Desmutagenicity of Commercial Cheese against the Trp-P1-induced Mutagenicity to Streptomycin-dependent Strain SD510 of *Salmonella typhimurium* TA 98

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Abstract Desmutagenicity of various types of commercial ripened cheese against the mutagenicity of an amino acid pyrolysate, Trp-P1, using the streptomycin-dependent strain (SD510) of *Salmonella typhimurium* TA 98, was studied. All kinds of cheese showed high desmutagenicity and were dose-dependent. The desmutagenicity of Camembert, Blue, Emmental, Gruyère and Pont-l'Évêque cheeses using secondary starter were higher than those of Cheddar, Gouda, Edam and Parmesan cheeses using only lactic acid bacteria as starter. Especially among the cheeses tested, the desmutagenicity of Camembert and Blue cheeses showed a rapid increase with increase in the amount of cheese suspensions, and reached a maximum of 100% at 150 and 250 μ l of cheese suspensions, respectively.

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Dietary inhibitions of mutagenesis and carcinogenesis are of particular interest because they may be useful for human cancer prevention. Lactic acid bacteria and some fermented milk products with lactic acid bacteria possess therapeutic, anticarcinogenic or desmutagenic properties^{3, 7, 9, 21, 22}. In recent years, much attention has been paid to desmutagenic properties of fermented milk

products. Hosono and coworkers^{10, 11, 14, 25-27, 32} reported that lactic acid bacteria and fermented milk possess a desmutagenic activity against the mutagenicity of amino acid pyrolyzates, potential chemical carcinogens and food-related mutagens.

Cheese is also a popular dairy product. The high nutritional value of cheese is made up of the nutrients of the milk and the metabolites

Salmonella typhimurium TA 98 から造成されたストレプトマイシン依存株 SD510 の Trp-P1 の変異原性に対する市販チーズの抗変異原性 : 山田正子・中澤勇二*・細野明義** (共立女子短期大学, 東京都千代田区 101-8433, * 共立女子大学大学院, 東京都千代田区 101-8433, ** 信州大学農学部, 長野県南箕輪村 399-4598)

produced during ripening by lactic acid bacteria, some mould or other bacteria in cheese manufacture. However, different types of cheese may have their respective physiological properties. We previously reported the inhibitory activity of Camembert cheese against the mutagenicity of 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P1)³¹⁾. This finding is very interesting because many of the physiological properties are unknown and only a few reports were published¹⁶⁾ regarding desmutagenic properties of cheeses.

The aim of this study was to investigate the desmutagenic effects of various types of cheese against Trp-P1 using the test strain SD510, a streptomycin-dependent strain of *Salmonella typhimurium* TA 98¹²⁾.

Materials and Methods

Materials

The following 9 kinds of cheeses, purchased from markets in Tokyo, were used in this study: Camembert and Blue cheeses as mould-ripened cheeses; Cheddar, Gouda, Edam and Parmesan cheeses as lactic acid bacteria-ripened cheeses; Emmental and Gruyère cheeses as propionic acid bacteria-ripened cheeses; and Pont-l'Évêque cheese as *Brevibacterium*-ripened cheese. As the mutagen, the amino acid pyrolysate, Trp-P1, was used and purchased from Wako Pure Chemical Co., Ltd, Osaka, Japan.

Bacterial cultures

Streptomycin-dependent strain (SD510) of *S. typhimurium* TA 98¹²⁾ used throughout this study was cultivated in SM20 medium at 37°C. The SM20 medium contained 25 g of Oxoid Nutrient Broth No. 2 (OX-medium) and 20 mg streptomycin in 1,000 ml of pure water.

Assay for desmutagenicity

Desmutagenicity was estimated by measuring the decrease in mutation induced by Trp-P1 and carried out according to the method of Ames *et al.*²⁾. In brief, a solution containing 150 µl of Trp-P1 in distilled water (2 mg/ml)

was mixed with 350 µl of cheese suspension, which was prepared by homogenizing of 2 g of whole cheese mixture with 98 ml of distilled water for 15 min using a homogenizer. The mixture was preincubated at 37°C for 60 min. After which, the mixture was sterilized by filtering the extract through a membrane filter (0.45 µm) (Fuji Film Co., Tokyo, Japan).

The test strain SD510 was grown in SM20 medium overnight in a shaking water bath at 37°C. The culture was diluted 1,000 fold with 1/15 M phosphate buffer (pH 6.98) and 100 µl of the diluted culture was laid on a plate of the OX-agar medium. Then, 40 µl aliquots of the filtered cheese suspension containing Trp-P1 were applied to a sterile 8 mm paper disk which was placed on the center of the OX-agar medium, and incubated at 37°C for 48 h. In case of the positive control, distilled water was used instead of the cheese suspension, while the negative control had no Trp-P1. All assays were repeated at least twice, with triplicates each time.

The desmutagenicity was estimated by measuring the decrease in mutation induced by the mutagen and expressed as percentage inhibition. Desmutagenicity was quantified in terms of inhibition percentage of mutagenicity (PI) and calculated:

$$PI(\%) = 100 - (A - C / B - C) \times 100,$$

where A is the number of revertants of test plate, B is the number of revertants of the positive control and C is the number of spontaneous revertants of the negative control that had no Trp-P1.

Chemical analyses of cheese

pH and acidity as lactic acid of cheese samples were analyzed by standard methods²³⁾. Water soluble nitrogen and total N were determined by the Kjeldahl method and calculated²⁴⁾:

$$\text{Ripening index (\%)} =$$

$$= (\text{Water soluble N} / \text{Total N}) \times 100$$

All the assays were repeated three times.

Results and Discussion

Analysis of general compositions

The cheeses were analyzed for general compositions : pH, lactic acidity and water soluble N as % of total N. The results obtained are given in Table 1. These values were suitable and were widely accepted for consideration of each cheeses composition^{8, 18, 20}. The pH and water soluble N as % of total N of Camembert, Blue and Pont-l'Évêque cheeses were higher than those of other cheeses : 6.60 and 77.4%, 6.64 and 51.3%, and 7.65 and 63.3%, respectively. The high values were due to proteolysis by

moulds such as *Penicillium camemberti*⁸⁾ for Camembert cheese and *Pen. roqueforti*⁸⁾ for Blue cheese, or *Brevibacterium*²⁸⁾ for Pont-l'Évêque cheese with strong proteolytic activity, and proteolysis of casein with the formation of some peptides and amino acids.

Desmutagenicity of cheeses

Changes in the desmutagenicity of cheeses to Trp-P1 on *S. typhimurium* SD510 by the amount of cheese suspensions are shown in Fig. 1. In order to establish the desmutagenic properties of the cheeses, cheese suspensions at a range of 50, 150, 250 and 350 μ l, were examined. All kinds of cheese exhibited high

Table 1. General characteristics of cheeses (n=4)

	Mould-ripened cheeses		Lactic acid bacteria-ripened cheeses				Propionic acid bacteria-ripened cheeses		<i>Brevibacterium</i> -ripened cheese
	Camembert cheese	Blue cheese	Cheddar cheese	Gouda cheese	Edam cheese	Parmesan cheese	Emmental cheese	Gruyère cheese	Pont-l'Évêque cheese
pH	6.60	6.64	5.48	5.69	5.04	5.54	5.28	5.26	7.65
Lactic acidity (%)	0.84	1.28	1.02	1.16	1.82	1.72	1.11	1.07	0.76
Ripening index (%)*	77.4	51.3	27.1	28.9	27.3	24.5	26.5	30.2	63.3

* Ripening index (%) means water soluble nitrogen (N) as % of Total N.

Fig. 1. Dose response of desmutagenicity of cheese suspensions to Trp-P1 on *Salmonella typhimurium* SD510 (n=4).

and dose-related desmutagenicity. Especially, Camembert and Blue cheeses showed rapid increase in desmutagenicity with increase in the amount of cheese suspensions, and reached a maximum of 100% with cheese suspensions of only a 150 and a 250 μ l, respectively. The desmutagenicity of other cheeses showed gradual increase with increasing amount of cheese suspensions, and a maximum with 350 μ l of cheese suspension. The scores of the others were as follows: Cheddar cheese 69.8%, Gouda cheese 64.6%, Edam cheese 61.1%, Parmesan cheese 74.7%, Emmental cheese 89.3%, Gruyère cheese 100.0% and Pont-l'Évêque cheese 93.0%.

Camembert, Blue, Emmental, Gruyère and Pont - l'Évêque cheeses exhibited higher desmutagenic effects than those of Cheddar, Gouda, Edam and Parmesan cheeses. These findings are likely to have some effects on the symbiotic actions by mixed strain starters both lactic acid bacteria and secondary starter. The main reason for the ripening of cheeses by the mixed strain starters is their difference in metabolites and ripening conditions. The desmutagenicity and ripening of cheeses are considered rather a complicated phenomenon, both experimentally and theoretically. Hosono *et al.*^(11,14) and Nadathur *et al.*⁽¹⁹⁾ reported the desmutagenicity of lactic acid bacteria or cultured milk with lactic acid bacteria, and also the desmutagenicity of lactic acid bacteria isolated from cheese^(6,32); the lactic acid bacteria such as *Lactococcus lactis* ssp. *lactis*, *Lc. lactis* ssp. *cremoris*, *Lc. lactis* ssp. *diacetylactis*, *Str. thermophilus* and *Lactobacillus helveticus*, are usually being used for cheesemaking. More over, Hosono *et al.* reported that the slimy substance consisting of sticky materials and cells of *Brevibacterium linens* exhibited remarkable antimutagenic activity towards Trp-P1 and 3-amino-1-methyl-5*H*-pyrido [4,3-*b*] indole (Trp-P-2)⁽¹⁵⁾. Vorobjeva *et al.* reported that dialysates of the cells of propionic acid bacteria contained several kinds of desmutagenic substances with different molecular

weights⁽³⁰⁾. From these findings, high desmutagenicity of cheeses could appear to influence synergistic effects between secondary starter and lactic acid bacteria.

On the other hand, the higher desmutagenicity of Camembert, Blue and Pont-l'Évêque cheeses could be correlated to higher value of water soluble N as % of total N as shown in Table 1. Such degradation might be affected by the enzymes released from bacteria such as lactic acid bacteria as starter, propionic acid bacteria, *Brevibacterium linens*, yeasts, and moulds like *Pen. roqueforti* and *Pen. candidum*. van Boekel *et al.*⁽²⁹⁾ reported on desmutagenicity of casein against mutagenicity induced by benzo[*a*] pyrene (B[*a*] P), *N*-metylnitrosourea and nitrosated 4-chloroindole, and pepsin-hydrolysed casein against mutagenicity induced by sodium azide and 4-nitroquinoline-1-oxide (4NQO) to *S. typhimurium* TA 100 and *Escherichia coli* K-12-343. Bosselaers *et al.*⁽⁵⁾ have shown that casein, its pepsin hydrolysis products have a protective effect against certain genotoxic compounds, where as other milk proteins like whey protein, β -lactoglobulin had no protective action. More over, Abdelali *et al.*⁽¹⁾ reported that the desmutagenic potential of casein increased with pepsin hydrolysis was due to the peptides formed, which interact with the mutagens. Nevertheless ripening period of Cheddar, Gouda, Edam and Parmesan cheeses were longer than that of other cheeses, which have low water soluble N as % of total N. In addition, since Cheddar, Gouda, Edam and Parmesan cheeses showed desmutagenic activity, the desmutagenicity of these cheeses could be partly due to the casein. The desmutagenic properties of casein, being a major constituent of milk products, have been demonstrated in salmonella/microsome test for nitroso compounds^(4,19,28) and on pepper extracts⁽¹³⁾. Thus the desmutagenicity of cheeses could be attributed to various micro-organisms, proteinases and other related enzymes produced by micro-organisms, and

casein with the formation of low molecular weight peptides and amino acid during ripening of cheese.

In this study, it was confirmed that various types of ripened cheese from markets had the desmutagenicity against the Trp-P1-induced mutagenicity to SD510. This conclusion was supported by the findings of Lê *et al.*¹⁷⁾ who reported that the risk of breast cancer was found to be positively associated with frequent cheese consumption in a case-control study, and Jongen *et al.*¹⁶⁾ who reported the inhibitory potential of Gouda cheese on the mutagenicity generated in feva beans after treatment with nitrite in acidic solution. However, more work has to be done to understand the exact mechanisms involved.

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