Inhibitory Effect of Milk Whey Cultured with *Flammulina velutipes* M-105 Mutant Strain on N-methyl-N'-nitro-N-nitrosoguanidine-induced Mutation in *Escherichia coli* B/r WP2 Trp

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With regard to possible economic benefits from converting the organic materials from milk whey into valuable food or feed products, many investigators have studied submerged cultivation of various micro-organisms¹⁻⁵). In a previous work, we investigated submerged growth of *Flammulina velutipes* in milk whey and isolated a mutant strain (M-105), which gives 4.4 times higher biomass than the parent strain by incubation at 25°C for 15 days⁶). The present investigation was undertaken to see if milk whey cultured with *F. velutipes* M-105 mutant strain could have a desmutagenic effect, because the mycellium of *F. velutipes* contains some antitumor polysaccharides⁷).

Materials and Methods

F. velutipes mutant strain

The *F. velutipes* M-105 mutant strain used in this experiment was obtained from *F. velutipes* (Curt. ex. Fr.) by UV radiation (3.49 J/m²/sec.) as described previously⁶).

Preparation and cultivation of milk whey

Acid whey was prepared by treatment of skimmed Holstein cow's milk by adjusting pH to 4.6 with concentrated hydrochloric acid. Forty milliliters of acid whey were dispensed in a 300 ml shaking flask, and inoculated with 10 ml of a seven-day culture of the M-105 mutant strain. Incubation was done on a reciprocal shaker (140 reciprocations per minute) for 15 days at 25°C.

Preincubation of supernatant fraction of the culture with MNNG

A supernatant fraction (pH 4.7) was obtained by centrifugation of the culture at 1,000×g for 20 min. A portion of the supernatant was poured into presoaked and washed Visking Cellulose Tubing, 27/32, having a molecular weight cutoff of 10,000⁸). The supernatant fraction was dialyzed against tap water overnight and the undia-

Flammulina velutipes M-105 変異株で培養したミルクホエーによるニトロングアニジン誘導体の変異原性の低下: 細野明義・梅原広子・鶴田文三郎 (信州大学農学部 長野県南箕輪村 399-45)

lyzable fraction was obtained.

One milliliter of the supernatant fraction (or the undialyzable fraction) was added together with 1 ml of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 1.5 mg/ml). They were kept at 36°C for 24 hrs, and the mutagenicity of these mixtures was examined. As a control, acid whey was used instead of the supernatant fraction (or the undialyzable fraction).

Desmutagenicity experiments

Escherichia coli B/r WP 2 *trp*⁻ was used as an indicator bacteria. This strain requires tryptophan for growth, and reversions of prototrophy were studied.

For counting *trp*⁺ revertants, 0.1 ml of the culture of the indicator bacteria grown in B-2 broth was plated onto a semi-enriched agar medium (MB agar plate); following that 40 μ l of the treated whey samples were spotted onto a paper disc (thick, 8 m/m dia.) which had been laid on the MB agar medium and the indicator bacteria. After incubation at 37°C for 2 days, the number of *trp*⁺ revertant colonies thus formed was scored.

B-2 broth medium consists of 10 g of beef extract (Kyokuto, Tokyo), 10 g of peptone (Mikuni) dry powder and 5 g of NaCl in 1,000 ml of distilled water, adjusted to pH 7.0. An MB agar plate contains 4 g of glucose, 15 g of agar (Difco) and 10 ml of Oxoid broth (8 g of Oxoid nutrient broth powder, No. 2 dissolved in 1,000 ml of distilled water) per liter of Vogler-Bonner medium. Vogler-Bonner minimal salts medium was prepared by dissolving 1 g of (NH₄)₂SO₄, 10 g of KH₂PO₄, 0.1 g of Mg SO₄ · 2 H₂O and 0.58 g of trisodium citrate · 2 H₂O in distilled water, neutralizing to pH 7.0, and finally making the volume to 1,000 ml with distilled water⁹.

Results and Discussion

Under the present assay conditions, colonies of *trp*⁺ mutants are easily distinguished from the back ground of *trp*⁻.

Results of the inhibitory effect of the treated samples on mutations induced in *E. coli* B/r WP 2 *trp*⁻ by MNNG are shown in Fig. 1. The number of revertant colonies (*trp*⁺) induced by MNNG which had been pre-incubated with the supernatant fraction of the whey culture of *F. velutipes* M-105 mutant strain decreased markedly, compared with those induced by MNNG treated either with the undialyzable fraction or with acid whey. Based on this observation, we think that desmutagenic components containing in the whey culture are of low molecular weight ($\leq 10,000$) and are not bound chemically to any large compounds, as they could be separated by dialysis.

Several studies have reported that milk and fermented milk products are possibly effective in suppressing various steps in the carcinogenic process. HIRAYAMA has observed that people drinking milk daily had the lowest incidence of stomach cancer¹⁰. REDDY *et al.*^{11,12} and AYEBO *et al.*^{13,14} have reported yoghurt has an inhibitory effect on the Ehrlich ascites tumor. In addition, lactobacilli-fermented products are known to contain antitumor components^{15,16}.

Fig. 1. Effect of the whey culture of *F. velutipes* M-105 mutant strain on MNNG-induced mutation in *E. coli* B/r WP 2 trp⁻. A: the undialyzable fraction-MNNG mixture, B: the supernatant fraction-MNNG mixture, C: acid whey-MNNG mixture

Milk whey has been used a substrate for the growth of various organisms. Submerged cultivation of *F. velutipes* in milk whey is especially of interest in the respect that *F. velutipes* has been used as food for many centuries and commercially cultivated in Japan. Additional studies are needed concerning further fractionation of the whey cultured with *F. velutipes* M-105 mutant strain to elucidate the nature of the component with desmutagenic effects.

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