

Bile and Acid Tolerance of Lactic Acid Bacteria Isolated from Dadih and Their Antimutagenicity against Mutagenic Heated Tauco*

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ABSTRACT : Antimutagenicity of milk cultured with lactic acid bacteria isolated from dadih on the mutagenicity of heated salty and sweet tauco was examined using streptomycin dependent (SD) 510 strain of *Salmonella typhimurium* TA 98 as a tester culture. Cultured milk samples exhibited widely antimutagenic activity against mutagenic heated salty and sweet tauco. *Lc. lactis* subsp. *lactis* R-22, *Lc. lactis* subsp. *casei* R-35, *Lc. lactis* subsp. *casei* R-52 and *E. faecalis* subsp. *liquefaciens* R-55 exhibited no inhibitory effect on the mutagenic heated salty tauco. Mutagenicity of heated sweet tauco was inhibited by cultured milks stronger than that of heated salty tauco. Milk cultured with *Lc. lactis* subsp. *cremoris* R-48, *Leuc. mesentroides* R-51 and *Lc. lactis* subsp. *casei* R-68 showed high inhibition against the mutagenicity of both heated salty and sweet taucos. Antimutagenic activity of the cultured milks against mutagenic heated tauco was attributed to the bacterial cells. Among the three strains which showed high antimutagenicity, only *Leuc. mesentroides* R-51 was tolerant to both acid and bile; so this strain can be used as probiotic in preventing the occurrence of mutagenesis caused by mutagenic heated food like tauco. (*Asian-Aust. J. Anim. Sci.* 2003, Vol 16, No. 11 : 1680-1685)

Key Words : Bile and Acid Tolerance, Antimutagenicity, Dadih, Tauco

INTRODUCTION

Several studies in the past decade have indicated that lactic acid bacteria (LAB) and their fermented products impart both nutritional and therapeutic benefits including antimutagenicity and anticarcinogenicity (Hosono et al., 1986; Hosono et al., 1990; Fernandes et al., 1987; Gilliland, 1990). Antimutagenic properties of LAB and their fermented milk products against some heterocyclic amines, *N*-nitroso compounds and fecal mutagens from various mammals have been intensively studied by Hosono and his co-workers (Hosono et al., 1986a,b,c; 1990; Thyagaraja and Hosono, 1993; Sreekumar and Hosono, 1998). Other investigators also have reported desmutagenic activity of fermented milks against mutagens and promutagens in various microbial and mammalian cell-based test systems (Bodana and Rao, 1990; Renner and Munzner, 1991; Nadathur et al., 1994).

Taucu is one of the traditional fermented foods which have a very good role in Indonesian diet as a source of protein especially in West Java. Taucu is usually made from yellow soybean and used as a flavoring agent in Indonesian cuisine due to its specific meat-like flavor (Usman and Hosono, 1996). Since taucu is consumed after heat treatment applied during cooking, we have carried out an experiment on taucu heated at various heating times and temperatures and observed that mutagenicity of taucu

increased with increase in time and temperature (Usman and Hosono, 1997).

LAB isolated from "dadih", an Indonesian traditional fermented had high antimutagenic activities toward various mutagens such as *N*-nitroso-dimethylamine (NDMA), *N*-nitroso-diethylamine (NDEA), *N*-nitroso-piperidine (NPIP) and *N*-nitroso-pyrrolidine (NPYR) as well as mutagenic heated foods such as terasi and terasi starter (Hosono et al., 1990; Surono and Hosono, 1996). To our knowledge, no finding has been published on the antimutagenicity of LAB isolated from dadih against the mutagenicity of heated taucu. To date, dadih is mainly consumed by people living in West Sumatera. Also this product is not commercially produced and marketed in other part of Indonesia yet. Moreover, many Indonesians do not know the potential health benefits of dadih and other fermented milk products. Hence it is necessary to study this matter since both dadih and taucu are traditional fermented products in Indonesia.

LAB for use as a probiotic culture or as food adjunct must be tolerant to acid and bile which enables a selected strain to survive, grow, and perform its therapeutic benefits in the intestinal tract (Gilliland and Walker, 1989; Salmunen and von Wright, 1993; Usman and Hosono, 1999). Some dadih LAB strains have been reported to perform *in vitro* acid and bile tolerance (Surono, 2003). Gilliland et al. (1984) reported that a culture of *L. acidophilus* with high bile tolerance is significantly better than a strain with low bile tolerance for increasing the number of facultative lactobacilli in the upper part of the small intestines of calves.

This study aims to assess the acid and bile tolerance of dadih's LAB and to evaluate the antimutagenicity of milk cultured with dadih LAB against mutagenic heated taucu.

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Table 1. Effect of bile salt on the growth rate of lactic acid bacteria isolated from dadih

Lactic acid bacteria	Time (h) required to increase absorbance at 620 nm by 0.3 unit		TL ¹
	MRS-THIO (without oxgall)	MRS-THIO (with oxgall)	
<i>Leuc. paramesentroides</i> R-51	5.61 ^{ab}	5.65 ^{bc}	0.05 ^d
<i>Lc. lactis</i> subsp. <i>lactis</i> R-22	5.50 ^{abc}	5.71 ^b	0.21 ^{cd}
<i>Lc. lactis</i> subsp. <i>casei</i> R-52	4.43 ^d	4.87 ^d	0.44 ^{cd}
<i>Lc. lactis</i> subsp. <i>casei</i> R-35	5.83 ^a	6.43 ^a	0.60 ^c
<i>Leuc. paramesentroides</i> R-62	5.02 ^{bc}	5.84 ^b	0.82 ^{bc}
<i>Lc. lactis</i> subsp. <i>lactis</i> R-63	4.03 ^d	4.85 ^d	0.82 ^{bc}
<i>E. faecalis</i> subsp. <i>liquefaciens</i> R-55	3.37 ^e	4.26 ^e	0.89 ^{abc}
<i>Lc. lactis</i> subsp. <i>cremoris</i> R-48	4.58 ^{cd}	5.58 ^{bc}	1.00 ^{abc}
<i>Lc. lactis</i> subsp. <i>casei</i> R-68	4.41 ^d	5.47 ^{bc}	1.06 ^{ab}
<i>E. faecalis</i> subsp. <i>liquefaciens</i> R-56	3.37 ^e	4.63 ^d	1.26 ^a

^{a, b, c, d, e} Means in same column followed by different superscript letters differ ($p < 0.05$).

¹ Difference in time lag (TL) required for the cultures to reach absorbance at 620 nm by 0.3 units in the media supplemented with or without 0.3% oxgall.

MATERIALS AND METHODS

Source and maintenance of cultures

The ten strains of LAB isolated from dadih used in this study were obtained from the stock culture collection of Animal Product Microbiology, Graduate School of Agriculture, Shinshu University, Japan. All cultures were maintained by subculture in MRS broth using 1% inocula and 18 to 20 h of incubation at 37°C; cultures were stored at 4°C between transfer. Each culture was subcultured twice in MRS broth prior to experimental use.

Assay for bile tolerance

To study the effect of bile salts on the growth rate of LAB, a method described by Walker and Gilliland (1993) was employed. All cultures were evaluated for growth in MRS-THIO broth (MRS supplemented with 0.2% sodium thioglycolate) with and without 0.3% oxgall. Freshly prepared cultures were inoculated (1%) into each medium, incubated at 37°C in a waterbath, and monitored hourly for growth spectrometrically at 620 nm. The growth was followed for 9 h or until a 0.3 unit difference in absorbance was reached. The effect was measured on the basis of 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 (h) between the culture media was considered as the lag time (LT).

Assay for acid tolerance

Washed cell pellets of LAB were resuspended in sterile distilled water and the absorbance at 625 nm was adjusted at 0.7 for each culture. Cell suspensions at the level of 2% were inoculated into each of 10 ml of 2% NDM that had been adjusted to pH 2.5 by 0.1 N HCl and pH 6.9 (control; without pH adjustment). The mixtures were incubated at 37°C for 2 h. Immediately after incubation period, 1 ml of suspended cells were diluted with 9 ml of 66 mM phosphate buffered saline (PBS), pH 6.8 and mixed uniformly with a

vortex mixer. Subsequent serial dilutions were made and plated by the spread-plate method with MRS agar. The plates were incubated at 37°C for 48 h before enumeration. The experiments were repeated two times with duplicates each time. The means of data from two trials were presented.

Assay for antimutagenic activity

Salmonella typhimurium SD 510, a strain derived from *S. typhimurium* TA 98 (1986) was used for antimutagenicity assay. The tester strain SD 510 was grown in Oxoid nutrient broth number 2 (Unipath, Basington, UK), fortified with streptomycin at a final concentration of 20 µg/ml (SM20 broth) overnight to an optical density of 1.3 at 660 nm (5×10^8 cfu/ml) in a shaking waterbath at 37°C. Antimutagenicity assay was performed according to the plate incorporation methods of Maron and Ames (1983) and Hosono et al. (1986). Briefly, 30 µl of tauco extracts were mixed with either (a) 100 µl of fermented milks or viable cells (2.0 mg/100 µl sterile distilled water), (b) 300 µl of PBS, and with (c) 100 µl of the tester culture (SD510 strain) diluted 10^{-4} with PBS. After preincubation at 37°C for 30 min, 2 ml soft agar (nutrient broth with 0.5% agar) maintained at 45°C were added, mixed by gently vortexing and poured onto an Oxoid plate (nutrient broth with 1.5% agar). All plates were incubated at 37°C for 48 h and the number of streptomycin-independent revertants was scored. Positive control contained the tester culture, distilled water instead of fermented milk and tauco extract. Negative control consisted of the tester culture, fermented milks of LAB, and only sterile ethanol instead of mutagen. The antimutagenicity of each fermented milk was estimated by measuring the extent of the decrease in mutation induced by tauco extract and expressed as percentage inhibition, calculated by the formula:

$$\text{Inhibition (\%)} = 100 - \frac{\text{revertants in plate of test sample}}{\text{revertants in plate of control}} \times 100$$

Table 2. Acid tolerance of lactic acid bacteria isolated from dadih

Lactic acid bacteria	log cfu/ml at pH	
	6.9	2.5
<i>Lc. lactis</i> subsp. <i>casei</i> R-35	6.05 ^b	ND ¹
<i>E. faecalis</i> subsp. <i>liquefaciens</i> R-55	6.87 ^a	ND
<i>Lc. lactis</i> subsp. <i>casei</i> R-52	6.92 ^a	ND
<i>Lc. lactis</i> subsp. <i>lactis</i> R-63	*6.84 ^{ab}	*0.74 ^d
<i>Lc. lactis</i> subsp. <i>cremoris</i> R-48	*6.22 ^b	*0.80 ^d
<i>Leuc. paramesentroides</i> R-51	*5.75 ^b	*2.39 ^c
<i>Lc. lactis</i> subsp. <i>lactis</i> R-22	*5.91 ^b	*2.63 ^c
<i>Leuc. paramesentroides</i> R-62	*5.98 ^b	*2.70 ^{bc}
<i>E. faecalis</i> subsp. <i>liquefaciens</i> R-56	*5.49 ^b	*3.37 ^b
<i>Lc. lactis</i> subsp. <i>casei</i> R-68	*6.19 ^b	*4.02 ^a

^{a, b, c, d} Means in same column followed by different superscript letters differ ($p < 0.05$). ^{*, **} Means in same row followed by different superscript letters differ ($p < 0.05$). ¹ ND = Not detected.

The experiments were repeated two times with duplicates each time. The means of the two trials were presented.

Statistical analysis

Bile and acid tolerance as well as antimutagenicity of LAB were analyzed by the ANOVA procedure from StatView (Haycock et al., 1992). The least significant difference procedure was used to determine whether statistically significant differences occurred among means.

RESULTS

Bile tolerance of dadih's lactic acid bacteria

Table 1 shows the effect of bile salt on the growth of the cultures arranged in order of decreasing LT. There was a significant variation in LT among the cultures. *Leuc. mesentroides* R-51 with LT of 0.05 h was the most bile-tolerant ($p < 0.05$) but was not ($p > 0.05$) more tolerant than *Lc. lactis* subsp. *lactis* R-22 and *Lc. lactis* subsp. *lactis* R-52. *E. faecalis* subsp. *liquefaciens* R-56 with an LT of 1.26 h was the most sensitive strain to bile acid but exhibited no significant difference from *E. faecalis* subsp. *liquefaciens*

R-55, *L. casei* subsp. *casei* R-68 and *Lc. lactis* subsp. *cremoris* R-48.

Acid tolerance of dadih's lactic acid bacteria

Acid tolerance of LAB isolated from dadih is shown in Table 2. After incubation for 2 h at pH 2.5, the viability of *Leuc. paramesentroides* R-51, *Lc. lactis* subsp. *lactis* R-22, *Leuc. paramesentroides* R-62, *E. faecalis* subsp. *liquefaciens* R-56 and *L. casei* subsp. *casei* R-68 decreased significantly ($p < 0.05$) by 2 to 4 log cycles while *Lc. lactis* subsp. *lactis* R-63 and *Lc. lactis* subsp. *cremoris* R-48 decreased ($p < 0.05$) by 5 to 6 log cycles. *Lc. lactis* subsp. *casei* R-35, *E. faecalis* subsp. *liquefaciens* R-55 and *Lc. lactis* subsp. *casei* R-52 could not survive at the same conditions.

Antimutagenicity of dadih's lactic acid bacteria

Antimutagenicity of milk cultured with LAB isolated from dadih against mutagenic heated sweet and salty tauco samples was examined. Tables 3 and 4 show the number of revertants and inhibition percentages when 24 h cultured milk was preincubated with ethanolic extract of heated sweet or salty tauco samples. In general, milk cultured with dadih's LAB displayed a higher antimutagenic activity against mutagenic heated sweet tauco than salty tauco. Milk cultured with *Lc. lactis* subsp. *cremoris* R-48, *Leuc. paramesentroides* R-51 and *L. casei* subsp. *casei* R-68 exhibited higher antimutagenicity in both heated sweet and salty taucos. Cultured milk of *Lc. lactis* subsp. *lactis* R-22, *Lc. lactis* subsp. *casei* R-35, *E. faecalis* subsp. *liquefaciens* R-55 and *Lc. lactis* subsp. *casei* R-52 had no inhibitory effect on heated salty tauco (Table 3), and all these LAB except *Lc. lactis* subsp. *casei* R-35 exhibited lower antimutagenic activity against heated sweet tauco (Table 4).

Since milk cultured with *Lc. lactis* subsp. *cremoris* R-48 displayed high antimutagenic activity against both heated sweet and salty taucos, this strain was chosen to study the component in cultured milk that was involved in the antimutagenic activity. Using 2.0 mg of freeze-dried cells of *Lc. lactis* subsp. *cremoris* R-48 that contained

Table 3. Antimutagenicity of milk cultured with lactic acid bacteria isoated from dadih against mutagenic heated salty tauco

Treatment	Number of revertants/plate	Inhibition (%)
SLT ¹ only	471	-
SLT+ <i>Lc. lactis</i> subsp. <i>lactis</i> R-22	595	NI ²
SLT+ <i>Lc. lactis</i> subsp. <i>casei</i> R-35	583	NI
SLT+ <i>E. faecalis</i> subsp. <i>liquefaciens</i> R-55	533	NI
SLT+ <i>Lc. lactis</i> subsp. <i>casei</i> R-52	516	NI
SLT+ <i>E. faecalis</i> subsp. <i>liquefaciens</i> R-56	202	57.1 ^b
SLT+ <i>Leuc. paramesentroides</i> R-62	178	62.2 ^b
SLT+ <i>Lc. lactis</i> subsp. <i>lactis</i> R-63	168	64.3 ^b
SLT+ <i>Lc. lactis</i> subsp. <i>casei</i> R-68	80	83.1 ^a
SLT+ <i>Leuc. paramesentroides</i> R-51	40	91.7 ^a
SLT+ <i>Lc. lactis</i> subsp. <i>cremoris</i> R-48	22	95.3 ^a

^{a, b} Means in same column followed by different superscript letters differ ($p < 0.05$). ¹ SLT=Salty tauco. ² NI=No inhibition.

Table 4. Antimutagenicity of milk cultured with lactic acid bacteria isolated from dadih against mutagenic heated sweet tauco

Treatment	Number of revertants/plate	Inhibition (%)
SWT ¹ only	1,023	-
SWT+ <i>Lc. lactis</i> subsp. <i>lactis</i> R-22	846	17.3 ^d
SWT+ <i>E. faecalis</i> subsp. <i>liquefaciens</i> R-55	534	47.8 ^{cd}
SWT+ <i>Lc. lactis</i> subsp. <i>casei</i> R-52	531	48.1 ^c
SWT+ <i>Lc. lactis</i> subsp. <i>casei</i> R-35	180	82.5 ^b
SWT+ <i>E. faecalis</i> subsp. <i>liquefaciens</i> R-56	175	83.0 ^{ab}
SWT+ <i>Lc. lactis</i> subsp. <i>casei</i> R-68	69	93.3 ^a
SWT+ <i>Lc. lactis</i> subsp. <i>lactis</i> R-63	46	95.6 ^a
SWT+ <i>Lc. lactis</i> subsp. <i>cremoris</i> R-48	30	97.1 ^a
SWT+ <i>Leuc. paramesentroides</i> R-62	28	97.3 ^a
SWT+ <i>Leuc. paramesentroides</i> R-51	1	99.9 ^a

^{a, b, c, d} Means in same column followed by different superscript letters differ ($p < 0.05$). ¹ SWT = Sweet tauco.

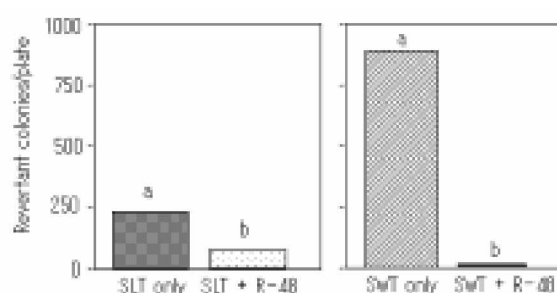


Figure 1. Antimutagenicity of freeze-dried cells of *Lc. lactis* subsp. *cremoris* R-48 against mutagenic heated tauco. SLT=salty tauco; SWT=sweet tauco.

approximately 1.9×10^5 cfu/ml of viable cells significantly ($p < 0.05$) inhibited the mutagenicity of both sweet and salty taucos (Figure 1).

DISCUSSION

We have reported in our previous study that the highest mutagenic activity were found for sweet tauco and salty tauco at a dose level of 30 mg/plate after heat treatment at 120°C for 45 min and 100°C for 60 min, respectively in the absence of metabolic activation (Usman and Hosono, 1997). Hence, these conditions were selected for the antimutagenicity assay. All milk cultured with dadih's LAB displayed desmutagenicity against mutagenic heated sweet tauco, although the degree of inhibitions toward heated salty and sweet tauco showed wide variation. Some strains such as *Lc. lactis* subsp. *casei* R-52, *E. faecalis* subsp. *liquefaciens* R-55 and *Lc. lactis* subsp. *lactis* R-22 which showed antimutagenic activity toward heated sweet tauco had no inhibitory against mutagenic heated salty tauco. This can be attributed to the characteristics of the strains and the difference in the mode of action or activation pathway of different mutagens and promutagens (Thyagaraja and Hosono, 1993; Nadathur et al., 1994). The mutagens produced during the heat treatment of the two taucos may also be different and their degree of mutagenicity was

different as can be seen from the difference in the number of revertants formed. Hosoda et al. (1992) have studied bacterial antimutagenicity of skim milk fermented by a number of lactic bacteria against *N*-methyl, *N*⁷-nitro, *N*-nitrosoguanidine, a direct-acting mutagen, and found that inhibition percentages greatly differed among the strains.

Results from the present study showed that freeze-dried cells strongly suppressed the mutagenicity of both taucos, indicating that LAB play an important role in inhibiting the mutagenicity. We have reported the same finding that freeze-dried cells of *L. acidophilus* had inhibitory effect on the mutagenicity induced by heated tauco (Usman and Hosono, 1998). Hosono et al. (1990) have shown antimutagenic effect of several LAB isolated from dadih against mutagenicity of volatile nitrosamines. Mutagenicities of various spice mutagens, heterocyclic amines and aflatoxins have been inhibited with several LAB isolated from "idly", a traditional cereal pulse product of India (Thyagaraja and Hosono, 1993). Hosono et al. (1987) suggested that antimutagenic substances reside in the cell wall of the LAB. Among three strains *Lc. lactis* subsp. *cremoris* R-48, *Leuc. paramesentroides* R-51 and *L. casei* subsp. *casei* R-68 which exhibited higher antimutagenic activity against both salty and sweet taucos, *Lc. lactis* subsp. *cremoris* R-48 and *Leuc. paramesentroides* R-51 reportedly have the same effect on various mutagens such as NDEA (Hosono et al., 1990), browned solution of cystein with glucose (Hosono and Shirai, 1996) and heated terasi and terasi starter (Surono and Hosono, 1996), suggesting that these strains may have relatively a broad spectrum of inhibition.

Bacteria would contact pH values ranging from 2.0 to 8.0 in the gastrointestinal tract if consumed (Hood and Zottola, 1988). Thus, probiotic cultures must survive in the environment with gastric and bile acids, when viable cells go through the gastrointestinal tract. Resisting at pH 3 for 2 h and growing in the medium containing 1,000 ppm of bile acids are considered as standards for acid and bile tolerance of probiotic culture (Itoh, 1992; Golran, 1994). Results from the present study showed that *Lc. lactis* subsp.

cremoris R-48 and *L. casei* subsp. *casei* R-68 showed higher inhibitory effect on the mutagenicity of both salty and sweet tauco. However these strains were sensitive to acid and bile. Thus, they could not be used as probiotic cultures. Meanwhile *Leuc. paramesentroides* R-51 was bile and acid tolerance, thus, this strain may survive under high acidity in the stomach and high concentration of bile components in the intestine when consumed. Further study is needed to evaluate *in vivo* experiments on the antimutagenicity of strain R-51.

Some compounds displaying mutagenic properties have been found and isolated from numerous cooked foods. These mutagenic compounds play an important role in the association shown to exist between diet and incidence of cancer in various digestive origins (Doll and Peto, 1981). Tauco is widely consumed as a flavoring agent in Java, the most populous island in Indonesia. On the other hand, average consumption of fermented milk products like dadih by the Indonesians is low due to the acidic taste of the products. Moreover, the beneficial health effect of dadih and its lactic acid bacteria is not understood well by some Indonesians yet. Therefore, results from the present study are expected to encourage Indonesians to consume more milk products, as it was proven that dadih exhibited antimutagenic activities against the mutagenicity induced by heated tauco.

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REFERENCES

- Bodana, A. R. and D. R. Rao. 1990. Antimutagenic activity of milk fermented by *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. *J. Dairy Sci.* 73:3379-3384.
- Doll, R. and R. Peto. 1981. The causes of cancer: quantitative estimate of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* 66:1195-1308.
- Fernandes, C. F., K. M. Shahani and M. A. Amer. 1987. Therapeutic role of dietary lactobacilli and lactobacillic fermented dairy products. *FEMS Microbiol. Rev.* 46:343-356.
- Gilliland, S. E. 1990. Health and nutritional benefits from lactic acid bacteria. *FEMS Microbiol. Rev.* 87:175-188.
- Gilliland, S. E., T. E. Staley and L. J. Bush. 1984. Importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. *J. Dairy Sci.* 67:3045-3051.
- Gilliland, S. E. and D. K. Walker. 1989. Factor to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. *J. Dairy Sci.* 73:905-911.
- Gohran, M. 1994. *Lactobacillus* with the ability of colonization and establishing in the intestinal tracts. Japan Patent 6-501624.
- Hood, S. K. and E. A. Zottola. 1988. Effect of low pH on the ability of *Lactobacillus acidophilus* to survive and adhere to human intestinal cells. *J. Food Sci.* 53:1514-1516.
- Hosoda, M., H. Hashimoto, H. Morita, M. Chiba and A. Hosono. 1992. Antimutagenicity of milk cultured with lactic acid bacteria against N-methyl, N'-nitro, N-nitrosoguanidine. *J. Dairy Sci.* 75:976-981.
- Hosono, A., T. Kashina and T. Kada. 1986a. Antimutagenic properties of lactic acid-cultured milk on chemical and fecal mutagens. *J. Dairy Sci.* 69:2237-2242.
- Hosono, A., E. Omote, Y. Izawa, F. Tokita and T. Kada. 1986b. Isolation of spontaneous induced-streptomycin dependent mutants of *Salmonella typhimurium* TA 98 and TA 100 strains and desmutagenicity tests *in vitro* of cultured milk. *Lebensm.-Wiss. u-Technol.* 19:161-163.
- Hosono, A., S. Sagae and F. Tokita. 1986c. Desmutagenic effect of cultured milk on chemically induced mutagenesis in *Escherichia coli* B/r WP2 trp- her-. *Milchwissenschaft* 41:142-145.
- Hosono, A., A. Yoshimura and H. Otani. 1987. Antimutagenic activity of cellular component of *Streptococcus faecalis* IFO 12965. *Neth. Milk Dairy J.* 41:239-245.
- Hosono, A., R. Wardoyo and H. Otani. 1990. Inhibitory effects of lactic acid bacteria from fermented milk on the mutagenicities of volatile nitrosamines. *Agric. Biol. Chem.* 54:1639-1643.
- Hosono, A. and H. Shirai. 1996. Antimutagenicity of milk cultured with lactic acid bacteria against the heated solution of cysteine-glucose. *Anim. Sci. Technol. (Jpn.)* 67:1076-1081.
- Itoh, T. 1992. Functional benefits from lactic acid bacteria used in cultured milk. *Anim. Sci. Technol. (Jpn.)* 63:1276-1289.
- Maron, D. M. and B. N. Ames. 1983. Revised methods for the Salmonella mutagenicity test. *Mutat. Res.* 113:173-215.
- Nadathur, A. R. and S. J. Gould and A. T. Bakalinsky. 1994. Antimutagenicity of fermented milk. *J. Dairy Sci.* 77:3287-3295.
- Renner, H. W. and R. Munzner. 1991. The possible role of probiotics as dietary antimutagens. *Mutat. Res.* 262:239-245.
- Salminen, S. and A. von Wright. 1993. Lactic acid bacteria. Marcel Dekker, Inc., New York, Basel, Hong Kong.
- Sreekumar, O. and A. Hosono. 1998. Antimutagenicity and the influence of physical factors in binding *Lactobacillus acidophilus* and *Bifidobacterium longum* cells to amino acid pyrolysates. *J. Dairy Sci.* 81:1508-1516.
- Surono, I. S. and A. Hosono. 1996. Antimutagenicity of milk cultured with lactic acid bacteria from dadih against mutagenic terasi. *Milchwissenschaft* 51:493-497.
- Surono, I. S. 2003. *In vitro* probiotic properties of indigenous dadih lactic acid bacteria. *Asian-Aust. J. Anim. Sci.* 16:726-731.
- Thyagaraja, N. and A. Hosono. 1993. Antimutagenicity of lactic acid bacteria from idly against food-related mutagens. *J. Food Prot.* 56:1061-1066.
- Usman and A. Hosono. 1996. Chemical and bacteriological composition of tauco, a traditional fermented food in Indonesia.

- Quest Tradit. Food 16:41-50.
- Usman and A. Hosono. 1997. Antimutagenic activity of Maillard reaction products against mutagenic heated tauco. Ital. J. Food Sci. 4:268-276.
- Usman and A. Hosono. 1998. Desmutagenicity of milk cultured with *Lactobacillus acidophilus* against mutagenic heated tauco. Food Chem. Toxicol. 36:805-810.
- Usman and A. Hosono. 1999. Bile tolerance, taurocholate deconjugation and binding of cholesterol by *Lactobacillus gasseri* strains. J. Dairy Sci. 82:243-248.
- Walker, D. R. and S. E. Gilliland. 1993. Relationship among bile tolerance, bile salt deconjugation, and assimilation of cholesterol by *Lactobacillus acidophilus*. J. Dairy Sci. 76:956-961.