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EFFECTS OF SUPPLEMENTING LACTIC ACID BACTERIA ON FECAL MICROBIOTA, TOTAL CHOLESTEROL,TRIGLYCERIDES AND BILE ACIDS IN RATS.

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Abstract

Background: Probiotics (LAB) are normal components of the intestinal micro-flora in both humans and animals and its ingestion in decreasing the risk of atherosclerosis. In addition, the potential health effects of LAB were investigated by monitoring changes in intestinal micro-flora and lipid metabolism in a rat model.

Materials and Methods: Rats were randomly assigned into four treatments and fecal samples were obtained on days 1, 3, 5, 9 and 14，to evaluate fecal micro biota, total cholesterol (TC), triglycerides (TG) and bile acids in rats (TBA).

Results: The results indicated that *Lactobacillus plantarum* strain L.p X3-2B increased fecal lactic acid bacteria(LAB) and Bifidobacterium while resisting the growth of harmful bacteria. Viable counts of LAB and Bifidobacterium reached 8 log cfu/mL after feeding for 14 days. Fecal pH in the control group was high in comparison with the treatments at all times. Treatment increased the excretion of triglycerides (TG), total cholesterol (TC) and total bile acid (TBA). The results indicate that LAB and Bifidobacterium induce a positive correlation with fecal TC, TG and TBA and a negative correlation with fecal Enterobacteriaceae, Enterococcus and Escherichia coli.

Conclusion: Lactic acid bacteria screened from Inner Mongolia native meat sausages in China had cholesterol-lowering effect.

Keywords: Lactic acid bacteria, fecal micro biota, TC, TG, TBA

Introduction

Cholesterol is the archetypical steroid in mammals. Hyper cholesterol associated with cardiovascular disease is one of the most important causes of death (Yıldız, Öztürk and Aslım 2011, Zhang et al. 2012). Elevated serum cholesterol level is a risk factor for ischemic heart disease and other lethal disease(Watanabe et al. 2013). Reduction in the serum cholesterol has been claimed for lactic acid bacteria (LAB) which are ingredient of several traditional fermented foods and dairy products. LAB have a long history of use in human foods and are naturally present among human gut flora (Szabo et al. 2011). Live microbial feed supplements beneficially affect the host by improving its intestinal microbial balance (Yıldız et al. 2011, Lim 2009). Probiotics (LAB) are normal components of the intestinal micro flora in both humans and animals.

Bile salt is the major route of eliminating cholesterol from the body. *Lactobacillus spp* were found to excrete bile salt hydrolase (BSH) , the enzyme that catalyzes the hydrolysis of glycine- and taurine-conjugated bile salts into amino acid residues and free bile salts (bile acids)(Liong and Shah 2005) splits glycine or taurine from the steroid moiety resulting in free (de-conjugated) bile salts. Bile-salt hydrolase activity is observed in some strains associated with the gastrointestinal tract (Hyeong-Jun Lim 2004, Zhang et al. 2008).

In a previous study (unpublished), we investigated the cholesterol-lowering effect of lactic acid bacteria screened from Inner Mongolia native meat sausages in China. We defined the cholesterol-lowering effect of *Lactobacillus plant arum* $X_{3\text{-}2B}$ (*L. p* $X_{3\text{-}2B}$)isolated from Inner Mongolia native meat sausages *in vitro*, and it was considered that the mechanism was incorporation of cholesterol into the *L. p* $X_{3\text{-}2B}$.

In this study, to verify our *in vitro* results and investigate the effectiveness of L. *p* $X_{3\text{-}2B}$ ingestion in decreasing the risk of atherosclerosis, we investigated the cholesterol-lowering effect of fecal micro biota, triglycerides (TG), total cholesterol (TC) and total bile acid, in rats to further characterize factors affecting and correlation of its ability to hydrolyze cholesterol.

Materials and Methods

Strain

L. p $X_{3\text{-}2B}$ was isolated from native fermented sausage of Inner Mongolia which showed a considerably cholesterol removal ability *in vitro*. *L. p* $X_{3\text{-}2B}$ was cultured in MRS broth and incubated at 30 °C for 24 h. The culture was diluted with 0.85% saline to obtain a preparation containing between 10^{10}, 5×10^{9} and 2.5×10^{9} cfu/mL, which served as the intra-gastric administration in rats.

Animals and Treatment

Sixty four healthy Kunming rats were obtained from the Laboratory Animal Center, Inner Mongolia University, China, with body weights between 16 and 18 g. The rats were individually housed using a 12h light/dark cycle. After a 7-day adaptation period, rats were randomly divided into 3 dose groups and a control group - 16 animals in each group, 8 from each sex. Each group received diet plus drinking water, group H (High doses group) supplemented with 1×10^{10} cfu mL^{-1} viable cells of *L. p* $X_{3\text{-}2B}$, group M (medium dose group) supplemented with 5×10^{9} cfu mL^{-1} viable cells of *L. p* X3-2B, group M (low-dose group) supplemented with 2.5×10^{9} cfu mL^{-1} viable cells of *L. p* X3-2B and group D (control group) with no bacterial supplement. During the experimental period lasting 2 weeks, food and water were available *ad libitum* for all rats (Jia et al. 2011).

Fecal Bacteriological Examination

Fecal samples were collected on days 1,3,5,9 and 14 after gastric perfusion in separate sterile tubes for microbial analysis. Each sample of 0.1g was homogenized with 10ml sterile saline. Subsequent 10-fold serial dilutions of each sample were plated in triplicate. MRS agar was used for total LAB, TYP agar was used for total *Bifidobacterium*. MacConkey agar was used for *E. coli*, violet red bile agar for coliform (Cotton and White, 1992) and *Enterococcus faecium* chromogenic medium for *Enterococcus.* Plates of total lactic acid bacteria, *Bifidobacterium, E. coli*, coliform and *Enterococcus* were incubated anaerobically at 37°C for 48 h (Shah 2006, Saxami et al. 2012, Usami et al. 2013, Wang et al. 2012a, Kazumasa Matsumoto and Toshihiko Takada 2010, Kumar 2013).

Fecal pH

Fecal samples were collected at 1, 3, 5, 9, and 14 days after treatment. Each 0.1g sample was homogenized in 10ml sterile Saline. The pH of the samples was measured with a pH meter (Wang et al. 2009)

Fecal TC, TG, TBA Concentrations

Fecal samples of 1,3,5,9,14 days respectively were weighed 0.1g and ground to a fine powder, and extracted with a 10-fold weight to volume ratio of saline homogenize at room temperature. The supernatant were then used for the analysis of fecal lipids by TC, TG and TBA commercial assay kits (Huang et al. 2013).

Statistical Analysis

Data analysis was carried out with SPSS Inc. software (version 17.0). A one-way ANOVA was used to check for any significant differences between means with a significance level of $P < 0.05$ and Pearson's correlation coefficient Test. Critical difference values were used to perform multiple comparisons between means. All data are presented as means with standard deviations.

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Results and Discussion

Fecal Micro Biota

The microbial population compositions of fecal samples in 1, 3, 5, 9, and 14 days respectively after gastric perfusion in different dose, as determined by counting on selective media, are presented in Table 1. There was a trend towards higher counts of lactic acid bacteria in each treated group, compared with the counts in the control, without reaching statistical significance. The counts of Lactobacillus in groups H increased compared with the control group, while the counts of coliform, *Enterococcus*, and *Escherichia coli* were reduced. The group of H and M were significantly higher than the control group after intake strain of *L. p* $X_{3\text{-}2B}$ for 5 days. The micro flora is a complex ecosystem that may vary with intake (Saxami et al. 2012). There was general agreement on the importance of probiotic micro flora in the intestine.

The intestinal micro biota of mammals is complex, numerous and strongly associated with the host's health (Frizzo et al. 2010). The micro biota, by virtue of its metabolic, trophic and protective functions, maintains the integrity of the intestinal barrier for which LAB are critical. LAB as beneficial bacteria normally associated with a balanced normal in the gut flora. Increase in the numbers of lactobacilli can show a normal occurrence in the development of intestinal flora of calves (Bayatkouhsar et al. 2013). In the present study, consumption of lactobacillus by rats not only resulted in increases in fecal numbers of *Lactobacillus* but also reciprocally suppressed the growth of coliform bacteria, *Enterococcus* and *Escherichia coli.*

Table 1: Fecal microbiota in rats fed different doses of L. plantarum X3-2B.

	Time(d)	H	M	L	D
C1	1	7.6825±0.3328^{aB}	7.3924±0.1069^{aAB}	7.4654±0.1949^{aAB}	7.1152±0.1630^{aA}
	3	8.2337±0.2614^{bC}	7.4268±0.1480^{aAB}	7.5396±0.0883^{aB}	7.0979±0.1388^{aA}
	5	8.1031±0.1411^{bC}	7.3997±0.1095^{aAB}	7.4998±0.0968^{aB}	7.1958±0.0703^{aA}
	9	8.2073±0.711^{bD}	7.9999±0.0123^{bC}	7.7504±0.1512^{aB}	7.2171±0.539^{aA}
	14	8.3322±0.0301^{bD}	7.8124±0.0793^{bC}	7.6674±0.0066^{aB}	7.1672±0.0597^{aA}
C2	1	7.8506±0.0346^{aB}	7.8574±0.0926^{cB}	7.6704±0.1517^{bB}	7.2891±0.3120^{abA}
	3	7.8429±0.4337^{aA}	7.4287±0.0685^{aA}	7.4204±0.1388^{aA}	7.2758±0.2430^{abA}
	5	7.8779±0.0995^{bcC}	7.6766±0.0463^{bB}	7.7132±0.0567^{bB}	7.1362±0.1511^{aA}
	9	8.2265±0.1802^{bcC}	8.2193±0.0370^{d}C	7.8775±0.0285^{bB}	7.5842±0.1442^{bA}
	14	8.3241±0.2739^{cB}	8.2375±0.0824^{dB}	8.2186±0.1842^{cB}	7.6223±0.0543^{bA}
C3	1	5.4271±0.3767^{bcA}	5.0731±0.1033^{aA}	5.2028±0.0991^{bA}	5.2037±0.0833^{bcA}
	3	5.7240±0.1999^{cC}	5.5281±0.3596^{aC}	4.6276±0.4619^{aA}	5.2734±0.0552^{bcB}
	5	4.8855±0.1694^{aB}	5.3197±0.1061^{aC}	4.8495±0.2129^{bB}	5.3238±0.2748^{bA}
	9	5.2648±0.1542^{bB}	5.2526±0.3572^{aB}	4.9702±0.3291^{bB}	5.3891±0.5502^{aA}
	14	5.3582±0.0861^{bB}	5.4331±0.1569^{aB}	5.6300±0.2483^{bB}	5.8878±0.0264^{cA}
C4	1	6.3236±0.1628^{cA}	6.3912±0.2701^{cA}	6.2745±0.1908^{cA}	6.4227±0.1234^{aA}
	3	5.8388±0.0856^{bA}	6.2613±0.1067^{bcB}	6.2328±0.0180^{abB}	6.5957±0.1455^{aC}
	5	5.8407±0.1046^{bA}	6.1600±0.0561^{bcBC}	6.0212±0.0058^{aBC}	6.3512±0.3532^{aC}
	9	5.4412±0.3204^{aA}	6.1245±0.0451^{bB}	6.3835±0.0866^{cB}	7.1269±0.3321^{bC}
	14	5.3418±0.1756^{aA}	5.7130±0.0881^{aB}	6.1453±0.0791^{abC}	6.5324±0.3459^{aC}
C5	1	4.3174±0.2482^{bA}	4.4432±0.1008^{bA}	4.0880±0.5914^{aA}	4.6505±0.2129^{aA}
	3	4.4774±0.1308^{bA}B	4.4068±0.0845^{bAB}	3.8010±0.6739^{aA}	5.0880±0.1569^{bB}
	5	3.6611±0.2602^{aA}	4.0777±0.3627^{aB}	4.6561±0.1079^{bcC}	5.3406±0.0724^{cD}
	9	4.3512±0.0410^{bA}	4.3613±0.0189^{bA}	4.5221±0.1282^{bcA}	5.4112±0.0690^{cB}
	14	4.2297±0.0362^{bA}	4.3847±0.0625^{bA}	5.2673±0.4117^{cB}	5.3203±0.0792^{cB}

a, b, c: different superscripts in the same column differ significantly ($p < 0.05$).

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A,B,C: different superscripts in the same horizontal differ significantly ($p < 0.05$).

C1,C2,C3,C4,C5:Viable counts (log cfu/mL) of Lactic acid bacteria, *Bifidobacterium*, *Coliform*, *Enterococcus*, *Escherichia coli*.H,M,L,D: High doses group, medium dose group, low-dose group, control group

Fecal pH

There was no significant effect of treatment on fecal pH (Table 2). That was in agreement with previous reports (Bayatkouhsar et al. 2013). Such a shift of the micro-ecological balance in favor of overall improved population ratios of micro flora may lead to healthier gastrointestinal tract conditions. These results may have been due to the production of high amounts of lactic acid or other organic acids during fermentation of carbohydrate, keeping the fecal pH low.

Table 2: Fecal pH of rats fed different doses of L. plantarum X3-2B on different days.

Time(d)	H	M	L	D
1	8.30	8.28	8.09	8.40
3	8.05	7.93	8.17	8.30
5	7.91	7.38	8.12	8.22
9	7.86	7.48	8.02	8.26
14	7.96	8.10	8.19	8.46

H,M,L,D: High doses group, medium dose group, low-dose group, control group

Fecal Lipid Analysis

Fecal cholesterol and triglyceride concentration of rats fed strain of L. Plant arum X3-2B increased after 5 days compared with the control group ($P < 0.05$) (Tables 3 and 4). Groups H and M showed higher cholesterol and triglyceride concentrations than did group L, and group D. Females showed higher cholesterol and triglyceride concentrations than males. LAB treatment leads to cholesterol reduction. These findings are in agreement with previous reports (Guo and Li 2013, de Almeida Jackix et al. 2013, Chiu et al. 2005).

Table 3: Fecal TC, TG, TBA excretion levels of male rats fed different doses ofL. plantarum X3-2B

	Time(d)	H	M	L	D
TC	1	0.4249 ± 0.0567^{aB}	0.3470 ± 0.0271^{aB}	0.3470 ± 0.0536^{aB}	$0．2125\pm0.02003^{aA}$
mmol/L	3	0.4603 ± 0.0536^{aB}	0.4249 ± 0.0000^{bB}	0.3966 ± 0.0567^{abB}	0.2833 ± 0.02313^{bA}
	5	0.5028 ± 0.0813^{abB}	0.5056 ± 0.0123^{cB}	0.4603 ± 0.0536^{bcB}	0.2762 ± 0.02712^{bA}
	9	0.5666 ± 0.0000^{bcD}	0.5099 ± 0.0000^{cC}	0.4816 ± 0.0001^{cB}	0.4320 ± 0.01416^{cA}
	14	0.6161 ± 0.0628^{cB}	0.5697 ± 0.0326^{dB}	0.4958 ± 0.0283^{cA}	0.4603 ± 0.02712^{cA}
TG	1	0.6332 ± 0.0124^{aB}	$0.6244\pm0. 0215^{aB}$	0.6859 ± 0.0124^{aC}	0.4583 ± 0.0000^{aA}
mmol/L	3	0.6507 ± 0.0000^{aB}	0.6244 ± 0.0000^{aB}	$0.7388\pm0. 0329^{aC}$	0.4286 ± 0.0124^{aA}
	5	0.6859 ± 0.0124^{bB}	0.7299 ± 0.0215^{bB}	0.8002 ± 0.0329^{abC}	0.4693 ± 0.0431^{aA}
	9	0.9321 ± 0.0329^{cB}	0.8970 ± 0.0249^{cB}	0.8916 ± 0.0125^{bcB}	0.4924 ± 0.0448^{aA}
	14	1.1960 ± 0.0249^{dC}	1.0992 ± 0.0000^{dBC}	0.9673 ± 0.1710^{cB}	0.5540 ± 0.0215^{bA}
TBA	1	37.3529 ± 1.2478^{aA}	35.6618 ± 0.7352^{aA}	36.0294 ± 2.8160^{aA}	33.8235 ± 4.1595^{aA}
μmol/L	3	69.1176 ± 1.2007^{bC}	44.1176 ± 0.0000^{bB}	43.3824 ± 0.8490^{bB}	33.4559 ± 0.7353^{aA}
	5	73.0392 ± 1.8341^{cB}	68.0147 ± 5.5513^{cB}	50.7353 ± 1.8985^{cA}	48.0392 ± 0.8490^{bA}
	9	85.2941 ± 2.4015^{dC}	67.2794 ± 3.8676^{cB}	51.1029 ± 1.4080^{cA}	50.9804 ± 1.6981^{bcA}
	14	86.7647 ± 3.6022^{dC}	85.2941 ± 6.8978^{dC}	67.1569 ± 0.8499^{dB}	52.2059 ± 0.8490^{cA}

a, b, c: different superscripts in the same column differ significantly ($p < 0.05$).

A,B,C: different superscripts in the same horizontal line differ significantly ($p < 0.05$).

C1,C2,C3,C4,C5:Viable counts (log cfu/mL) of Lactic acid bacteria, Bifidobacterium, Enterobacteriaceae, *Enterococcus*, *Escherichia coli*.H,M,L,D: High doses group, medium dose group, low-dose group, control group

Fecal TBA Concentrations

The fecal chloric acid levels differed significantly among the experimental groups ($P < 0.05$) (Tables 3 and 4). Bile acids are amphipathic molecules synthesized from cholesterol in the liver. Bile acid synthesis is a major pathway for endogenous cholesterol metabolism in humans and other animals. Enhancement of faecal bile acid excretion is important for reducing serum cholesterol levels. We found that the H, M, L groups showed considerable increases in the excretion of cholic acid in their feces. After 14 days of feeding, the maximum fecal cholic acid excretion (87 mmol/L) was recorded in the H group, whereas in the D group, the corresponding increases in fecal cholic acid occurred in male rats. However, after 14 days of feeding, the maximum fecal cholic acid excretion (93 mmol/L) was recorded in the H group in female rats. In another study, both the low and high-dose lactobacilli fed rats showed significantly more increased levels of daily faecal TBA excretion similar to our findings (Guo and Li 2013).

Table 4: Fecal TC, TG, TBA excretion levels of female rats fed different dose of L. plantarum X3-2B

	Time(d)	H	M	L	D
TC	1	0.3245 ± 0.0000^{aC}	0.3820 ± 0.0000^{aB}	0.2060 ± 0.0473^{aA}	0.2279 ± 0.0101^{aA}
mmol/L	3	0.5120 ± 0.0144^{aD}	0.4075 ± 0.0000^{abC}	0.3372 ± 0.0250^{bB}	0.2560 ± 0.0125^{abA}
	5	0.5471 ± 0.0091^{bC}	0.4330 ± 0.0000^{bB}	0.4246 ± 0.0204^{cB}	0.2810 ± 0.0125^{bA}
	9	0.5994 ± 0.0000^{cB}	0.4371 ± 0.0322^{bA}	0.4584 ± 0.0000^{cA}	0.4246 ± 0.0456^{cA}
	14	0.6931 ± 0.0125^{dC}	0.6182 ± 0.0124^{cB}	0.5858 ± 0.0000^{dB}	0.4246 ± 0.0353^{cA}
TG	1	0.5698 ± 0.0148^{aB}	0.5826 ± 0.0724^{aB}	0.7427 ± 0.0128^{aC}	0.7973 ± 0.0125^{aA}
mmol/L	3	0.8515 ± 0.0181^{bB}	0.8903 ± 0.0169^{bB}	0.8323 ± 0.0567^{bB}	0.7619 ± 0.0210^{aA}
	5	1.0244 ± 0.0128^{cC}	0.9667 ± 0.0000^{cB}	0.9219 ± 0.0322^{cA}	0.9392 ± 0.0000^{bAB}
	9	1.1545 ± 0.0169^{cB}	$1.0606\pm0.0391d^{AB}$	1.0308 ± 0.0905^{dA}	1.0692 ± 0.553^{cA}B
	14	1.5728 ± 0.1989^{dB}	1.5173 ± 0.0331^{eB}	1.1447 ± 0.0000^{eA}	1.1035 ± 0.0153^{cA}
TBA	1	52.4510 ± 0.6932^{aC}	52.4510 ± 0.0693^{aC}	49.0197 ± 1.3865^{aA}	50.4902 ± 0.6932^{aB}
µmol/L	3	55.3922 ± 1.3865^{bB}	52.6176 ± 0.4575^{aA}	50.4902 ± 2.4995^{aA}	50.1961 ± 1.8341^{aA}
	5	73.0392 ± 0.6932^{cD}	68.1373 ± 0.6932^{bC}	59.3137 ± 1.3865^{bB}	53.8235 ± 2.4015^{aA}
	9	85.2941 ± 2.0797^{dC}	91.6667 ± 0.6932^{cD}	65.1961 ± 0.6932^{cB}	53.1373 ± 4.0919^{aA}
	14	93.1373 ± 2.4995^{eD}	85.2941 ± 2.0797^{dC}	68.1373 ± 0.6932^{dB}	50.4902 ± 1.3865^{aA}

a, b, c: different superscripts in the same column differ significantly ($p < 0.05$).

A,B,C: different superscripts in the same horizontal line differ significantly ($p < 0.05$).

C1,C2,C3,C4,C5:Viable counts (log cfu/mL) of Lactic acid bacteria, Bifidobacterium, Enterobacteriaceae, *Enterococcus*, *Escherichia coli*.H,M,L,D: High doses group, medium dose group, low-dose group, control group

Correlation Net Works among Fecal Micro Biota and Fecal TC, TG, TBA Concentration

Correlations among fecal micro biota and fecal TC, TG, TBA concentrations are shown in Fig 1.. LAB indicated positive correlation ($r=0.942$, $p=0.017$) with fecal TBA and negative correlation ($r=-0.901$,$p=0.037$) with fecal *Enterococcus* while *Bifidobacterium* indicated positive correlation ($r=0.962$,$p=0.009$) with fecal TG in the H1 group. LAB and *Bifidobacterium* indicated positive correlation with fecal TC, TG and TBA while indicated negative correlation with fecal Enterobacteriaceae and *Enterococcus*. Fecal triglyceride, total cholesterol and total bile

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acid indicated positive correlation within the four groups.

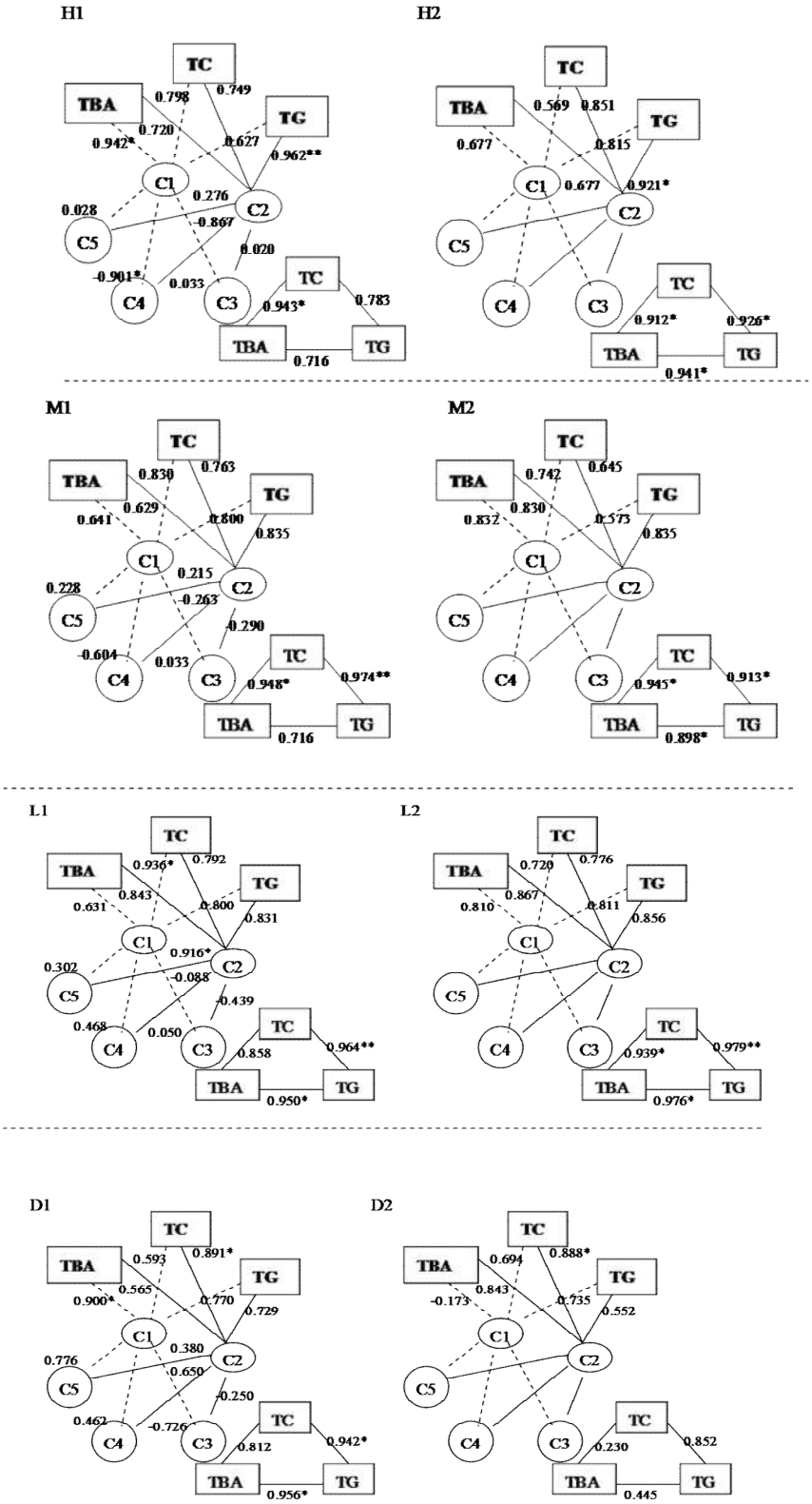

Figure 1: Correlation networks among fecal microflora, total cholesterol (TC), total triglyceride (TG) and Total bile acid (TBA) in rat of different lactic acid bacteria. Solid lines indicate C2 correlations and dotted lines indicate C1 correlations. H1: High doses group of Male rat. H2: High dose group of female rat. M1: medium dose group of Male rat. M2: medium dose group of Female rat. L1: low-dose group of Male rat. L2:

low-dose group of Female rat. D1: Control group of male rat. D2: Control group of Female rat. *p < 0.05 and **p < 0.01; C1, Lactic acid bacteria; C2, *Bifidobacterium*; C3, Enterobacteriaceae; C4, E*nterococcus*; C5, *Escherichia coli*.

Wang et al. (2009) suggested that *L. plantarum* resulted in a reduction of serum TC, LDL-C, and TG levels of rats fed high-cholesterol diets (Wang et al. 2009). De-conjugating bile acids produce free bile acids, which are more likely to be excreted (Wang et al. 2009), therefore, fecal cholesterol levels reflect in part the reducing serum cholesterol ability of *L. p* $X_{3\text{-}2B}$. The bacteria facilitate elimination of cholesterol in feces and inhibit absorption of cholesterol by binding with cholesterol (Wang et al. 2010).

An increased excretion of bile acids would decrease the amount of bile acids reaching the liver for secretion back into the intestine in enterohepatic circulation. Bile salts are de-conjugated and cholesterol is co-precipitated with free bile acids (Yıldız et al. 2011). The convention of cholesterol into bile acids plays a vital role for clearance of cholesterol, which was one of the main pathways regulating cholesterol homeostasis (Zhu et al. 2013). To maintain the necessary levels of conjugated bile acids for the enterohepatic circulation, excreted bile acids are replaced by synthesis of new ones from cholesterol and free bile acids do not support the absorption of lipids, including cholesterol, from the intestines, thus providing the potential to reduce cholesterol level (Wang et al. 2012b, Lye et al. 2012).

The beneficial effects of LAB are, probably, due to their growth in the intestinal tract, which creates a microbiological barrier against the development of pathogenic bacteria, therefore, LAB indicated a negative correlation with Enterobacteriaceae and *Enterococcus* which are in accordance with the findings of Sgnorini et al. (Signorini et al. 2012). LAB plays an important role in reducing cholesterol. There was insignificant correlation between de-conjugating sodium glycocholate and cholesterol precipitation (Baccigalupi et al. 2005). De-conjugation of conjugated bile salts by bile salt hydrolases was an important detoxifying modification resulting in the formation of free bile acids. Free bile salts are less absorbed in the intestine and escape the enterohepatic circulation; hence, more cholesterol was utilized which minimizes hypercholesterol associated cardiac arrest l(Mandal, Sen and Mandal 2009, Menghe Bilige 2009)

Conclusions

Feeding probiotic *L. p* $X_{3\text{-}2B}$ to rats increased fecal LAB and Bifidobacterium while resisting the growth of harmful bacteria. Fecal pH in the control group was high in comparison to other treatments. It seems that is necessary to achieve an effect of increasing excretion of TC, TG and TBA in test group. The results indicate that LAB and Bifid bacterium induce a positive correlation with fecal TC, TG and TBA and negative correlation with fecal Enterobacteriaceae, *Enterococcus* and *Escherichia coli.*

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