微生物によるニトロサミンの分解 I

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Microbial Degradation of Nitrosamines-I

Inducible Breakdown of Nitrosamines*1

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In order to investigate the microbial degradation of nitrosamines, the degrading activity of three strains of non-pathogenic microorganisms, *i. e.*, *Rhizopus oryzae*, *Streptococcus cremoris*, and *Sccaromyces rouxii* was examined.

These microorganism, were active in degrading NDMA, NDEA, NDPA, NPYR, and NPIP, NDPA being degraded more rapidly than the other nitrosamines. *Rhizopus oryzae* degraded 804 of NDPA, 40% of NPIP, 25% of NDEA, 20% of NPYR, and 10% of NDMA (precentages of amounts added to the medium). *Streptococcus cremoris* and *Saccharomyces rouxii* degraded 70% and 50% of NDPA respectively; the other nitrosamines were degraded to a smaller extent.

NDPA was also degraded faster in the suspensions of cells of these three strains precultured in the medium containing the nitrosamine than in the control cultures, *i. e.*, the suspensions of cells harvested from the medium which did not contain NDPA.

The cell-free extract of *Rhizopus oryzae* grown in the medium containing either NDMA or NDPA was active in degrading these two nitrosamines.

These results suggest that the degradation of nitrosamines is conducted by an inducible enzyme.

Since the early works conducted by English and Canadian researchers, it has been known that small amounts of volatile amines are produced in the muscle of a variety of fish and shellfish during their refrigerated storage[1]. Therefore, it is conceivable that these amines react with nitrite blended, whether as a food additive or not, and most potent carcinogenic nitrosamines are produced during food processing. Indeed, the formation of nitrosamines in sea foods has recently aroused a great deal of concern among scientists.

The initial discovery of nitrosamines was made in nitrite-treated fish meal by ENDER *et al.*[2] This discovery led to the investigations of possible formation of these compounds in various sea foods. SEN *et al.*[3] reported that NDMA was formed in the smoked and canned fish with nitrite added. SAKAI *et al.*[4], KAWABATA *et al.*[5], NAKAMURA *et al.*[6], and GADBOIS *et al.*[7], detected NDMA from some kinds of processed fish and roe treated with nitrite. In addition, FONG *et al.*[8], NAKAMURA *et al.*[6], and IYENGAR *et al.*[9] found NDMA and NDEA from some of fresh and processed fish which could not be suspected as nitrate and/or nitrite-treated foods.

Based on these findings, much attention has been paid to the prevention of nitrosamine formation in foods and model sytems. MIRVISH *et al.*[10], FAN *et al.*[11], FIDDLER *et al.*[12], KAWABATA *et al.*[13], and TOZAWA *et al.*[14] demonstrated the effectiveness of ascorbic acid for the prevention of nitrosamine formation. Besides ascorbic acid, other reducing agents such as cysteine and glutathion, and phenol compounds, *i. e.*, tannin, tocopherol and propylgallate, were reported to be also effective[15,16]. On the other hand, HARADA and YAMADA[10] showed that some strains of non-pathogenic microorganisms possessed the ability to decompose NDMA, and suggested that the microbial degradation could be applicable to eliminate the nitrosamines formed in foods.

The present report describes the inducible breakdown of nitrosamines by cells and cell-free extract of *Rhizopus oryzae* and other microorganisms.

Materials and Methods

Chemicals, Microorganisms, and Media

Nitrosamines used, *i. e.*, NDMA, NDEA, NDPA, NPYR and NPIP were kindly supplied by Dr. A. MIRNA, Bundesanstalt für Fleischforschung, Kulmbach, Germany.

Microorganisms used, *i. e.*, *Rhizopus oryzae*

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The following abbreviations are used: NDMA, nitrosodimethylamine; NDEA, nitrosodiethylamine; NDPA, nitrosodipropylamine; NPYR, nitrosopyrrolidine; NPIP, nitrosopiperidine.

IFO 4705, *Saccharomyces rouxii* IFO 0495, and *Streptococcus cremoris* IFO 3427 were obtained from the Institute for Fermentation, Osaka, Japan.

Malt extract and potato infusion medium for the mould and yeast was purchased from the Nisui Chemical Co. and the tomato juice medium for the bacterium was obtained from the Eiken Chemical Co.

Preparation of Precultured Cells in Nitrosamine-containing Medium

Strains of the microorganisms were grown to mid-exponential phase in their respective media containing NDPA (0.1 μmol/m*l*), and the grown cells were centrifuged at 16,000$\times g$ for 15 min at 0°C. The suspended fluid was removed and the cells were washed with 15 m*l* of distilled water. The washed cells were centrifuged twice and finally a thick cell suspension (10^{10} cells/m*l* for bacterium and yeast, 0.01 g/m*l* for mould) was obtained. All the procedures were conducted under aseptic condition.

Preparation of Cell-free Extract

The cells of *Rhizopus oryzae* were harvested from the growth medium containing nitrosamine and a portion of 3 to 4 g of the wet cells was thoroughly ground in a porcelain mortar with a small amount of fine quartz. The raptured cells were poured into 10 m*l* of Tris buffer (pH 8.5). The suspension was centrifuged at 16,000$\times g$ for 15 min at 0°C. The suspended fluid was filtered through the membrane filter whose pore size was 0.45 μm. The filtrate thus obtained was used as cell-free extract.

Degradation of Nitrosamine

The ability of growing cells, precultured cells, and cell-free extract to degrade nitrosamines was investigated in the below-mentioned methods.

A loopful stock culture of the test microorganisms was grown statically in 25 ml of their respective media containing each nitrosamine (0.1 μmol/m*l*) at 30°C under aerobic condition. After regular time intervals (2–8 days), nitrosamine in the medium was assayed.

The precultured cells suspended in 10 m*l* of the medium containing NDPA (0.1 μmol/m*l*) were incubated for 2 days at 30°C. After the incubation, nitrosamine was assayed.

Either NDPA or NDMA was added to 4 m*l* of the cell-free extract (0.1 μmol/m*l*) and the mixture was incubated for 3 hours at 30°C. After the incubation, nitrosamine was assayed.

Assay of Nitrosamine

To 2 m*l* of dichloromethane, 1 m*l* of the test solution was added and the mixture was shaken vigorously for each about 2.5 min. The residual nitrosamine in the aquous fraction was re-extracted with the same volume of the soluvent twice. The combined extract was treated with 1 m*l* of 0.15% hydrobromic acid and the nitrite released was determined by the method of ADRIAANSE *et al.*[18]

Results and Discussion

Degradation of Nitrosamine by Growing Cells

The growing cells of the test microorganisms were investigated for the ability to degrade nitrosamines. The proportion of the growing cells capable of degrading nitrosamines is shown in Fig. 1. In any strains of the microorganisms, it should be noted that the decomposition rate of NDPA was highest. The rate of the other nitrosamines, however, varied more or less between the strains.

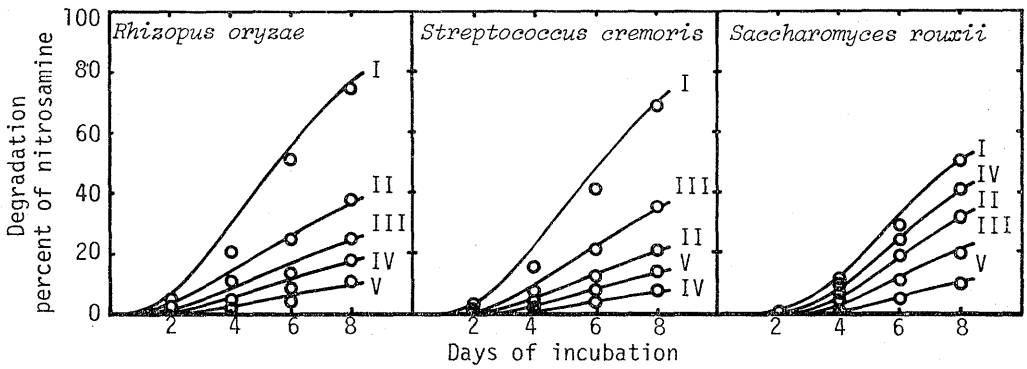

Fig. 1. Degradation of nitrosamines by growing cells. I, NDPA; II, NPIP; III, NDEA; IV, NPYR; V, NDMA.

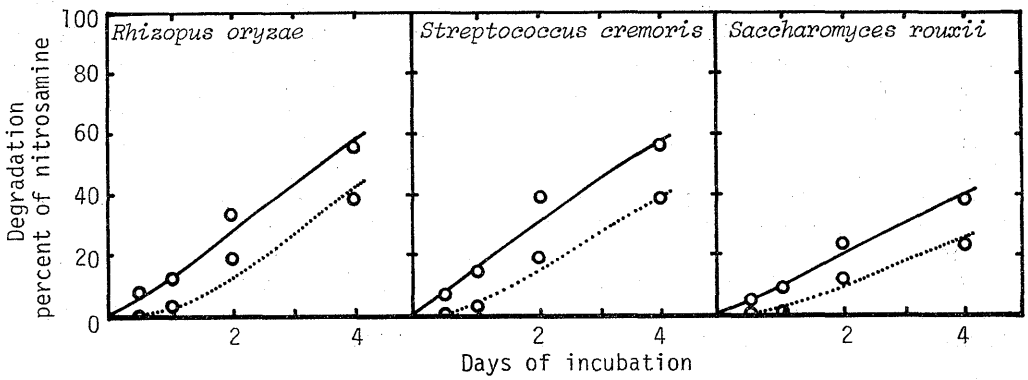

Fig. 2. Degradation of NDPA by precultured cells. The dotted line indicates the result from control cultures.

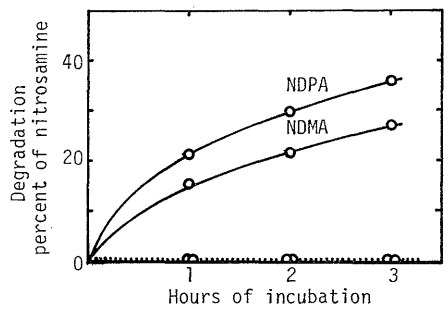

Fig. 3. Degradation of nitrosamines by cell-free extract. The dotted line indicates the result from control cultures.

This may reflect the different cell permeability of these compounds tested.

Degradation of Nitrosamine by Precultured Cells

To test the possibility that an inducible enzyme might be involved in the degradation of nitrosamine, the precultured cells in the medium containing NDPA were subcultured in the same medium and the degraded nitrosamine was assayed. The results obtained is shown in Fig. 2. The breakdown of NDPA was faster in the case where the precultured cells were used than in the case of control cultures having no prior exposure to NDPA. This suggests that an inducible enzyme takes a part in the degradation of NDPA. In contrast to our finding, ROWLAND *et al.*[19] demonstrated that the inducible enzyme participating in nitrite liberation from nitrosodiphenylamine was not involved in any strains of *E. coli* tested.

Degradation of Nitrosamine by Cell-free Extract

In order to elucidate the formation of an ind-n cible enzyme for degrading nitrosamines in the growing cells of microorganisms, the ability of cell-free extract of *Rhizopus oryzae* to degrade nitrosamines was investigated. As shown in Fig. 3, the cell-free extract obtained from the cultures grown in nitrosamine-containing medium was active in degrading nitrosamines, as opposed to that from the control cultures.

When the cell-free extract was heated at 100°C for 10 min, its ability to degrade nitrosamines was lost.

From these findings, it may be concluded that an inducible enzyme for degrading nitrosamines is involved in the cells exposed previously to nitrosamine.

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