

Isolation and Identification of Lactic Acid Bacteria from Human Fecal Samples and Determination of Their Some Probiotic Properties

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ABSTRACT

The possibility of using lactic acid bacteria to maintain health, and to prevent or treat disease is an important topic for microbiology. The research of novel probiotic strains is important in order to satisfy the increasing request of the market and to obtain functional products in which the probiotic cultures are more active and with better probiotic characteristics than those already present on the market. In this study, the probiotic potential of *Lactobacillus* and *Enterococcus* strains isolated from human feces was investigated.

In this research, 107 strains of lactic acid bacteria were isolated from fecal samples of 19 adult volunteers who have stomach problems and fecal samples of 12 healthy adult volunteers. The strains were identified by 16S rRNA analyses and examined for resistance to gastric acidity (pH 3.5) and bile salts (0.3% bile salt). The strains antibiotic susceptibility of chloramphenicol (C, 30 µg), erythromycin (E, 15 µg), kanamycin (K, 30 µg), nalidixic acid (NA, 30 µg), penicillin G (P, 10 units), tetracycline (TE, 30 µg) and vancomycin (VA, 30 µg) was also determined.

The counts of lactic acid bacteria in fecal samples were ranged from 1.7x10⁷ to 3.1x10¹⁰ cfu/g. The species present in those samples were found to be *Lactobacillus plantarum*, *L. fermentum*, *Enterococcus faecium* and *E. durans*. Results are indicated that *L. plantarum* (AA1-2, AA17-73, AC18-88, AK4-11 and AK7-28), *L. fermentum* (AB5-18, BB16-75 and AK4-180), *E. faecium* (AB20-98 and BK11-50) and *E. durans* (AK4-14 and BK9-40) showed good probiotic characteristics. These strains could be potential candidates as health-promoting bacteria.

Keywords: Feces, lactic acid bacteria, probiotic

INTRODUCTION

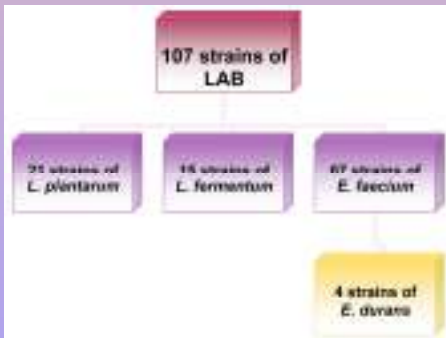
A probiotic is a culture of living microorganisms which beneficially affects the health of the host when ingested in sufficient quantities (Erkkilä and Petäjä 2000). The acid and bile tolerances are two fundamental properties that indicate the ability of a probiotic microorganism to survive the passage through the gastrointestinal tract, resisting the acidic conditions in the stomach and the bile acids at the beginning of the small intestine (Prasad et al. 1999). Probiotic strains should also have desirable antibiotic resistance and sensitivity patterns. The purpose of the present study was to identification of lactobacilli from fecal samples of humans, determination of the survival of lactic acid bacteria isolates in vitro when exposed to low pH, bile and antibiotics.

MATERIALS AND METHODS

In this research, one hundred and seven strains of lactic acid bacteria were isolated from fecal samples of 31 adult volunteers. These strains were evaluated for some potential probiotic properties. They were examined in vitro for resistance to pH 3.5 and 0.3% bile salts. Their antibiotic resistance was also determined against to 7 different antibiotics (Oxoid) by disc diffusion method (Başyigit, 2004). The strains were identified by 16S rRNA sequencing analyses (Başyigit Kılıç, 2009).

RESULTS AND DISCUSSION

IDENTIFICATION OF LAB STRAINS



In this study, the 16S rRNA of the one hundred and seven strains of LAB were sequenced and they were identified by alignment as 21 strains of *L. plantarum* (accession numbers in GenBank are GQ332648 to GQ332657 and GQ332692 to GQ332702), 15 strains of *L. fermentum* (FJ966274 to FJ966288), 67 strains of *E. faecium* (GQ332660 to GQ332691 and GQ332705 to GQ332739) and 4 strains of *E. durans* (GQ332658, GQ332659, GQ332703, GQ332704).

ACID and BILE SALT TOLERANCE

In this research, according to the viable counts of bacteria on MRS agar, it was shown that 76% of *L. plantarum* and 73% of *L. fermentum* strains were able to survive and/or grow in MRS broth (pH 3.5). It was determined that 90% of *L. plantarum*, 93% of *L. fermentum*, 71% of *E. faecium* and 100% of *E. durans* strains tested in this study could tolerate 0.3% bile salt and the increase and decrease in the viable counts of the strains was approximately 1 log unit (Table 1).

Table 1. Effect of the exposure in MRS at pH 3.5 and MRS with bile salt (0.3%) on the survival of selected LAB (Log CFU g⁻¹)

Strains	Resistance (log ₁₀ CFU/g)			
	Low pH(3.5)		Bile salt	
	Initial	Final	Initial	Final
<i>L. plantarum</i> AA1-2	7.34	7.84	6.68	6.81
<i>L. plantarum</i> AA17-73	6.75	6.86	7.17	7.87
<i>L. plantarum</i> AC18-88	5.69	5.91	6.91	7.82
<i>L. plantarum</i> AK4-11	7.19	7.34	7.12	7.31
<i>L. plantarum</i> AK7-28	7.11	7.45	6.61	7.0
<i>L. fermentum</i> AB5-18	6.33	6.27	6.87	7.84
<i>L. fermentum</i> BB16-75	6.81	7.20	7.17	7.32
<i>L. fermentum</i> AK4-180	5.32	5.17	5.17	6.25
<i>E. faecium</i> AB20-98	3.68	4.5	6.33	6.85
<i>E. faecium</i> BK11-50	5.3	6.1	7.14	7.21
<i>E. durans</i> AK4-14	6.06	6.56	6.07	7.0
<i>E. durans</i> BK9-40	4.28	6.27	5.62	5.69

ANTIBIOTIC SUSCEPTIBILITY

All *L. plantarum* and *L. fermentum* strains exhibited generally sensitive to chloramphenicol, erythromycin, penicillin G and tetracycline. The results demonstrated that *L. fermentum* strains are more sensitive to penicillin G than that of *L. plantarum* strains. The strains were resistant to kanamycin, nalidixic acid and vancomycin, while only *L. plantarum* (AA1-2, AA17-73 and AC18-88) and *L. fermentum* AB5-18 was sensitive to vancomycin. Lactobacilli strains generally showed high resistance to vancomycin, 89% of all lactobacilli were resistant to vancomycin. In this study, among enterococci strains while 100% of them were sensitive to chloramphenicol and tetracycline, 100% of the strains were resistant to kanamycin, nalidixic acid and 20% of the strains were resistant to erythromycin. Antibiotic resistance test results indicated that only *E. faecium* (AB4-6, AC3-9, AC3-11, AB6-24 and AB16-66) and *E. faecium* (AB7-30 and BC11-45) were resistant to penicillin G and vancomycin, respectively. 93% of the strains were sensitive to penicillin G and 97% of the enterococci strains were sensitive to vancomycin (Table II).



Table 2. Antibiotic susceptibility of selected *Lactobacillus* and *Enterococcus* strains

Strains	C	E	K	NA	P	TE	VA
<i>L. plantarum</i> AA1-2	S	R	R	R	R	S	S
<i>L. plantarum</i> AA17-73	S	S	R	R	S	S	S
<i>L. plantarum</i> AC18-88	S	S	R	R	S	S	S
<i>L. plantarum</i> AK4-11	S	S	R	R	R	S	R
<i>L. plantarum</i> AK7-28	S	I	R	R	I	S	R
<i>L. fermentum</i> AB5-18	S	I	R	R	S	S	S
<i>L. fermentum</i> BB16-75	S	S	R	R	S	S	R
<i>L. fermentum</i> AK4-180	S	S	R	R	S	S	R
<i>E. faecium</i> AB20-98	S	I	R	R	S	S	S
<i>E. faecium</i> BK11-50	S	R	R	R	S	S	S
<i>E. durans</i> AK4-14	S	I	R	R	S	S	S
<i>E. durans</i> BK9-40	S	I	R	R	S	S	S

R: Resistant

I: Intermediate

S: Susceptible

C: Chloramphenicol (30 µg), E: Erythromycin (15 µg), K: Kanamycin (30 µg), NA: Nalidixic acid (30 µg), P: Penicillin G (10 units), TE: Tetracycline (30 µg) and VA: Vancomycin (30 µg).

CONCLUSIONS

In recent years, studies on probiotics and gastrointestinal flora have been emphasized to isolate possible probiotics from microbial flora of different countries. Natural intestinal flora shows differences depending on the cultural differences, geographical region, physiological conditions of the host (aging, stress, health status, ethnical environment) and composition of the diet. In this research the diversity of total fecal microbiota as well as the species composition of LAB has been shown no differ between patients and controls. The identified strains are *L. plantarum*, *L. fermentum*, *E. faecium* and *E. durans*. Results indicated that *L. plantarum* (AA1-2, AA17-73, AC18-88, AK4-11 and AK7-28), *L. fermentum* (AB5-18, BB16-75 and AK4-180), *E. faecium* (AB20-98 and BK11-50) and *E. durans* (AK4-14 and BK9-40) showed good probiotic characteristics. They survived under low pH conditions for 3 h and they tolerated well the bile salt under in vitro conditions. The low resistance of these strains to the most common antibiotics could be an additional criterion for selecting this strain as a potential probiotic. We therefore conclude that these LAB possess some probiotic properties which make them potentially good candidates for probiotics. These strains will be investigated in more depth for such probiotic activities in further investigations.

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