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Note

Conversion of Volatile Sulfides by *Aspergillus awamori* var. *kawachii*

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In order to improve the odor of foods containing volatile sulfur compounds, we screened 198 molds and yeasts, and found 14 molds that provided an 80% decrease in 1,000 ppm didecyl sulfide in MY medium after 4 days of culture shaking at 25°C. Of the 14 molds, 13 of which were molds of the *Aspergillus niger* group, *Asp. awamori* var. *kawachii* No. 91 most effectively decreased the amount of didecyl sulfide. This mold oxidized dibutyl sulfide into dibutyl sulfoxide and dibutyl sulfone. The optimum pH for conversion of dibutyl sulfide was approximately pH 6.5, and the optimum temperature was approximately 30°C. If used to treat food, it could weaken the pungent odor of onion and garlic.

Keywords: sulfide, *Aspergillus*, microbial conversion

The olfactory threshold of volatile sulfur compounds is very low, and even trace amounts have a strong and characteristic odor, resulting in an unpleasant odor that damages food quality. The application of microorganisms for the oxidation of sulfides has been undertaken (Dodson *et al.*, 1962; Auret *et al.*, 1966, 1968; Ohta *et al.*, 1984, 1985). These reports of such applications all describe the conversion of aryl or aryl-alkyl sulfides, but almost all of the sulfides that give rise to undesirable odors are alkyl sulfides. Zhang *et al.* (1991) found a strain of *Pseudomonas* from a peat biofilter that could oxidize dimethyl sulfide. However, little work has been done on the screening of strains having the conversion ability of sulfide from molds and yeasts related to foods. In this study, therefore, the conversion of alkyl sulfides by molds and yeasts was investigated.

Table 1 shows the screening results of microorganisms for didecyl sulfide conversion activity; didecyl sulfide was selected as the substrate in the assays that took 15 h or longer, and dibutyl sulfide was used in the shorter assays. In all, 172 strains of molds and 26 strains of yeasts from the collection of Takara Shuzo Central Research Laboratories were cultured in 5 ml of MY medium (3 g of malt extract, 3 g of yeast extract, 5 g of polypeptone, and 10 g of glucose in 1 *l* of distilled water) containing 1,000 ppm didecyl sulfide. Yeasts were cultured with shaking at 25°C for 3 days, and molds were cultured for 4 days. After the cultivation, 5 ml of ethyl acetate was added to each test tube, and the tubes were shaken for 1 min. The concentration of the didecyl sulfide extracted by ethyl acetate was then assayed using a gas chromatograph (model GC-7AG, Shimadzu) equipped with a flame photometric detector. The liquid phase of a 2.1 m×3 mm i.d. glass column was 2% SE-30. Isothermal analyses were done at 230°C, and the flow rate of the carrier gas, N_2, was 30 ml/min. A calibration curve was obtained using authentic didecyl sulfide, and the peak heights were used in the exponential calculations done with a chromato-integrator. The conversion rate of sulfide was calculated by the decrease in the concentration of sulfide after cultivation compared with that without microorganisms.

From a total of 198 strains, 14 strains (all molds) consumed 80% or more of the didecyl sulfide. Thirteen of these were *Aspergillus* molds that were originally obtained from fermented food. Of 74 strains of *Aspergillus* spp. tested, none of the *Aspergillus oryzae* group (*Asp. oryzae, Asp. sojae, Asp. tamarii*) had didecyl sulfide converting activity while many of the molds having such activity were of the *Aspergillus niger* group (*Asp. niger, Asp. awamori, Asp. usamii*). Molds isolated from dried bonito (*katsuobushi*) and yeasts for fermentation or bread making did not give high conversion rates.

Next, these 14 selected strains were cultured with shaking, and three kinds of preparations, fresh, homogenized, and freeze-dried, were allowed to react with didecyl sulfide in 0.1 M phosphate buffer (pH 6.5). We found that *Aspergillus awamori* var. *kawachii* No. 91, which is used in the preparation of fermented foods in the form of malted rice (*kome koji*), had the highest activity in all three forms of strains. It was then selected as the sulfide-converting microorganism to be studied further.

First, 40 mg of the freeze-dried mycelia of strain No. 91 was allowed to react with dibutyl sulfide at a concentration of 100 ppm in 2 ml of 0.1 M phosphate buffer at pH 2 to 10 and at 15 to 50°C for 3 h. Strain No. 91 converted over 70% of the starting sulfide at pH 4 to 7 at 30°C. The optimum pH for conversion was at around 6.5. The optimum temperature was at around 30°C, and strain No. 91 converted about 40% of the starting sulfide even at 15°C.

Table 2 shows the reaction products from dibutyl sulfide treated with strain No. 91. Here, 0.05 g of freeze-dried mycelia of strain No. 91 was allowed to react with 100 ppm (0.683 mM) of dibutyl sulfide in 5 ml of 0.1 M phosphate buffer at pH 6.5 and 30°C, followed by extraction with 5 ml of ethyl acetate

Table 1. Screening for didecyl sulfide converting activity of microorganisms.

Organisms	Number of strains	
	Tested	With activity[a]
Aspergillus oryzae	19	0
sojae	4	0
tamarii	2	0
niger	12	6
awamori	6	2
usamii	7	1
repens	8	0
Other *Aspergillus*	16	4
Penicillium	11	0
Monascus	3	0
Rhizopus	9	0
Schizophyllum	10	0
Fusarium	7	0
Molds of dried bonito	20	0
Other molds	38	1
Molds (total)	172	14
Yeasts	26	0

[a] Conversion rate of didecyl sulfide >80%.

Table 2. Generation of oxidized products of dibutyl sulfide by strain No. 91.

Time (h)	Dibutyl sulfide (μM)	Dibutyl sulfoxide (μM)	Dibutyl sulfone (μM)
0	683.5 (683.5)	0 (0)	0 (0)
3	410.1 (392.3)	8.6 (0)	0 (0)
6	77.9 (48.5)	27.7 (0)	1.7 (0)
12	8.8 (6.8)	31.5 (0)	4.0 (0)
24	3.4 (4.1)	44.4 (0)	12.3 (0)
48	0 (3.4)	29.6 (0)	33.1 (0)

Values in parentheses are in the absence of strain No. 91.

and analysis by gas chromatography. The gas chromatographic conditions were as follows: column, 10% polyethylene glycol 20 M, 2.1 m × 3 mm i.d.; column temperature, programmed to increase from 170 to 230°C at the rate of 8°C/min; carrier gas, N_2 at 40 ml/min; and injection temperature, 250°C. The peak heights were used in the exponential calculations done using a chromato-integrator. The continuous decrease in dibutyl sulfide by vaporization prevented quantitative examination, but the oxidized products, dibutyl sulfoxide and dibutyl sulfone, were detected, while a reduced product, dibutyl mercaptan, was not.

These results suggest that the conversion of sulfides by strain No. 91 resulted, at least in part, from an oxidative reaction involving oxygenase.

The ability of strain No. 91 to convert alkyl disulfides was investigated. A 200 mg sample of fresh mycelia of strain No. 91 was allowed to react with 100 ppm of disulfides in 4 ml of 0.1 M phosphate buffer (pH 6.5) at 30°C for 1 h. The disulfide remaining was extracted with 2 ml of ethyl acetate and assayed by gas chromatography. The converted ratio of diallyl disulfide, dipropyl disulfide, dioctyl disulfide, and didecyl disulfide was 73.1, 58.1, 81.1, and 49.8%, respectively. This result suggests that strain No. 91 has the ability to convert alkyl disulfides.

The ability of strain No. 91 to weaken the odor of sulfur compounds in foods was investigated. By mixing 20 mg of freeze-dried mycelia and 5 g of an onion or garlic paste in 4 ml of 0.1 M phosphate buffer (pH 6.5), and by incubating the mixture at 30°C for 15 to 20 h, we found an overall decrease in volatile sulfur compounds based on gas chromatography; for example, diallyl disulfide, the most characteristic flavor compound of garlic, was 43% of its initial amount, while diallyl disulfide in the buffer solution without mycelia was 93% of its initial amount. The reaction products from diallyl disulfide treated with strain No. 91 were not identified; diallyl disulfide was probably oxidized to allicin, which was so labile as to be easily decomposed. The other possible pathway was reduction followed by methylation of the disulfide (Fukushima *et al.*, 1978). The odor from onion or garlic after incubation was less pungent than the odor without mycelia, and a sweet smell was generated. Both the onion and garlic extracts have antimicrobial activity, which only slightly affected the sulfide and disulfide converting activity of strain No. 91. The use of strain No. 91 may be an effective method to improve food flavor.

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