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Lactococcus lactis subsp. lactis J525-2の抗変異原性
Antimutagenic property of *Lactococcus lactis* subsp. *lactis* J525–2.

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Abstract

The purpose of this study was to evaluate a factor of an antimutagenic activity of *Lactococcus lactis* subsp. *lactis* J525–2. A fermented milk prepared with this strain showed antimutagenic activity against food-borne mutagens such as 3-amino-1,4-dimethyl-5H-pyrido indole (Trp-P–1), 2-amino-6-methylpyrido imidazole (Glu-P–1), and 2-amino-3,4-dimethyl-imidazo quinoline (MeIQ). Cultures of strain J525–2 showed high antimutagenic activity, but cell free culture showed little activity. These results suggest that extracellular products of strain J525–2 have no antimutagenic activity and the cells play major part of the antimutagenic activity. The binding of Trp-P–1 to cells of strain J525–2 was confirmed. Mutagen binding occurred instantaneously and reached maximum at 10 min incubation. Prolongation of incubation time (up to 50 min) had no influence on the binding. The binding ability was strongly affected by pH and the highest percentage binding was noted at pH 6.0. Cell of *Lactococcus lactis* subsp. *lactis* J525–2 was suggested to play a major role in the antimutagenic activity, and the cell could bind food-borne mutagens.

Introduction

Lactic acid bacteria (LAB) are used in many fermented foods, particularly fermented dairy products such as cheese, buttermilk, and fermented milk. Fuller1) was the first to propose the term “probiotic,” and recently, its definition was further refined to “living microorganisms, which upon ingestion in certain numbers, exert health benefits by improving intestinal microbial balance.” Probiotic foods belong to the functional food sector and probiotic LAB are a representative of live food ingredients that exert a beneficial effect on the host's health.

It is well known that mutagens of food origin are very common in our regular meals2). Various pyrolysis products from amino acids, proteins, and proteinaceous foods formed during heating are highly mutagenic3). Antimutagenic actions of fermented milk and LAB have been studied since 1980s. Antimutagenic activities of fermented milks were reported and some studies indicated that cell walls of some LAB strains could inactivate mutagenicities by binding with the mutagens4,5,6,7,8). The binding mode of LAB cells and mutagens was suggested to be ionic and/or hydrophobic bonds9,10,11,12). On the other hand, antimutagenic activities of slimy substance, polysaccharide, acetone extracted fermented milks, and LAB live cells were reported13,14,15,16,17). The products generated by some LAB can play a major role in antimutagenic actions.

We detected a strain that the fermented milk prepared with showed an antimutagenic activity, strain J525–2, in our screening of LAB strains isolated from dahi. Dahi is yogurt-like traditional fermented milk produced from cow and Buffalo milk in India and Bangladesh. The production method, quality, and microflora of dahi in Bangladesh have been studied previously at our laboratory18,19). The purpose of this study was to evaluate a factor of an antimutagenic activity of *Lactococcus lactis* subsp. *lactis* J525–2. This paper presents characteristics of the strain, the antimutagenic activity against food-borne mutagens of culture and cell free culture and the binding of amino acid pyrolysates to cells of strain J525–2.

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Materials and Methods

1. Bacterial strains

*Lactococcus lactis* subsp. *lactis* J525–2 isolated from dahi was used. This strain was incubated in TYLG broth (tryptone 10 g/l, yeast extract 5.0 g/l, lactose 5.0 g/l, glucose 5.0 g/l, Tween 80 1.0 g/l, and L–cysteine HCl monohydrate 0.1 g/l, pH 6.8 ± 0.2) and stocked in 10% reconstituted skim milk at −20°C. *Salmonella typhimurium* TA98 was used for Ames test. An inoculum of 1% was used in all tests.

2. Ames Test

For an Ames test, fermented milks, M–17 culture and cell free M–17 culture were prepared as follows: 10% (w/v) reconstituted skim milk inoculated with strain J525–2 was incubated for 72 h at 30°C. M–17 culture was incubated 24 h at 30°C and this filtrate (pore size: 0.20 µm) and cell free M–17 culture were prepared as samples. Cell suspension was prepared as follows: 10% (w/v) reconstituted skim milk and TYLG broth substituted for suspensions were run as positive controls. Percentage binding was calculated with the following equation.

\[
\text{Percentage binding (\%)} = \frac{\text{peak area of Trp-P-1 solution with samples/peak area of positive control}}{1} \times 100
\]

After incubation, the mixture was poured onto minimal glucose agar plates [glucose 20 g/l, agar 15 g/l, 50× Vogel-Bonner solution (MgSO₄·7H₂O 10 g, citric acid·H₂O 100 g, K₂HPO₄ anhydrous 500 g and NaNH₄HPO₄·4H₂O 175 g in distilled water 670 ml) 20 ml/l] with 2 ml of molten top agar (0.75%, containing histidine 0.05 mM and biotin 0.05 mM), and was incubated at 37°C for 48 h. Antimutagenic activity was calculated with the following equation.

\[
\text{Antimutagenic activity (\%)} = \frac{1 - (C - S)}{(C - B)} \times 100
\]

C: Number of mutagen-induced revertants observed without sample; S: Number of mutagen-induced revertants observed in the presence of sample; B: Number of spontaneous revertants observed without mutagen.

3. Binding of Mutagen

Trp–P–1 was used to investigate binding properties. Cell suspension was prepared as follows: strain J525–2 was incubated in M–17 broth (Difco) for 24 h at 30°C, and the cells were harvested on a filter (pore size: 0.45 µm) by suction filtration. The cell was washed with phosphate buffer (pH 7.0), and then it was suspended in phosphate buffer equal volume to M–17 culture.

Mixtures of 0.1 ml of mutagen solution (0.1 mg/ml) and 0.9 ml of cell suspension were incubated at 37°C for 30 min, and then they were filtered (pore size: 0.20 µm, Minisalt). Mutagens in the filtrate were quantified with a reverse-phase HPLC system (column: Shim-Pack CLC-ODS, Shimadzu, Kyoto, Japan. A mobile phase of 0.1 M citrate–0.2 M disodium hydrogen phosphate (pH 3.0), acetonitrile, and triethylamine (60: 40: 0.05) was employed and the absorbance was measured at 254 nm. Mixtures in which phosphate buffer was substituted for suspensions were run as positive control. Percentage binding was calculated with the following equation.

Percentage binding (\%) = \frac{\text{area of Trp-P-1 solution with samples/peak area of positive control}}{1} \times 100.

Furthermore, effects of incubation time (0, 10, 40, and 50 min) and pH (3.0, 4.0, 5.0, 6.0, and 7.0) of phosphate buffer on the binding were investigated. All assays were performed at least three times.

Results and Discussion

1. Antimutagenic activity

Characteristics of *Lactococcus lactis* subsp. *lactis* J525–2 were shown in Table 1. This strain was isolated from traditional fermented milk in Bangladesh, and it was mesophilic and homo fermentative cocci, 2.0–7.0 µm and occurring singly or in pairs. Produced lactic acid isomer was L. Growth in 6.5% NaCl, and acid production from maltose and ribose were observed.
Antimutagenic activities of fermented milk prepared with strain J525-2 against Trp-P-1, MeIQ and Glu-P-1, and those of M-17 culture and cell free M-17 culture were investigated (Figs. 1 and 2). Antimutagenic activities of fermented milk against Trp-P-1, MeIQ and Glu-P-1 were 67.5%, 75.1% and 41.2%, respectively. M-17 culture had antimutagenic activity (76.1%). Cell free M-17 culture, however, had little activity (3.6%). These results suggest that extracellular products of strain J525-2 have no antimutagenic activity and the cells play major part of the antimutagenic activity.

2. Binding of mutagen

The binding ability to Trp-P-1 of cell of strain J525-2 was investigated (Fig. 3). Cells bound Trp-P-1 (76%). The effects of incubation time (0, 10, 30, 40, and 50 min) and pH (3.0, 4.0, 5.0, 6.0, and 7.0) on the binding ability were investigated. Mutagen binding occurred at 0 min and reached maximum at 10 min (Fig. 4). Prolongation of incubation time (up to 50 min) had no influence on the binding. The binding ability was strongly affected by pH of phosphate buffer (Fig. 4). The highest percentage binding was noted at pH 6.0.

Antimutagenic activity was not owed to extracellular filtrate of Lactococcus lactis subsp. lactis J525-2. Cells of this strain showed a binding ability, and
the binding was pH dependent, occurred instantaneously. This result agreed with earlier reports. Some studies have shown that LAB cells and peptidoglycan could bind food-borne mutagens such as heterocyclic amines, and the binding mechanisms were thought to be involved with ionic and hydrophobic bond. Dahi is a traditional fermented milk in Bangladesh and India, and the function of dahi is paid attention. A LAB strain in dahi has antimitagenicities as mentioned in this paper. This fact has importance on explanation about the function of dahi. Although mutagenicity of mutagens bound LAB cells - the binding part and binding mechanism of strain J525–2 remain to be investigated, the fermented milk prepared with this strain is expected to protect from mutagens produced by heating proteinous food.

References

Lactococcus lactis subsp. lactis J525-2の抗変異原性

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本研究では、バングラデシュのダヒから分離した乳酸菌J525-2株の抗変異原性を調べた。ダヒ "dahi" は、インドおよびバングラデシュなどで伝統的に作られている発酵乳製品である。本菌株はホモ型発酵形式でL-乳酸を生産する中温性の乳酸球菌であり、糖類発酵性試験の結果などからLactococcus lactis subsp. lactisと同定した。この菌株で調製した発酵乳は食品変異原物質であるヘテロサイクリックアミン（Trp-P-1、Glu-P-1、MeIQ）に対して抗変異原性を示した。抗変異原性の要因を検討するために培養液と菌体を除去した培養濁液の抗変異原性を調べたところ、培養濁液では抗変異原性が見られなかったことから、菌体が抗変異原性に関与していると考えられた。そこで、変異原物質の菌体への吸着を調べたところ、菌体は変異原物質を吸着していた。この吸着活性に及ぼすpHおよび反応時間の影響を調べたところ、pH 6.0で最大となり酸性側では低くなった。また、吸着は即時的に起きており、反応時間10分後にはほぼ最大となり50分後まで吸着量はほとんど変化しなかった。以上のことから、このJ525-2株で調製した発酵乳製品が示す抗変異原性は菌体によるものであることが示唆され、菌体は変異原物質を結合する事がわかった。

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