

Growth inhibition of foodborne microorganism by lactic acid bacteria isolated from newborn baby feces and *Dongchimi*

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Introduction

Lactic acid bacteria (LAB) are generally recognized benefit organisms. They belong to the normal intestinal flora in humans and are also widely used in dairy and other fermented foods.

Several studies have reported that LAB can inhibit the growth of food-borne pathogens and spoilage organisms. In most cases, the inhibitory action during the organisms growth is attributable to the production of acid, hydroperoxide, or bacteriocins by LAB.

In this experiment, LAB were isolated from newborn baby meconium and feces and *Dongchimi* (fermented vegetable in brine by lactic acid bacteria). Selection criteria employed in the ability of strains to withstand environmental condition such as low pH, high bile concentration, and oxygen. The antimicrobial activity and characteristics of 16 lactic acid bacteria strains was investigated against six food-borne microorganisms (*Listeria monocytogenes* ATCC 19111, *Bacillus cereus* ATCC 11778, *Salmonella* Typhimurium ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* O157:H7 ATCC 43894, *Salmonella* Enteritidis KCCM 12021).

Material Methods

Source of strains from fecal sample and *Dongchimi*

The meconium and feces from 61 clinically healthy Korean newborn baby (male 31, female 30) were obtained from Hana obstetrics and gynecology hospital in Jeonju, Korea. And these samples were incubated on LBS agar.

Culture supernatants preparation

LAB strains were incubated in MRS broth for 24 hour. Supernatants of LAB strains were obtained by centrifugation at $10,000 \times g$ for 10 min, and then filtered through $0.22 \mu\text{m}$ membrane filters.

Measurement of antimicrobial activity

Antimicrobial activity of supernatants of LAB strains with food-borne pathogens culture was measured with the absorbance by Bioscreen C (Labsystem, Oy, Helsinki, Finland) for 72 h.

Interaction in YS-medium

LAB strains and *S. Typhimurium* or *E. coli* O157:H7 were co-cultured in YS-medium (0.1% yeast extract and 10% skim milk) at optimal temperature for 72 hour. *E. coli* O157:H7 was enumerated on Violet red bile agar(OXOID), and *S. Typhimurium* was enumerated on brilliant green agar(OXOID).

Identification of inhibitory substance

In this experiments, the correlation between acid and/or hydrogen peroxide production and the inhibition of food-borne microorganisms was determined.

Results and Discussions

Supernatants of sixteen LAB strains (20% added in broth medium) inhibited the growth of six food-borne microorganisms. The pH and titratable acidity values by LAB strains supernatants(20%) were about 4.0-5.0 and 0.32-0.43%, respectively. LAB strains supernatants(20%) showed more strong inhibition than that of lactic acid at 0.50% level.

When LAB strains and *S. Typhimurium* or *E. coli* O157:H7 were co-cultured in YS-medium, food-borne microorganisms were completely inactivated within 48 hour of incubation by D2, F35-2 strains (10% added in YS-medium) and 72 hour by D2, F35-2 strains (5% added). The pH values of co-cultured medium by LAB strains and *S. Typhimurium* or *E. coli* O157:H7 were about 3.8 at 72 hour of incubation.

The antimicrobial substance of LAB strains supernatants were uneffect at pH 6-7, and stable at 50-90°C for 30 min and 121°C for 15 min. On treating LAB strains supernatants with catalase, only F20-3 strains was slightly lost its antimicrobial activity. This inhibitory effect could be assigned to undissociated form of organic acid produced during the incubation. The isolated strains were D2 and F35-2 identified as *L. plantarum* and F20-3 identified as *L. fermentum*.

Table 1. Inhibitory effects of isolated species against food-borne microorganisms.

strains	<i>L.monocyto-</i>	<i>B.cereus</i>	<i>S.Typhimurium</i>	<i>S.aureus</i>	<i>E.coli</i> O157H:7	<i>S.Enteritidis</i>
	<i>genes</i>	20%	20%	20%	20%	20%
D1	99.311)	98.08	98.99	95.81	99.00	99.81
D2	100	98.49	99.35	96.59	99.03	99.73
F20-1	87.87	96.98	98.40	58.77	40.11	63.76
F20-2	91.12	97.50	98.47	46.13	37.34	51.35
F20-3	86.78	97.45	98.53	54.04	28.56	50.29
F20-4	76.99	97.14	98.31	56.14	45.47	60.57
F26-2	54.42	60.74	77.13	58.82	29.08	57.99
F35-1	100	97.92	98.76	93.76	99.77	80.62
F35-2	100	97.61	98.66	96.88	98.86	75.13
F35-3	91.39	96.62	97.78	68.85	35.23	71.79
F35-4	100	97.19	98.57	95.85	97.92	88.98
F35-6	86.01	70.98	81.85	69.96	31.56	15.75
F42-1	84.12	94.54	92.73	71.07	36.4	73.47
F42-2	87.52	96.46	96.94	69.29	39.17	73.67
F42-3	99.65	98.02	98.21	71.69	97.61	70.71
F42-4	91.28	97.71	98.40	62.56	30.47	66.34

1)Growth inhibition rate(%)=[1-(T1/T2)] × 100

T1 : Total area of growth curve of treatment by Biosceen C for 72hr incubation

T2 : Total area of growth curve of control by Biosceen C for 72hr incubation

Table 2. Inhibitory effects of lactic acid bacteria on *Salmonella* Typhimurium ATCC 14028 in YS medium and change of pH level in medium.

strains	inoculum concentration	Viable cell counts (log CFU/ml)				pH			
		0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr
control		6.13	8.81	8.84	8.85	6.44	5.95	6.00	5.99
D2	1%	6.13	8.31	8.09	8.79	5.88	4.63	4.20	4.10
	3%	6.13	7.43	7.14	5.89	5.54	4.17	4.02	3.88
	5%	6.13	5.82	2.00	N	5.29	4.10	3.81	3.73
	10%	6.13	0.78	N	N	4.78	3.83	3.70	3.67
F20-3	1%	6.13	8.42	8.79	8.82	6.18	4.75	4.39	4.21
	3%	6.13	7.87	7.20	6.29	6.07	4.56	4.12	4.10
	5%	6.13	7.00	6.65	5.28	5.99	4.47	4.06	3.98
	10%	6.13	6.47	5.36	2.29	5.70	4.31	3.96	3.81
F35-2	1%	6.13	8.36	8.13	8.06	5.99	4.10	4.33	4.21
	3%	6.13	7.45	7.23	5.99	5.43	3.98	3.76	3.73
	5%	6.13	6.91	6.38	5.00	5.15	3.92	3.74	3.70
	10%	6.13	0.98	N	N	4.72	3.83	3.72	3.66

1)N: viable cell growth was not detected.

Table 3. Influence of neutralized pH and catalase on the antimicrobial activities of lactic acid bacteria strains supernatant.

	isolated strains	supernatant (20%)	neutralized pH			catalase (30 u/mL)
			5	6	7	
<i>L. monocytogenes</i>	D2	99.111)	27.19	15.97	13.59	99.08
ATCC 33844	F20-3	77.89	21.75	10.90	6.89	57.84
	F35-2	98.84	32.39	15.48	12.28	98.80
<i>B. cereus</i>	D2	99.57	97.94	25.20	21.61	99.34
ATCC 11778	F20-3	98.87	78.61	19.71	6.89	57.53
	F35-2	99.03	97.45	41.96	18.18	99.34
<i>S. Typhimurium</i>	D2	99.98	97.37	44.24	37.30	99.65
ATCC 14028	F20-3	99.46	93.24	24.97	20.95	58.85
	F35-2	98.66	98.21	43.26	38.16	98.63
<i>S. aureus</i>	D2	98.78	50.00	38.59	22.70	98.26
ATCC 25923	F20-3	63.83	26.22	21.21	17.80	51.28
	F35-2	98.74	41.85	33.19	21.67	98.04
<i>E. coli</i> O157:H7	D2	98.80	22.70	15.44	3.64	98.75
ATCC 43894	F20-3	67.37	18.63	15.23	6.63	55.79
	F35-2	98.74	19.60	18.37	7.45	98.58
<i>S. Enteritidis</i>	D2	98.91	37.67	27.20	24.31	98.07
KCCM 12021	F20-3	72.17	19.73	13.80	13.09	59.04
	F35-2	98.86	32.87	27.56	21.62	98.80

1)see footnote on Table 1.

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