Journal of Agricultural Technology 2013 Vol. 9(7):1849-1862
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Bile and acid tolerance of lactic acid bacteria isolated from tempoyak and their probiotic potential

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Usman Pato and Ingrid S. Surono (2013) Bile and acid tolerance of lactic acid bacteria isolated from tempoyak and their probiotic potential. Journal of Agricultural Technology 9(7):1849-1862.

Supplementation of diet with fermented or lactic acid bacteria-containing food products have shown to reduce serum cholesterol levels in humans and animals, and many findings on hypocholesterolemic effect of these products have been published. In this study, we evaluated the *in vitro* cholesterol-binding and bile salt-deconjugating abilities as well as acid and bile tolerance of lactic acid bacteria isolated from tempoyak. Tempoyak is an Indonesian and Malaysian traditional fermented food made of durian fruit. Results of this study showed that *Enterococcus* sp. were the dominant lactic acid bacteria followed by *Lactobacillus* sp. obtained during spontaneous fermentation of durian into tempoyak. Almost all 44 isolates were tolerant to acid, however, only 3 isolates namely *Enterococcus* sp. strains UP-9, UP-11 and UP-14 were tolerant to bile. Among these 3 isolates, only *Enterococcus* sp. UP-11 exhibited cholesterol-binding activity. *Enterococcus* sp. UP-9 showed relatively higher sodium taurocholate-deconjugating ability followed by strain UP-11, while strain UP-14 could not deconjugate sodium taurocholate. Based on the 16S rRNA gene sequences of *Enterococcus* sp. retrieved from GenBank and aligned with the taxonomically related bacteria compared with GenBank, *Enterococcus* sp. UP-9 was identified as *Enterococcusgallinarum* UP-9, and *Enterococcus* sp. UP-11 was assigned as *Enterococcusfaecalis* UP-11.

Keywords:*Enterococcus*, bile and acid tolerance, natural probiotic, hypocholesterolemic effect, tempoyak

Introduction

Lactic acid bacteria (LAB) are normal components of the intestinal microflora in both humans and animals and have been associated with various health-promoting properties. LAB for use as a probiotic culture or as food adjunct must be tolerant to acid and bile, which enables selected strains to survive, grow and perform its therapeutic benefits in the intestinal tract (Boke

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et al., 2010; Gilliland and Walker, 1989; Ljungh and Wastrom, 2006; Salminen and Von Wright, 1993; Usman and Hosono, 1999). Some LAB have performed *in vitro* acid and bile acid tolerance, which were isolated from Indonesian dadih(Pato, 2003; Surono, 2003). Fermented milk has been reported to posses a range of beneficial properties to humans, including assimilation of cholesterol (Al-Saleh *et al*., 2006; Ziarno *et al*., 2007; Guo *et al*., 2011) and deconjugation of bile salts (Pato *et al*., 2004, Begley *et al*., 2006). For this reason, there has been much interest in developing food products containing these bacteria as dietary adjuncts.

Tempoyak is a traditional spontaneous fermented condiment made from the raw pulp of the durian fruit (*Durio zibethinus*), a kind of tropical fruit. This product is popular among people living in Riau Province, Indonesia as well as in Malaysia. Interestingly, even though the fresh durian pulp is fermented without any heat application, there is no record of food-borne illness caused by the consumption of tempoyak. Hence, there must be some important factors in the indigenous tempoyak lactic acid bacteria that contribute in preventing growth of pathogenic microorganisms.

Tempoyak has a long history of safe consumption, in Riau, Sumatra. Several LAB found to be involved in the fermentation of tempoyak are *Lactobacillus plantarum, Leuconostoc mesentroides* subsp. *mesentroides* and *Streptococcus faecalis* (Ohhira *et al*., 1990), *Leu. mesentroides, L. brevis, L. mali* and *L. fermentum* (Leisner *et al*., 2000), *L. durianis* (Leisner *et al*., 2002). Probiotic is strain specific, and no publication is available yet on the probiotic and hypocholesterolemic properties of LAB isolated from tempoyak.

Coronary heart disease (CHD) is a multifactorial disease characterized by long-term degeneration changes in the wall of arteries, resulting in a narrowing of the lumen of blood vessels, limiting the blood supply to vital organs such as the heart. In general there are two ways in reducing serum total and LDL cholesterol levels, namely by the use of drugs and diet control (Lipid Research Clinics Program, 1984). Control of diet is considered to be a more natural method by changing the lipid composition of diet such as decreasing saturated fatty acids and cholesterol, and increasing polyunsaturated fatty acids resulted in lowering serum total cholesterol levels and reduce the incidence of CHD (Hughes, 1995). Supplementation of diet with fermented dairy products or nonfermented dairy products have also the potential to reduce serum cholesterol in humans and animals. The present study reports on the molecular identification and *in vitro* assessment of indigenous tempoyak LAB strains that could be used as potential probiotic with the ability in lowering cholesterol serum levels.

Materials and methods

Tempoyak Sample

Fresh tempoyak was purchased from a traditional market at the city of Pekanbaru, Riau Province, Indonesia. The pH of the samples were measured immediately, and the samples were kept in a refrigerator (8 to 10°C). Microbiological analysis was carried out within 24 hours.

Isolation of Lactic Acid Bacteria

Five grams of tempoyak sample were pipetted into 45 ml of 66 mM phosphate buffered saline (PBS), pH 6.8 and shaken vigorously for 5 min. Suitable decimal dilution were prepared with PBS, then 0.1 ml portions of the diluted samples were spreaded on MRS agar and Nutrient Agar (NA) plates. The plates were incubated aerobically at 30°C for 24 hours, and 83 colonies were randomly picked and isolated. The colonies on the MRS agar and NA plates were purified by streaking on Bromo Cresol Purple Plate Count agar, Nissui (BCP). Fifty one of purified single colonies that appeared as yellow colonies were picked up and streaked on MRS agar slant for further identification.

Identification of Lactic Acid Bacteria

Taxonomic properties of isolates were examined and identified according to *Bergey's Manual of Determinative Bacteriology*, 8th Edition and *Testing Methods in Food Microbiology* (Kiss, 1984). For genus identification, Gram staining, motility test, catalase production, formation of acid and gas from glucose, growth in various temperature, growth in 6.5% NaCl, production of ammonia from arginine and formation of acid or acid and gas from lactose were carried out. After performing these tests, 44 out of 51 isolates were identified as lactic acid bacteria. The confirmed genus of LAB then further evaluated for their probiotic properties. The best two potential probiotic strains, with good hypocholesteromic properties, namely UP-9 and UP-11 which were identified as *Enterococcus* species were further identified to species level by molecular identification using PCR and the nucleotide sequence was compared with Gen Bank sequences.

Protocol for PCR light cycler 1.5 system

Bacterial strains and growth conditions

The strains used in this study were *Enterococcus* strain UP-9 and UP-11. Strains were grown in MRS broth or MRS agar (Oxoid, Basingstoke, UK) and incubated at 37°C in aerobic conditions.

Genomic DNA preparation

Bacterial cultures were grown in MRS broth, and centrifuged at 2000 x g for 5 minutes. The pellet was resuspended in 200 □l TE buffer and 4 ml (5□g/1 mlTE) Lysozyme, and incubated at 37^{O}C for 10 minutes. DNA was extracted and isolated by using reagent LC FastStart DNA Master plus SYBR Green I Roche Applied Science, High Pure PCR Template (Roche, Manheim) according to the protocol supplied by the manufacturer.

PCR and melting curve analysis

Amplification of the DNA was performed with primers Ec-ssu1'Forward and reverse Ec-ssu1R in a PCR light Cycler 1.5 apparatus (Roche). The total volume of each reaction mixture was 50 □l. PCR amplification was performed using a real-time PCR system (Light-Cycler 1.5; Roche). The PCR was prepared as follows: 1 cycle of 95°C for 10 min for denaturation, 45 cycles of amplification (10 s at 95°C, 10 s at 52°C, and 10 s at 72°C). This step was followed by a melting curve analysis from 40 to 95°C and afterwards cooling to room temperature. The PCR product was purified and sequenced using an automated sequencer.

Sequence analysis of Enterococcus sp. 16S rRNA gene

The 16S rRNA gene sequences of *Enterococcus* sp. retrieved from GenBank and aligned with the taxonomically related bacteria compared with GenBank. Alignment was done using Blast program of GenBank

Assay for Acid Tolerance

Acid tolerance of LAB isolated from tempoyak was carried out according to Pato *el al*. (2004). Washed cell pellets of tempoyak's LAB were resuspended in sterile distilled water and the absorbance (625 nm) is adjusted to 0.7 for each

culture. Cell suspensions were inoculated into each of 10 ml of 2% skim milk that had been adjusted to pH 3.0 with concentrated HCl. The mixtures were incubated at 37°C for 2 h. Immediately after incubation, 0.5 ml of suspended cells were diluted with 4.5 ml of 66mM phosphate buffered saline, pH 6.8 and is mixed uniformly with a vortex mixer. Subsequent serial dilutions were made and plated by the spread-plate method on MRS agar. The plates were incubated at 37°C for 48 hours before enumeration.

Assay for Bile Tolerance

To study the effect of bile salts on the growth rate of LAB isolated from tempoyak, a method described by Walker and Gilliland (1993) was employed. All cultures were evaluated for growth in MRS-THIO broth with and without 0.3% oxgall. Freshly prepared cultures were inoculated into each medium, incubated at 37°C in a waterbath, and monitored hourly for growth spectrophotometrically at 620 nm. The growth was followed for 9 hours or until a 0.3-unit difference in absorbance was reached. The effect was measure on the basis of the time required to increase the absorbance at 620 nm by 0.3 units both in MRS-THIO broth with and without 0.3% oxgall. The difference in time (hour) between the culture media was considered as the lag time (LT).

Analysis for Sodium Taurocholate-Deconjugating Activity

Deconjugation of sodium taurocholate is assessed in MRS-THIO broth (MRS plus 0.2% sodium thioglycholate) supplemented with 0.2% sodium taurocholate. The broth medium was inoculated with 1% of active culture and incubated at 37°C for 18 hours. Analysis for the free cholic acid released during deconjugation was carried out according to the method of Walker and Gilliland (1993).

Assay for Cholesterol-Binding Activity

Sheep serum was added to MRS broth containing 2% sodium thioglycholate and 0.3% oxgall (MRSO broth) to obtain a cholesterol concentration of 100 µg/ml. 5 ml of MRSO broth were distributed into sterile tubes and then inoculated individually with 100 µl of active cultures. After incubation at 37°C for 18 hours, cells were removed by centrifugation at 12,000 rpm for 10 minutes. The spent broth was collected and analyzed for cholesterol using the method described by Rudel and Morris (1973).

Results

Tempoyak is composed of protein, fat and carbohydrate, and has an acid taste due to low pH (Table 1). Eighty three bacteria were picked up randomly from MRS agar and Nutrient Agar and inoculated in BCP agar. After 24 h incubation, only 51 isolates could grow on BCP agar and was further characterized for their taxonomic properties. Of the 51 BCP colonies picked, 44 isolates were identified as lactic acid bacteria.

The 44 LAB were then screened for further taxonomic properties for identification to the genus level as shown in Tables 2 and 3. Morphological and biochemical characteristics revealed that 12 isolates classified as genus *Lactobacillus* sp. and the other 32 isolates belonged to *Enterococcus* sp.

Almost all LAB isolated from tempoyak were relatively resistant to acid as indicated by the reduction in the number of colonies between 0.76 and 2.82 log cycles at pH 3.0 after 2 hours incubation (Table 4). Isolate no. UP-57 was very resistant to acid indicated by the reduction of colonies of only 0.76 log cycles at pH 3.0 for 2 h. The number of colony reduction in the range of 1.0 to 2.0 log cycles were found in isolates no. UP-1, UP-2, UP-6, UP-7, UP-9, UP-12, UP-13, UP-14, UP-16, UP-20, UP-23, UP-24, UP-25, UP-26, UP-29, UP-31, UP-32, UP-35, UP-37, UP-44, UP-57, UP-60, UP-61, UP-66 and UP-71. The other isolates showed colony reduction of more than 2 log cycles when grown at pH 3.0 for 2 hours.

Among the 44 strains tested, only isolates no. UP-9, UP-11 and UP-14 were tolerant to bile (Table 5). Isolates no. UP-3, UP-61 and UP-64 grew in MRS-THIO without oxgall but the absorbance did not increase by 0.3 units after 9 h in the same medium supplemented with oxgall. The absorbance of other isolates did not increase by 0.3 units in the medium with or wihout oxgall.

Among 44 isolates, only 3 isolates were tolerant to bile. These 3 isolates were further screened for their ability to deconjugate taurocholic acid and to bind cholesterol. Among the three isolates, strain No. UP-9 could deconjugate taurocholic acid by releasing relatively higher amounts of cholic acid. Strain No. UP-11 released very low amount of cholic acid, and strain UP-14 could not deconjugate taurocholic acid at all (Table 6).

Table 7 shows the results obtained for binding of cholesterol by LAB isolated from tempoyak. Among three strains, only isolate No. UP-14 exhibited cholesterol-binding ability by removing 17.86% cholesterol from the medium broth.

Only strains UP-9 and UP-11 which show high cholesterol binding and/or taurocholate-deconjugating abilities were further identified to the species level. Identification of two strains were based on the 16S rRNA gene sequences by

PCR amplification using 16S rRNA specific primers and sequencing the amplified region by using an automated sequencer. *Enterococcus* sp. 16S rRNA retrieved from GenBank and aligned with the taxonomically related bacteria compared with GenBank showed that *Enterococcus* sp. UP-9 had 97% homology to *Enterococcus gallinarium* UP-9, and *Enterococcus* sp. UP-11 was assigned as *Enterococcusfaecalis* UP-11 as it had 97% homology to *Enterococcus faecalis reference strain.*

Discussions

Elevated serum cholesterol in human is generally a risk factor correlated with development of coronary heart disease. Modification of diets such as supplementation of diet with fermented dairy products or LAB-containing fermented foods is a way that may be helpful in reducing serum cholesterol. Present study shows that *Enterococcus* and *Lactobacillus* were the predominant genus in tempoyak. This finding is contradictory to the previous report by Leisner *et al*. (2000) who found that genus *Lactobacillus* were dominant LAB flora in tempoyak. The variation is due to the natural fermentation of tempoyak. These LAB play an essential role in preserving raw food materials i.e. durian and contribute to the nutritional, organoleptic and health properties of tempoyak. Factors that should be considered in selecting LAB as a probiotic culture or as a food adjunct is acid and bile tolerances to be able to survive, grow and perform therapeutic activity in the gastrointestinal tract (Salminen and von Wright, 1993). Thus, probiotic must survive to acid conditions during gastrointestinal tract transit. Survival at pH 3 for 2 h and growing in mendium containing 1,000 ppm of bile acids are considered as standard assay for acid and bile-tolerance of probiotic cultures (Itoh, 1992; Gohran, 1994). The present study shows that all the isolates were relatively resistant to acid as the isolates were originated from tempoyak (pH around 3.69). However, only 3 isolates namely *Enterococcus* sp. strains UP-9, UP-11 and UP-14 could survive in the medium containing 1,000 ppm of oxgall (bile). This is contradictory to our previous finding that all 28 strains of *Lactobacillus gasseri* isolated from human intestinal tract were tolerant to bile (Usman and Hosono, 1990). This is maybe due to the difference in the living environmental condition of those lactic acid bacteria.

Therefore these 3 isolates were chosen to be assessed further for their sodium taurocholate-deconjugating and cholesterol-binding abilities. The results show that only isolate no. UP-11 was able to bind cholesterol. Hosono and Tono-oka (1995) reported that binding of cholesterol to cells of LAB from various fermented milk products varied among the strains and species, and

suggested that differences in binding abilities were due to the chemical and structural properties of their cell-wall peptidoglycans.

Deconjugation of bile acids may help reduced serum cholesterol in human because deconjugated bile acids are excreted more rapidly than the conjugated forms (Chikai *et al*., 1987). Sodium taurocholate is a major bile salt in human and carnivores (Uchida *et al*., 1977); therefore, we interested in studying the bile salt-deconjugating ability of tempoyak's LAB. It was found that isolate No. UP-9 could deconjugate taurocholic acid by deliberating relatively higher amount of cholic acid than that of isolates No. UP-11 and UP-14. The results from the present study is contradictory to the previous findings by some researchers who reported that all LAB isolated from human and animal feces or small intestine had the ability to deconjugate sodium taurocholate, eventhough their bile salt-deconjugating activities varied among the species and strains (Gilliland and Walker, 1989; Gopal *et al*., 1996; Usman and Hosono, 1999; Al-Saleh *et al*., 2006).

The present study suggests that consumption of tempoyak made from *Enterococcus gallinarum* UP-9 and *Enterococcus faecalis* UP-11 by different mechanisms, namely, deconjugating taurocholic acid and cholesterol binding, respectively, which are potential probiotic in reducing human serum cholesterol level. To validate this conclusion, *in vivo* animal model experiments and clinical trial on subjects with high total cholesterol and LDL cholesterol levels are necesssary.

Table 1. Chemical Composition of Tempoyak

Composition	**Number (%)**
Carbohydrate	6.279
Protein	8.099
Fat	6.645
Ash	1.834
Moisture content	74.950
pH	3.69

Table 2. Cultural characters of genus *Lactobacillus* isolated from tempoyak

Culture No.	**Ammonia from arginin**	**Gas & acid from glucose**	**Growth at 10°C**	**Growth at 15°C**	**Growth at 45°C**	**Growth in 6.5% NaCl**	**Reaction in litmus milk**
UP-3	-	-	-	-	-	+	Soft clot
UP-5	-	-	-	-	-	+	Soft clot
UP-7	-	-	-	-	-	+	Hard clot
UP-13	-	-	-	-	-	+	Hard clot
UP-15	-	-	-	-	+	+	Hard clot

UP-18	-	-	-	-	-	+	Hard clot
UP-38	-	-	-	-	-	+	Hard clot
UP-43	-	-	-	-	-	+	Hard clot
UP-64	-	-	-	-	+	+	Hard clot
UP-65	-	-	-	-	+	+	Hard clot
UP-71	-	-	-	-	-	+	Hard clot
UP-78	-	-	-	-	-	+	Hard clot

Table 3. Cultural characters of genus *Enterococcus* isolated from tempoyak

Culture No.	Ammonia from arginin	Gas & acid from glucose	Growth at 10°C	Growth at 45°C	Growth in 6.5% NaCl	Reaction in litmus milk
UP-1	-	-	-	-	+	Hard clot
UP-2	-	-	-	-	+	Hard clot
UP-6	-	-	-	-	+	Soft clot
UP-9	-	-	-	-	+	Hard clot
UP-11	-	-	-	-	+	Hard clot
UP-12	-	-	-	-	+	Hard clot
UP-14	-	-	-	-	+	Hard clot
UP-16	-	-	-	+	+	Soft clot
UP-20	-	-	-	+	+	Hard clot
UP-21	-	-	-	-	+	Hard clot
UP-22	-	-	-	-	+	Hard clot
UP-23	-	-	-	-	+	Hard clot
UP-24	-	-	-	-	+	Hard clot
UP-25	-	-	-	-	+	Hard clot
UP26	-	-	-	-	+	Hard clot
UP-28	-	-	-	-	+	Hard clot
UP-29	-	-	-	+	+	Hard clot
UP-30	-	-	-	-	+	Hard clot
UP-31	-	-	-	+	+	Hard clot
UP-32	-	-	-	-	+	Hard clot
UP-33	-	-	-	-	+	Soft clot
UP-35	-	-	-	-	+	Hard clot
UP-36	-	-	-	+	+	Hard clot
UP-37	-	-	-	-	+	Hard clot
UP-39	-	-	-	-	+	Hard clot
UP-41	-	-	-	-	+	Hard clot
UP-42	-	-	-	-	+	Hard clot
UP-44	-	-	-	-	+	Hard clot
UP-57	-	-	-	-	+	Hard clot
UP-60	-	-	-	-	+	Hard clot
UP-61	-	-	-	+	+	Hard clot
UP-66	-	-	-	-	+	Hard clot

Table 4. Acid tolerance lactic acid bacteria isolated from tempoyak

Culture no.	Number of colony at pH 6.9 (log_{10} cfu/ml)	Number of colony at pH 3.0 (log_{10} cfu/ml)	Reduction of colony at pH 3.0 (log_{10} cfu/ml)
UP-1	7,03	5.43	1.60

Culture no.	Number of colony at pH 6.9 (log_{10} cfu/ml)	Number of colony at pH 3.0 (log_{10} cfu/ml)	Reduction of colony at pH 3.0 (log_{10} cfu/ml)
UP-2	8.65	6.65	2.00
UP-3	8.08	6.04	2.04
UP-5	7.29	4.94	2.35
UP-6	6.68	4.74	1.94
UP-7	7.57	5.96	1.61
UP-9	8.06	6.66	1.40
UP-11	8.04	5.44	2.60
UP-12	7.58	5.77	1.81
UP-13	7.68	5.77	1.94
UP-14	7.05	5.37	1.68
UP-15	7.48	5.16	2.32
UP-16	7.61	6.19	1.01
UP-18	8.51	6.15	2.36
UP-20	8.12	6.38	1.74
UP-21	7.85	5.58	2.27
UP-22	7.50	5.12	2.38
UP-23	7.89	6.15	1.74
UP-24	7.10	5.18	1.92
UP-25	7.44	5.67	1.77
UP-26	7.84	6.17	1.67
UP-28	7.82	5.31	2.51
UP-29	7.13	5.20	1.93
UP-30	8.08	6.05	2.03
UP-31	7.29	5.63	1.66
UP-32	7.39	5.45	1.94
UP-33	7.55	5.40	2.15
UP-35	7.32	6.05	1.27
UP-36	7.35	4.59	2.76
UP-37	6.68	4.90	1.78
UP-38	7.67	5.29	2.38
UP-39	7.69	4.87	2.82
UP-41	8.31	5.86	2.42
UP-42	7.31	5.21	2.10
UP-43	7.73	5.38	2.35
UP-44	8.22	6.56	1.66
UP-57	6.72	5.96	0.76
UP-60	7.88	6.24	1.64
UP-61	8.04	6.09	1.95
UP-64	8.44	6.05	2.39
UP-65	8.36	6.34	2.02
UP-66	7.26	6.24	1.02

Culture no.	Number of colony at pH 6.9 (log_{10} cfu/ml)	Number of colony at pH 3.0 (log_{10} cfu/ml)	Reduction of colony at pH 3.0 (log_{10} cfu/ml)
UP-71	6.86	5.41	1.45
UP-78	7.78	5.07	2.71

Table 5. Bile tolerance of lactic acid bacteria isolated from tempoyak

	Time required (h) to increase A_{620} nm by 0.3 units		
Culture no.	MRS-THIO without oxgall	MRS-THIO with oxgall	**LT* (h)**
UP-1	>9.0	>9.0	NM**
UP-2	>9.0	>9.0	NM**
UP-3	8.56	>9.0	NM**
UP-5	>9.0	>9.0	NM**
UP-6	>9.0	>9.0	NM**
UP-7	>9.0	>9.0	NM**
UP-9	4.34	6.96	2.62
UP-11	8.45	9.00	0.55
UP-12	>9.0	>9.0	NM**
UP-13	>9.0	>9.0	NM**
UP-14	7.52	7.95	0.43
UP-15	>9.0	>9.0	NM**
UP-16	>9.0	>9.0	NM**
UP-18	>9.0	>9.0	NM**
UP-20	>9.0	>9.0	NM**
UP-21	>9.0	>9.0	NM**
UP-22	>9.0	>9.0	NM**
UP-23	>9.0	>9.0	NM**
UP-24	>9.0	>9.0	NM**
UP-25	>9.0	>9.0	NM**
UP26	>9.0	>9.0	NM**
UP-28	>9.0	>9.0	NM**
UP-29	>9.0	>9.0	NM**
UP-30	>9.0	>9.0	NM**
UP-31	>9.0	>9.0	NM**
UP-32	>9.0	>9.0	NM**
UP-33	>9.0	>9.0	NM**
UP-35	>9.0	>9.0	NM**
UP-36	>9.0	>9.0	NM**
UP-37	>9.0	>9.0	NM**
UP-38	>9.0	>9.0	NM**
UP-39	>9.0	>9.0	NM**
UP-41	>9.0	>9.0	NM**
UP-42	>9.0	>9.0	NM**
UP-43	>9.0	>9.0	NM**
UP-44	>9.0	>9.0	NM**
UP-57	>9.0	>9.0	NM**

Culture no.	Time required (h) to increase A_{620} nm by 0.3 units		LT* (h)
	MRS-THIO without oxgall	MRS-THIO with oxgall	
UP-60	>9.0	>9.0	NM**
UP-61	7.17	>9.0	NM**
UP-64	>9.0	>9.0	NM**
UP-65	8.58	>9.0	NM**
UP-66	>9.0	>9.0	NM**
UP-71	>9.0	>9.0	NM**
UP-78	>9.0	>9.0	NM**

*Difference in lag time (LT) required for the cultures to reach absorbance at 620 nm by 0.3 units in MRS-THIO broth supplemented with 0.3% oxgall or without oxgall; **Absorbance not measured because time required for the cultures to reach absorbance at 620 nm by 0.3 units in MRS-THIO broth supplemented with 0.3% oxgall or without oxgall was more than 9 h.

Table 6. Deconjugation of taurocholic acid by lactic acid bacteria isolated from tempoyak

Culture no.	Broth pH*	Cholic acid released**(µmol/ml)
UP-9	4.52 ± 0.34	0.26 ± 0.35
UP-11	4.23 ± 0.05	0.04± 0.25
UP-14	4.06 ± 0.05	-0.04 ± 0.18

*pH of medium after incubation at 37°C for 18 h; **All cultures were grown in MRS-THIO broth (MRS broth supplemented with 0.2% sodium thioglycholate) supplemented with 0.2% sodium taurocholate

Table 7. Binding of cholesterol by lactic acid bacteria isolated from tempoyak

Culture no.	Broth pH*	Binding of cholesterol**(%)
UP-9	4.44 ± 0.03	-1.26 ± 8.03
UP-11	5.27 ± 0.13	17.86 ± 3.86
UP-14	4.51 ± 0.11	-0.07 ± 3.58

*pH of medium after incubation at 37°C for 18 h; **All cultures were grown in MRSO broth (MRS broth supplemented with 0.2% sodium thioglycholate and 0.3% oxgall) supplemented with sheep serum to obtain a concenntration of cholesterol of 100 µg/ml.

Acknowledgements

We sincerely thank the International Foundation for Science (IFS) for the research grant. We also thank Armanusah, Rahmayuni, Ahmad Tarmizi, Joni HAS and Jamsiah for their laboratory assistance.

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(Received 30 September 2013;accepted 22 December 2013)