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Cholesterol-Lowering Lactic Acid Bacteria from Kimchi

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Abstract

Lactic acid bacteria are dominant microflora in fermented kimchi. In this study, lactic acid bacteria were isolated from 5 kinds of fermented kimchi and determined their physiologic property. Total 64 of LAB were isolated from kimch samples. In this study, 8 strains of them were selected by pH and bile salt tolerance test. HFI 58, HFI 40, and Yeulmu E strain had significant cholesterol lowering effect *in vitro* test. These were made of special feed of chicken by WooJin B&G. A Diet was tested for 5 weeks. The feed of special material supplement HFI58 groups had significant lower cholesterol concentration in egg yolk.

Introduction

High levels of cholesterol are considered to be a major risk factor for vascular disease including coronary heart disease[1]. It has also known to be the cause of colon cancer. Lipid research clinic program in USA reported that the higher total serum cholesterol is in humans, the greater is the risk for the emergence of coronary heart disease which cause human death (1). Much attention is recently being given to further elucidate beneficial functions of lactic acid bacteria for human health as probiotics, including antimicrobial, antitumor, antimutagenic, and antigenotoxigenic activities[2,3]. Kimchi has also been attracted for its beneficial effect on promoting human health including assimilation cholesterol and many functional lactic acid bacteria have been isolated. This research was motivated to isolate lactic acid bacteria which have cholesterol lowering effect from various kimchi and to use to make functional feed for pig, poultry and cow [9].

Method

Five traditional fermented kimchi samples were collected from local grocery stores. Ten gram of fermented kimchi samples was homogenized with 90ml of 0.85% (w/v) sterile

physiological saline in a stomacher. One hundred microliters of the appropriated dilution spread plated on MRS Agar. Single colony was inoculated on MRS broth and incubated at 30℃ for 24 hours. In order to assess bile salt tolerance of bacteria, they were incubated in MRS broth (pH 7.0) at 30℃ for 48hr. MRS (Difco) broth was supplemented with 0.3% (w/v) oxgall (Difco)[4]. All bacteria were inoculated as 50μl volume and incubated at 30℃ for 48hours. Bacteria were spread onto MRS (Difoc) agar plate to discriminate the survival of bacteria. To assess low pH tolerance, the isolates were incubated in MRS broth adjusted to pH 3.0, respectively, by using HCl(Japan Junsei). According to the result we were selected survival and proliferation strains in extreme condition. The MRS-THIO broth was prepared the day of experimental use supplementing lactobacilli MRS broth (Difco) with 0.2% sodium thilglycolate (Sigma). The broth was further supplemented with 0.004M sodium taurocholate (Sigma) or 0.3% oxgall (Difco)[6]. From the prepared MRS (Difco) broth 1% inoculated in THIO-MRS broth contained cholesterol 1mg/10ml and incubated at 30℃ for 48hours. The o-phthalaldehyde (Sigma) method for measuring cholesterol described by Rudel and Morris[5]. Three strains were selected for *in vivo* test. The selected LAB was made feed for chicken of bacteria (WooJin B&G). The feed was checked how many bacteria still alive in supplement with other material. And 2-month-old chickens randomly assigned for cholesterol *in vivo* test. Chickens were divided into four group dependant of LAB supplement in special feed and were fed once daily almost 10^7 cfu with other supplement. Water was available at all time. After 4 and 5 weeks, we were

Table 1. pH and Bile salt tolerance

Strain No	pH 3.0 Growth	0.5% oxgall Growth
부추 A	+	+
열무 E	+	+
HFI 12	−	−
HFI 21	+	+
HFI 31	+	+
HFI 32	+	+
HFI 40	+	+
HFI 40	+	+
HFI 58	+	+

64 strains were isolated from fermented kimchi.
Determined survival property in low pH and bile salt condition. +: survive, −: none.
7 strains were selected pH and bile salt tolerance effect.

gathered more than 40 eggs each strain. And cholesterol concentration was assayed with same method using ethanol extraction. All experiments were conducted in triplicate.

Results

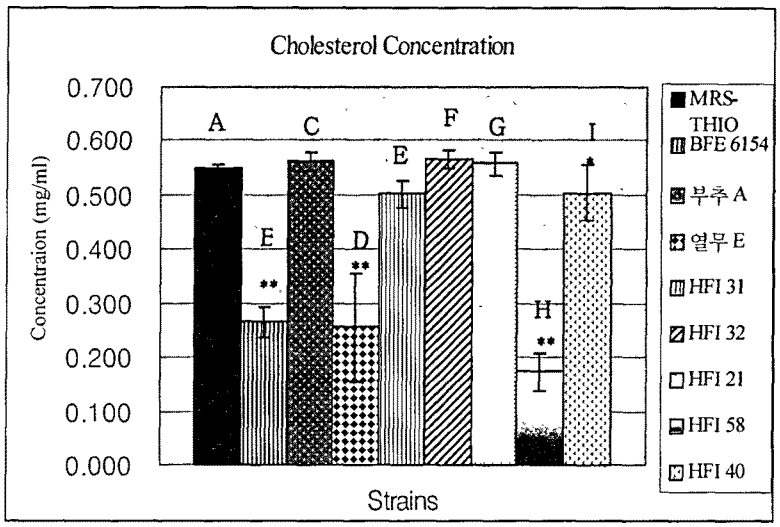

Fig. 1. Cholesterol lowering in broth culture.

Candidate strains were 1% inoculated in THIO-MRS broth supplemented cholesterol. A: THIO-MRS broth, B~I : Bacteria inoculated in THIO-MRS broth at 30℃ for 48hr. Cholesterol concentration was determined by Rudel and Morris method[5].

HFI 58, Yeulmu E, and HFI 40 are significant decreasing Cholesterol concentration *in vitro*.

Results are shown as mean ± standard deviations (n=3). (*$p<0.05$, **$p<0.01$)

Table 2. Alived LAB in specialized chicken feed

Strain	Dilution factor	Count	Number
58	10^7	43	4.3×10^8
열무 E	10^5	39.5	3.9×10^6
40	10^5	169	1.7×10^7

LAB was spreading on MRS agar plate, incubated at 30℃ for 48hr.

Plates are triplication and then make the mean value.

Decide feed CFU according to these result.

10^7 cfu LAB were administrated to chicken once in a day.

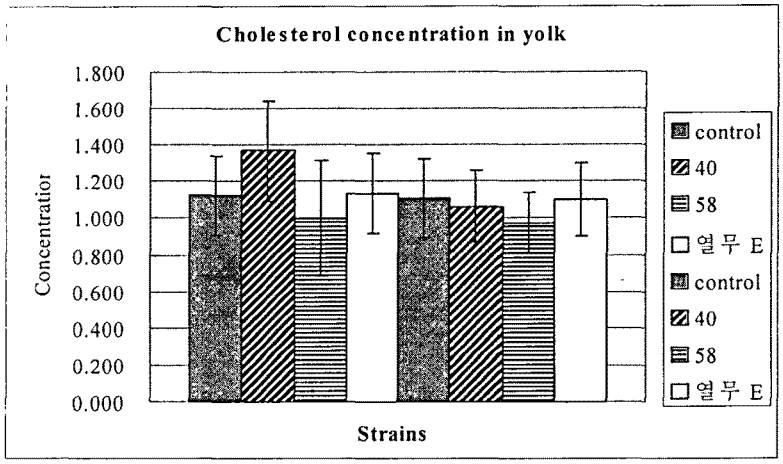

Fig. 2. Determined cholesterol concentration in egg yolk.

All treated chicken group sampled more than 40 eggs, measured weight each of eggs, and determined cholesterol concentration in yolk by ethanol extraction. Results are shown as mean + standard deviation (n=40). ($*p<0.05$).

Conclusion

In this study of cholesterol lowering, lactic acid bacteria as probiotic revealed remarkable difference in behavior among the three species. Yeulmu E strain had a good lowering activity in THIO-MRS broth test *in vitro*. But this strain didn't have significant *in vivo* test. The treated HFI 58 strain observed amounts of cholesterol in the egg yolk were significantly decreased oral administration for 5 weeks.

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