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Detection of *Lactobacillus acidophilus* in Feces of Humans, Pigs, and Chickens[1]

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Lactobacilli in fecal material from humans, pigs, and chickens were enumerated on lactobacillus selective agar (LBS). In all samples, higher numbers of lactobacilli were detected when plates were incubated in a system flushed with CO_2 rather than in air. Much higher numbers of bacteria from human feces were detected when the LBS agar plates were incubated anaerobically in a hydrogen-carbon dioxide atmosphere (GasPak) than when incubated in CO_2. The bacteria from human feces isolated on LBS agar incubated anaerobically were predominately bifidobacteria. Cultures from all three sources isolated on LBS agar incubated under CO_2 were lactobacilli, including *Lactobacillus acidophilus*. Differences were observed in biochemical characteristics of some of the *L. acidophilus* isolated from all three sources. Guanine plus cytosine base ratios of deoxyribonucleic acid isolated from *L. acidophilus* cultures from humans were lower, in most cases, than those from pigs and chickens.

Many reports have been published regarding the importance of lactobacilli in maintaining balanced intestinal flora necessary for the health of humans (for a review, see reference 18). Therapy involving the consumption of viable *Lactobacillus acidophilus* has been beneficial in treating certain gastrointestinal disorders (18). There are indications that the biotypes of lactobacilli present in the intestinal tract vary among different host animals (11). A possible "host specificity" for intestinal microorganisms has been suggested (12). For these and other reasons there is a need for more selectivity in the quantitation of lactobacilli in the intestinal tract; also more detailed information is needed on lactobacilli found in the intestines of different hosts.

In the present study, lactobacillus selection (LBS) agar incubated under different conditions was used in efforts to differentiate various groups of lactobacilli. Characteristics of cultures of *L. acidophilus* isolated from the feces of humans, pigs, and chickens also were observed.

MATERIALS AND METHODS

Source and maintenance of cultures. *L. acidophilus* NCFM (of human origin) was from the culture collection in the Food Science Department of North Carolina State University. *L. acidophilus* 4962 was from the American Type Culture Collection, Rockville, Md. *L. acidophilus* strains CNRZ 216 and CNRZ 218 were from the Centre National de Recherches Zootechnique (Jouy-en-Josas, France). Other lactobacilli were isolated from fecal material of either humans, pigs, or chickens and are so designated. All *L. acidophilus* strains were routinely propagated in sterile lactobacillus MRS broth (Difco Laboratories, Detroit, Mich.) by using a 1% inoculum and incubation at 37 C for 18 h. At least three successive transfers were made prior to characterization experiments. The cultures were stored at 5 C between transfers. The *Bifidobacterium* cultures were propagated in a similar manner except that the tubes were incubated and stored between transfers at 5 C in a GasPak system (BBL, Cockeysville, Md.).

Enumeration procedures. The fecal samples were diluted with sterile 1% peptone, and duplicate plates were prepared for each dilution. The plates were poured with LBS agar (BBL) prepared from individual ingredients, and an overlay of the same medium was added. Some plates were placed in a plastic bag, flushed for 1 min with carbon dioxide, and sealed (LBS-CO_2 counts). For incubation in an atmosphere of hydrogen and carbon dioxide, plates were placed in a GasPak system (LBS-GP counts). This atmosphere contained approximately 7% CO_2 (BBL). In addition, some plates were incubated aerobically. All plates were incubated for 48 h at 37 C.

Isolation procedures. Colonies were picked from countable plates of LBS-CO_2 and LBS-GP and inoculated into tubes containing 10 ml of sterile MRS broth. The tubes containing isolates from the LBS-CO_2 plates were incubated aerobically, and those from the LBS-GP plates were incubated anaerobically in a GasPak system. Cultures that grew within 24 to 48 h were diluted and plated by the pour plate method with MRS agar (MRS broth plus 1.5% agar). Isolated colonies from these plates were picked and maintained in MRS broth for characterization test.

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Classification of isolates. Only those isolates that were gram-positive rods and catalase negative were considered for further identification. Tests for catalase were made by adding 5 ml of 3% hydrogen peroxide to the cell pellet obtained by centrifuging (12,000 × *g* for 10 min) 10 ml of an MRS broth culture. Cultures were considered catalase negative if no visible gassing was observed.

Cultures were examined for growth at 15 and 45 C in MRS broth and for gas production by the methods of Rogosa et al. (17); for the latter, 5 ml of 1.5% sterile agar was used as the overlay.

MRS broth with glucose and beef extract omitted (19) was the basal medium used for determination of ammonia production from arginine, esculin hydrolysis, and carbohydrate fermentations. Esculin hydrolysis and ammonia production were determined as described by Davis (4). Ability of cultures to ferment various carbohydrates was evaluated as described by Rogosa and Sharpe (16), except that amygdalin, melibiose, and raffinose were not employed. Cultures of anaerobic organisms were incubated in a GasPak system.

To check for branched cellular forms of the anaerobic isolates, the cultures were grown in the low-calcium medium of Kojima et al. (7) in which yeast extract (0.5%) was substituted for the beef liver infusion. Cellular morphology was determined by examining, with a microscope, methylene blue stains of the cultures.

Determination of DNA base composition. Deoxyribonucleic acid (DNA) was isolated from representative lactobacilli and bifidobacteria by the method of Marmur (9). The percentage of guanine plus cytosine (G+C) in the DNA samples was determined from the thermal melting point of the DNA by using procedures described by Marmur and Doty (10). The change in absorbance of the heated DNA was measured automatically by using a Beckman DU spectrophotometer equipped with a Gilford model 2000 automatic recording photometer (Gilford Laboratories, Inc., Oberlin, Ohio). The cuvette chamber was heated by a Haake model FE constant-temperature circulation bath (Gilford Laboratories, Inc., Oberlin, Ohio) filled with ethylene glycol.

RESULTS

Enumeration of lactobacilli. *L. acidophilus* NCFM did not form colonies on LBS agar incubated under aerobic conditions (Table 1). However, the culture grew equally well on LBS-CO_2 and LBS-GP. Colonies developed from chicken and pig feces plated on LBS agar and incubated aerobically. Few differences were observed between the colony counts on LBS-CO_2 and those on LBS-GP. The LBS-CO_2 and LBS-GP counts were approximately 2.5 and 5.5 times greater than the LBS-aerobic counts for chickens, and pigs, respectively.

Marked differences in counts were obtained when human feces was plated on LBS-CO_2 and LBS-GP (Table 2). In all subjects studied, much higher numbers were observed when the fecal material was plated on LBS-GP. Aerobic conditions were not evaluated because of the poor growth of *L. acidophilus* incubated aerobically on LBS agar. Furthermore, the lactobacilli from pig and chicken feces grew less well on LBS agar incubated aerobically than on LBS-CO_2 and LBS-GP.

Isolation of lactobacilli. Cultures isolated from the highest dilutions of human fecal material on LBS-GP were anaerobic and had characteristics of *Bifidobacterium* species (Table 3). All of the isolates, with the exception of BA1,

TABLE 1. *Enumeration of lactobacilli by using LBS agar*[a]

Type of count	*L. acidophilus* NCFM[b] (CFU/g)	Chicken feces (CFU/g)	Pig feces (CFU/swab)
LBS-aerobic	NG	2.0×10^8	1.3×10^8
LBS-CO_2	5.5×10^{10}	4.9×10^8	7.5×10^8
LBS-GP	5.5×10^{10}	4.7×10^8	9.7×10^8

[a] CFU, Colony-forming units; NG, no growth.
[b] Concentrated culture.

TABLE 2. *Enumeration of lactobacilli in human feces by using LBS agar*

Sample	CFU[a]/g	
	LBS-CO_2	LBS-GP
A	2.5×10^7	8.6×10^8
M	2.5×10^7	3.8×10^8
S	5.8×10^5	3.6×10^8
W	1.5×10^6	2.6×10^8

[a] Colony-forming units.

TABLE 3. *Characterization of isolates from human feces plated on LBS agar and incubated in a GasPak system*

Isolate	Branching	Biochemical reactions of *Bifidobacterium* sp.	mol% G+C
BA1	[a]	+	
BA2	[b]		
BA3	[b]		
BS1	+	+	57.8
BS2	+	+	
BS3	+	?	60.9
BW1	+	+	
BW2	+	+	60.9
BW3	+	+	59.0
BW4	+	+	58.5
BW5	+	+	

[a] Curved rods, no definite branching.
[b] Unable to subculture after initial isolation.

formed branched rods in the medium of Kojima et al. (7); this is typical for this species. Furthermore, all of the isolates from human feces plated on LBS-GP (with the exception of BS3) had biochemical characteristics of *Bifidobacterium* species (15). The G+C contents of five strains (including BS3) tested were within the range reported for *Bifidobacterium* (1).

Cultures isolated from human, pig, and chicken fecal material plated on LBS-CO_2 were all classified as lactobacilli. Of the 20 isolated from human feces, 6 possessed characteristics closely resembling *L. acidophilus*. Three additional isolates did not closely fit the fermentation pattern described by Rogosa and Sharpe (16) for *L. acidophilus*; however, their characteristics were closer to those of *L. acidophilus* than to any other organism of the thermobacterium group. Of 12 lactobacilli isolated from pigs, none was identical to *L. acidophilus*. However, eight of the isolates had characteristics more nearly similar to *L. acidophilus* than to any other lactobacilli. Of 12 isolates obtained from chickens, 3 had characteristics closely resembling *L. acidophilus* and 2 additional isolates more closely resembled *L. acidophilus* than any other lactobacilli in the thermobacterium group.

Complete fermentation patterns for five representative isolates characterized as *L. acidophilus* from humans, pigs, and chickens are presented in Table 4. Four laboratory strains of *L. acidophilus* were included for comparison. The fermentation patterns for these strains conformed closely with the characteristics of *L. acidophilus* (15) with the exception of CNRZ 216. Isolates HA3 and HM2 (human origin) possessed the same characteristics as *L. acidophilus*. Isolate HA1 was similar except for an apparent variation in the ability to ferment maltose and galactose. Isolate HA2 differed from *L. acidophilus* in that it failed to ferment trehalose. Isolate HM6 was variable with respect to the fermentation of cellobiose and salicin, and failed to ferment trehalose or hydrolyze esculin.

All of the isolates of pig origin differed from the fermentation pattern of *L. acidophilus* because of negative or variable actions on cellobiose, salicin, and trehalose. All except PA3 were also variable with respect to the hydrolysis of esculin.

Of the five representative isolates from chickens, isolates C1, C2, and C3 possessed characteristics identical to those of *L. acidophilus*. Isolate C7, however, was variable on mannitol, and isolate C11 fermented mannitol but did not attack esculin, cellobiose, or salicin.

G+C content of DNA. The G+C content for isolates identified as *L. acidophilus* ranged from 36.7 to 43.7 mol%, except for isolate C11, which was 29.8 mol% (Table 4), and therefore could not be considered as *L. acidophilus*. The G+C contents for two of the three isolates of human origin (HA3 and HM2) were closer to the percentages observed for the laboratory strains than were those for the isolates obtained from chicken and pig fecal material. Isolate PA 19 (of pig origin) also appeared to have a G+C content close to that observed for the laboratory strains. In general, G+C contents for the isolates (except PA19 and C11) obtained from the chicken and pig fecal material were higher than those observed for the laboratory strains. Isolate HA1 (of human origin) also possessed a higher G+C content than those observed for the laboratory strains.

DISCUSSION

In evaluating the effect(s) of feeding *L. acidophilus* on the microbial flora of the intestinal tract, the selection of the proper medium for detection of the organism being fed is of great importance. In addition, it appears that selection of an *L. acidophilus* strain compatible with the host is also important. LBS agar has highly selective qualities for lactobacilli. The conditions under which LBS agar is incubated have been shown in this study to yield different counts for the same sample of fecal material, indicating a possible means of more selectively enumerating the lactobacilli. Although colonies from chicken and pig feces were observed on LBS agar when incubated aerobically, *L. acidophilus* NCFM failed to show growth under these conditions. Rogosa and Sharpe (16) indicated that high concentrations of acetate caused by evaporation during aerobic incubation may be inhibitory to some lactobacilli. *L. acidophilus* NCFM and other strains may be sensitive to these higher levels of acetate caused by evaporation. Thus, this medium should not be used aerobically for the enumeration of *L. acidophilus*. On the other hand, incubation under strictly anaerobic conditions (GasPak system) enables anaerobic *Bifidobacterium* species to grow and completely prevents the detection of *L. acidophilus* from some sources (i.e., human feces). Anaerobic incubation of LBS plates would provide a method of enumerating bifidobacteria. Not all organisms detected on LBS-CO_2 were classified as typical *L. acidophilus*; therefore, this method of incubation cannot be used to enumerate *L. acidophilus* selectively. Such results also indicate that lactobacilli other than *L. acidophilus* are present in the intestinal flora of humans, pigs, and chickens.

Comparison of the fermentation patterns of

TABLE 4. *Characteristics of L. acidophilus isolates from fecal material*

Characteristic	Laboratory strains				Human					Pig					Chicken				
	NCFM	4962	CNRZ216	CNRZ218	HA1	HA2	HA3	HM2	HM6	PA3	PA12	PA19	P18	P47	C1	C2	C3	C7	C11
Growth at 15 C	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Growth at 45 C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gas	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
NH_3 from arginine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Esculin	+	+	+	+	+	+	+	+	−	+	±	±	±	±	+	+	+	+	−
Cellobiose	+	+	+	+	+	+	+	+	±[a]	+	+	±	−	−	+	+	+	+	−
Galactose	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	+
Rhamnose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Salicin	+	+	−	+	+	+	+	+	±	±	±	+	−	−	+	+	+	+	−
Sorbitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	−	+	+	−	+	+	−	−	−	−	−	±	+	+	+	+	+
mol% G+C[b]	38.4	37.9			43.5		38.6	36.7		43.7	41.6	36.8		43.3	40.0	41.3	40.3	41.3	29.8

[a] ±, Weak positive reaction was obtained in one of the duplicate tests for each isolate. Such reactions are referred to as "variable" in the text.
[b] Guanine plus cytosine content in DNA.

isolates identified as *L. acidophilus* indicate that different biotypes exist. Mitsuoka (11) reported that different biotypes of *L. acidophilus* existed which were host specific. Morishita et al. (12) described a similar phenomenon in that a strain of *L. acidophilus* of human origin would not become established in the intestines of chickens. The differences observed in the fermentation patterns and G+C content of the DNA among the isolates of the present study also lends support to the theory that relationships exist between the host and the strain or biotype of *L. acidophilus* that can become established in the intestine. Thus, it appears that care must be taken in selecting strains of *L. acidophilus* for use in dietary preparations intended as a source of lactobacilli for establishment in the intestinal tract.

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