspended culture grown at 37°C for 18 h. The bacteria were plated onto MRS media and enumerated after 24 h of growth at 37°C. The bile tolerance for each concentration of bile acids was expressed as follows: (Final log number of viable cells) (Initial log number of viable cells). The amount of bile salts (sodium glycocholate and sodium taurocholate; Sigma Chemical Co.) deconjugated by *L. acidophilus* strains were determined using HPLC (Waters, Milford, MA, USA) on Nova Pak C_{18} column (Waters) as described by Corzo and Gilliland [7]. Deconjugation activity was based on the percent reduction of sodium glycocholate and sodium taurocholate from the original medium.

Cells grown to an O.D=1 were used as an inoculum in the experiments. Skim milk (11% w/v) with filter sterilized (0.22um, Milipore, Millipore Corp., Bedford, MA, USA) linoleic acid solution (0.1g/L final) was used to test CLA production. Samples were extracted and methylated as previously described by Kim and Liu (1999).

Results

Comparison of probiotic characteristics

Table 1 summarizes the comparison of cholesterol assimilation, bile tolerances (0.3 and 0.5%), acid tolerance, deconjugation activities and CLA production by the 3 groups. The high cholesterol assimilation group showed a higher tolerance to 0.3% and 0.5% oxgall than either the medium and low assimilation groups ($P<0.05$). Whereas the low cholesterol assimilation group showed a higher acid tolerance than high and medium assimilation groups ($P<0.05$). After incubation in bile acid supplemented media for 24 h, the viable cell counts in the high assimilation group showed a slight increase with 1.48 log and 0.93 log in

Table 1. Comparison of cholesterol assimilation, bile and acid tolerances, and reduction of glycocholate and taurocholate by *Lactobacillus acidophilus* strains.

	High Group [n=36]	Medium Group [n=12]	Low Group [n=24]
Cholesterol assimilation	57.98^a (6.152)*	47.704^b (4.08)	31.736^c (7.996)
0.3% Bile tolerance	1.479^a (0.447)	1.156^{ab} (0.532)	1.03^b (0.923)
0.5% Bile tolerance	0.93^a (0.846)	1.179^a (0.702)	0.176^b (1.136)
Acid tolerance	-0.177^a (0.172)	-0.207^a (0.111)	0.03^b (0.397)
Reduction % of glycocholate	30.54^a (5.482)	31.38^a (5.397)	40.86^a (12.66)
Reduction % of taurocholate	44.86^a (5.839)	50.98^a (8.649)	57.53^a (9.3)
CLA production (mg/mulk fat g)	4.55^a (0.354)	4.1^a (0.141)	4.26^a (0.357)

*Means with standard error in parentheses.

^{a,b,c} Means with the same superscript are not significantly different ($P<0.05$).

Comparisons are made within same row for means.

Table 2. Correlation coefficients of probiotic characteristics of *Lactobacillus acidophilus*

CLA	Cholesterol assimilation	0.3% Bile tolerance	0.5% Bile tolerance	Acid tolerance	Reduction of glycocholate	Reduction of taurocholate	Reduction of Production
Cholesterol assimilation	1.0 (0.0)*	0.212 (0.075)	0.333 (0.004)	-0.346 (0.003)	-0.138 (0.422)	-0.327 (0.051)	0.009 (0.977)
0.3% bile tolerance		1.0 (0.0)	0.601 (0.001)	-0.45 (0.001)	0.119 (0.49)	-0.1 (0.577)	-0.102 (0.752)
0.5% Bile tolerance			1.0 (0.0)	-0.494 (0.001)	0.215 (0.208)	0.122 (0.461)	0.024 (0.94)
Acid Tolerance				1.0 (0.0)	-0.134 (0.436)	-0.02 (0.909)	-0.101 (0.754)
Reduction of glycocholate (0.704)					1.0	0.22 (0.0)	-0.123 (0.197)
Reduction of taurocholate (0.497)						1.0	0.217 (0.0)

* The value in parentheses are *P*-value.

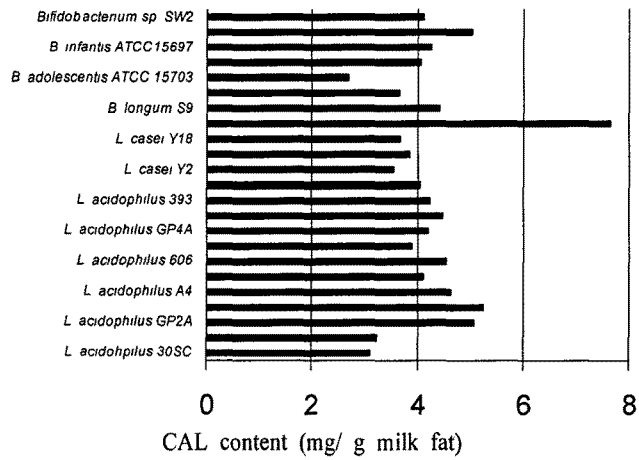

Fig. 1. Concentration of conjugated linoleic acid by lactic acid bacteria in 11% skim milk medium.

0.3% and 0.5% bile acids, respectively. All 12 strains of *L. acidophilus* studied were shown to be able to deconjugate glycocholate and taurocholate. There were no significant differences in the reduction of glycocholate and taurocholate among the three cholesterol assimilation groups of *L. acidophilus* strains. However, the high assimilation group showed the lowest reduction of glycocholate and taurocholate.

CLA production

The ability for CLA production by lactic acid bacteria was previously suggested. In this study, 12 strains

of *L. acidophilus* that possessed cholesterol assimilation activity as well as and other LAB strains including *Bifidobacterium* species, were selected to assess CLA production. The results from the comparison of *L. acidophilus* cultures for CLA production are shown in Fig. 1. *L. acidophilus* strains were capable of producing CLA in the range of 3.24 to 5.04 mg/g fat.

Summary

Twelve strains of *Lactobacillus acidophilus* isolated from feces of human or animal sources were tested for probiotic properties such as cholesterol assimilation, bile and acid tolerances, and CLA production. Although the cultures showed some variation with respect to each test, the 12 strains could be classified into 3 groups based on their ability to assimilate cholesterol. The cholesterol assimilation showed positive correlation with bile tolerance and negative correlation with acid tolerance. The cholesterol assimilation of L. *acidophilus* strains may not be related to the deconjugation activity, but may in fact be attributed to its bile tolerance. CLA production by lactic acid bacteria (LAB) exhibited a wide variation that ranged from 2.69 to 7.64 mg/g fat. CLA production of Bifidobacterium longum ATCC 15707 was the highest among the LAB tested, but there was no evidence for differences in CLA production between genus and species.

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