Jurnal Sains Kesihatan Malaysia Isu Khas 2018: 23-26
DOI : http://dx.doi.org./10.17576/JSKM-2018-04

Mutagenicity and Antimutagenic Activities of Lactic Acid Bacteria (LAB) Isolated from Fermented Durian (Tempoyak)

(Aktiviti Mutagenik dan Antimutagenik Bakteria Asid Laktik yang Dipencilkan daripada Fermentasi Durian (Tempoyak))

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ABSTRACT

Mutagenic and antimutagenic activities of lactic acid bacteria (LAB) Lactobacillus plantarum isolated from the local fermented durian (tempoyak) was determined by Ames test (Salmonella/microsome mutagenicity assay). Our study also involved pre-incubation assay against Salmonella typhimurium TA 98 and TA 100 bacterial strain in the presence and absence of metabolic activator S9 system. It was found that the L. plantarum showed no mutagenic activity on both S. typhimurium strain TA 98 and TA 100 in the presence and absence of metabolic activator. Significant antimutagenic activity ($p < 0.05$) was observed in both cell-free supernatant and bacterial cell suspension of L. plantarum as compared to the mutagenicity induced by 2-Aminoanthracene in the presence of metabolic activator. Meanwhile, in the absence of metabolic activator, only the bacterial cells of L. plantarum showed antimutagenicity acitivity against Sodium Azide and 2-Nitrofluorene. In conclusion, L. plantarum could play a vital role as chemopreventive agent by binding to mutagens and suppressing mutagenesis. Thus, L. plantarum could be consider as a good candidate for functional food development as a supplement product to prevent development of colon cancer.

Keywords: Mutagenicity; antimutagenicity; Lactobacillus plantarum; lactic acid bacteria; Ames test; tempoyak

ABSTRAK

Aktiviti mutagenisiti dan antimutagenisiti bakteria asid laktik (LAB) Lactobacillus plantarum yang dipencilkan daripada durian yang difermentasi (tempoyak) telah dinilai melalui Ujian Ames (Salmonella/asai mutagenisiti mikrosom). Kajian kami juga melibatkan asai pra-inkubasi dengan Salmonella typhimurium TA 98 dan TA 100 dengan keadaan kehadiran dan ketidakhadiran sistem pengaktifan metabolik S9. Hasil kajian kami menunjukkan L. plantarum tidak mempunyai sebarang kesan mutagenik terhadap kedua-dua TA 98 dan TA 100 dalam kedua-dua keadaan ujian. Aktiviti antimutagenik yang signifikan ($p < 0.05$) dilihat pada kedua-dua supernatan bebas sel dan juga suspensi sel bakteria L. plantarum berbanding mutagenisiti yang diaruh oleh 2-aminoanthracene pada asai dengan kehadiran sistem pengaktifan metabolik. Manakala pada keadaan ketidakhadiran sistem pengaktifan metabolic pula, hanya suspensi sel bakteria L. plantarum menunjukkan kesan antimutagenisiti terhadap Sodium Azide dan 2-Nitrofluorene. Sebagai kesimpulan, L. plantarum boleh memainkan peranan penting sebagai ejen kemoprevensi dengan mengikat mutagen dan menghalang mutagenesis. Oleh itu, L. plantarum berpotensi untuk dibangunkan sebagai makanan berfungsi seterusnya produk suplementasi bagi mencegah perkembangan kanser usus.

Kata kunci: Mutagenisiti; antimutagenisiti; Lactobacillus plantarum; bakteria asid laktik; Ujian Ames; tempoyak

INTRODUCTION

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries (WHO 2008). Colon cancer is one of the leading causes of cancer death too. Dietary intake pattern that is high red meat and low in vegetables are highly associated with colon cancer (Slattery et al. 1998). The complex web of gut microbiota in the human intestines may play a crucial function for both human health and diseases. Production of microbial enzymes such as azoreductase, nitroreductase and β-glucuronidase by the microbiota are capable to mediate carcinogen production in human colon through metabolic activation of dietary component (Commane et al. 2005). Probiotic defined as live microbial food ingredients (supplements) confer beneficial health effects to the host upon ingestion in adequate amounts (Salminen & Wright 1998). They are beneficial bacteria that may alter the gut microbiota by promoting good digestion, inhibiting the growth of harmful bacteria as well as by reducing levels of microbial enzymes, boosting immune function and increase resistance towards food-borne infection (Helland et al. 2004). Previous *in vitro* studies suggested that some probiotic strain might exert potential to protect against colon cancer, through several mechanisms such as, modifications of metabolic activities of gut microbiota, alteration of gut microbiota, reduction of possible pro-carcinogens in colon, production of anti-tumor and anti-mutagenicity compounds (Kumar et al. 2010). Studies had reported the antimutagenic activities

of lactic acid bacteria from various fermented food sources (Ahmadi et al. 2014; Park et al. 1998; Asahara et al. 1992). The aim of the present study was to investigate the antimutagenic potential of *Lactobacillus plantarum* isolated from tempoyak (fermented *Durio zibethinus*) a traditional condiment in Malaysia against Sodium Azide, 2-Aminoanthracene and 2-nitroflourene in TA 98 and TA 100 strain of *Salmonella typhimurium* using Ames test.

MATERIALS AND METHODS

BACTERIAL STRAIN AND GROWTH CONDITIONS

Lactobacillus plantarum was isolated from fermented durian (*Durio zibethinus*) at Department of Chemical & Process Engineering, Faculty of Engineering & Built Environment, Universiti Kebangsaan Malaysia, Bangi Selangor, Malaysia. Strain was stored at -80°C in de Man Rogosa (MRS) broth (Oxoid Ltd, Hampshire, England), supplemented with 20% (v/v) glycerol. As the routine analysis, *L. plantarum* was subcultured twice in MRS broth (Oxoid Ltd, Hampshire, England) and MRS agar (de Man Rogosa, Merckmilipore, Darmstadt, Germany) at 37°C for 16 hours to get 8.0 log cfu /ml before every experiment was conducted. Bacterial cells were then harvested and washed by centrifugation (10000 rpm, 10 min) and then re-suspended in phosphate buffer saline (PBS, 2M, pH 7.4), while the cell-free supernatant (CFS) was filtered using filter membrane 0.22µM. These samples were then immediately subjected to mutagenicity and antimutagenicity assay.

Salmonella typhimurium strain TA 98 and TA 100 were used for the Ames assay. Test of histidine requirement, *rfa* mutation, *uvr*B mutation and R-factor were also performed to confirm the genotypes of both as previously described by Maron and Ames (1983). *Salmonella typhimurium* TA 98 is a frame shift strain which contain the his3052 mutation and *Salmonella typhimurium* TA 100 contain the base-pair substitution mutation hisG46. Prior to each mutagenicity and antimutagenicity test, *Salmonella typhimurium* strain TA 98 and TA 100 was grown in fresh nutrient broth No. 2 (Oxoid, Basingstoke, Hamsphire, England) at 37°C for 12-16 hour.

MUTAGENS AND S9 MIX

Sodium Azide (NaN_3), 2-Nitrofluorene (2-NF) and 2-aminoanthracene (2-AA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and dissolved in dimethyl-sulfoxide (DMSO). An S9 microsomal fraction (S9 mix) of rat liver obtained from MOLTOX, Inc. (USA).

MUTAGENICITY AND ANTIMUTAGENICITY ACTIVITY ASSAY

Mutagenic and antimutagenic activities of *L. plantarum* were determined using the pre-incubation method of Maron and Ames (1983) with minor modification. For antimutagenicity assay, overnight culture of *Salmonella typhimurium* strain TA 98 or TA 100 (0.1 ml) in a tube was added with cell suspension or culture medium of *L. plantarum* (0.1 ml), PBS (0.5 ml) (without microsomal activation) or S9 mix (microsomal activation) following 20 minutes of incubation at 37°C in the rotary shaker. Then 2.0 ml top agar supplemented with 0.5 mM L-histidine/D-biotin and mutagen such as 2-AA, 2-NF and NaN_3 were mixed with the tube content. The tubes were vortexed and poured onto the minimal glucose agar plates and the reverted colonies were counted after 48 hours of incubation at 37°C.

Percent of inhibition of mutagenicity (%) was determined by the following (Negi et al. 2003).

$$\text{Inhibition rate (\%)} = 1 - (T/M) \times 100\ \%$$

Where T, is the number of revertants per plate in the presence of mutagen and bacterial sample and M is the number of revertants per plate in positive control. No antimutagenic effect was considered to give a value smaller than 25%, a moderate effect value between 25 and 40% and a strong antimutagenicity value greater than 40%. Mutagenicity of *L. plantarum* was examined under the condition described for antimutagenicity testing except without the addition of mutagens.

STATISTICAL ANALYSIS

The results were expressed as mean ± SEM of three independent experiments. Data analysis was carried out by the Independent t-test. P values < 0.05 were considered to be significant.

RESULTS AND DISCUSSION

As shown in Table 1, the number of revertants per plate with and without the presence of metabolic activation system were less than twice that of spontaneous revertants (negative control). Both bacterial cells and cell-free supernatant (CFS) of *L. plantarum* had no mutagenic effect on both *Salmonella typhimurium* TA 98 and TA 100. The absence of mutagenic activities from *L. plantarum* indicated that it would be developed further for the use in nutraceuticals.

As shown in Table 2. The antimutagenic activity of *L. plantarum* with and without the presence of activation system against NaN_3, 2-NF and 2-AA. The percentage of antimutagenicity *L. plantarum* against 2-NF in TA 98 and NaN_3 in TA 100 on assay without the presence of metabolic activation were 28.2% and 72% for bacterial cells, while there was no inhibition by CFS in TA 98 and only 18% inhibition rate in TA 100. Meanwhile, in the presence of metabolic activation, the antimutagenic activity against 2-AA in both TA 98 and TA 100 were 93% and 50% for bacterial cells and 91.7% and 36% for CFS.

TABLE 1. Mutagenic activities of *L. plantarum* without metabolic activation S9 (- S9) and with metabolic activation S9 (+ S9)

Strain	Samples	Number of Revertants per plate	
		Mean ± SEM (-S9)	Mean ± SEM (+S9)
TA 98	Control (PBS)	15.33 ± 1.45	16.44 ± 2.62
TA 98	Bacterial Cells	19 ± 2.08	13.33 ± 0.33
TA 98	Control (MRS)	25.33 ± 4.25	23.44 ± 0.22
TA 98	CFS	39.67 ± 0.88	25.11 ± 3.63
TA 100	Control (PBS)	68.67 ± 4.33	169.22 ± 9. 63
TA 100	Bacterial Cells	50.15 ± 1.15	170.33 ± 1.07
TA 100	Control (MRS)	84 ± 7.23	234.11 ± 2.38
TA 100	CFS	55.33 ± 3.71	205.11 17. 46

Results are presented as mean ± SEM for three plates. (n = 3)

TABLE 2. Antimutagenic activities of *L. plantarum* without metabolic activation S9 (- S9) and with metabolic activation S9 (+ S9)

Strain	Samples	Mean ± SEM (-S9)	Inhibition rate (%)	Mean ± SEM (+S9)	Inhibition rate (%)
TA 98	Positive Control	374 ± 12	-	332 ± 13	-
TA 98	Bacterial Cells	263 ± 19a	28.2	23 ± 5a	93
TA 98	Positive Control	335 ± 42	-	340 ± 8	-
TA 98	CFS	409 ± 38	ND	28 ± 5a	91.7
TA 100	Positive Control	471 ± 9	-	245 ± 16	-
TA 100	Bacterial Cells	413 ± 20a	72	121 ± 4a	50
TA 100	Positive Control	478 ± 14	-	236 ± 18	-
TA 100	CFS	203 ± 9	18	150 ± 17a	36

Results are presented as mean ± SEM for three plates.(n=3) [a] Statistically significant differences compared to positive control value at $p < 0.05$, ND; not detected

Colon carcinogenesis is a multistep process which starts from initiation process with the occurrence of DNA mutation (Cooper 2000). DNA mutation either happens spontaneously or is chemically induced and leads to development of carcinogenesis if left untreated (Carr 1948). Ames mutagenicity assay is the basic test or model used to detect the mutagenic properties of a chemical or drug of interest. The bacterial strain of *Salmonella typhimurium* used in the assay carried mutation in histidine operon, thus are histidine dependent. Upon introduction of mutagenic substances, this bacterial strain will revert back into the wildtype strain. Meanwhile, non-mutagenic substances would not be able to revert back those strains. The strains used both carry different type of mutation. TA 98 carried frameshift mutation while TA 100 carried base pair mutations (Mortelmans & Zeiger 2000). In the present study, the mutagenicity and antimutagenicity activity of isolated lactic acid bacteria isolated from the fermented durian (tempoyak) had been reported. Our study was the first finding on the potential chemopreventive mechanism involved in lactic acid bacteria isolated from fermented durian (tempoyak).

Mutagenicity and carcinogenicity are often correlated (Griffiths 2000). Thus, mutation and cancer occurrence that arises from diet are actually highly preventable by consumption of dietary antimutagens (Ferguson 1994). Probiotic are considered as a potential antimutagen diet to help prevent colon carcinogenesis (Lim 2014). Mechanisms of antimutagenic activity of probiotic remain unclear. Previous study reported that the antimutagenic activity of LAB are highly related to the binding interaction between cell wall and cells' components with the mutagen and its metabolites (Lo et al. 2004). In *Lactobacillus plantarum* KLAB21, the antimutagenic activities was mediated by its extracellular excretion which consist of glycoproteins (Rhee & Park 2001). Meanwhile, LAB isolated from fermented cabbage (kimchi) a type of Korean traditional dish showed that the antimutagenic activities were mainly contributed to cell wall fraction rather than the cytosolic fraction (Park et al. 1998). The antimutagenic activities of LAB was also dependent to bacterial strain and mutagen used. Some *Lactobacillus* sp. exhibit inhibitory effect on 2-amino-3-methylimidazo[4,5-f]quinoline compared to *Streptococcus thermophilus* and *Bifidobacterium adolescentis* while in other experiment *Lactobacillus alimentarius* has a percentage of antimutagenicity of 65% against SA and 41% against benzo[a]pyrene (Tavan et al. 2002; Apas et al. 2014).

CONCLUSION

Our results demonstrated that *Lactobacillus plantarum* isolated from fermented durian (tempoyak) did not exerts any mutagenic effect towards both *Salmonella typhimurium* strains. It also had strong antimutagenic activity when co-incubated with 2-AA and NaN_3. The antimutagenicity of probiotic *Lactobacillus plantarum* observed in the present study implied its potential to be developed as a putative functional food as a chemopreventive agent.

ACKNOWLEDGMENTS

This study was supported by Fundamental Research Grant Scheme (FRGS/1/2014/SG03/UKM/02/2) granted by Ministry of Higher Education Malaysia (MOHE).

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Received: August 2017
Accepted for publication: January 2018