Journal of Microbiology and Biotechnology
Vol. 3, No. 1, 61-63, 1993

NOTE

Dimethyl Trisulfide Produced by *Bacillus* sp. in Cooked Soybean

JI, WON DAE, SANG JUN LEE AND JONG KYU KIM*

Department of Applied Microbiology, Yeungnam University, Kyongsan 712-749, Korea

The neutral fraction of whole volatile flavor compounds produced by *Bacillus licheniformis* SSA3 and *Bacillus subtilis* PM3 in cooked soybean was identified by using GC/MS and Kovats retention index. The presence of dimethyl trisulfide, which emits characteristically Korean soy sauce-like odor in traditional Korean soy sauce, in identified volatile flavor components was confirmed. Dimethyl trisulfide may be produced by *Bacillus licheniformis* SSA3 and *Bacillus subtilis* PM3 in cooked soybean.

Dimethyl trisulfide (DMTS) is usually an off-flavor component in vegetables.

Several reports about it have been published. Buttery *et al.* (2) identified DMTS in Cabbage, Broccoli and Cauliflower, and proposed that it is one of the off-flavor components in these vegetables.

Maruyama (11) reported that although it exists in the Brassicaceous vegetables less than dimethyl disulfide (DMDS) and other sulfur-containing compounds DMTS is an important off-flavor component because of its unpleasant quality. Whitfield *et al.* (16) also reported that DMTS is an important off-flavor component in the royal red prawn (*Hymenopenaens sibagae*) of crustaceans.

Furthermore, DMTS was found in samples of potable water ranging from 5 to 250 ng/liter. Its presence in concentrations higher than 10 ng/liter was associated with the objectionable odor from water samples (15).

On the other hand, DMTS was reported to be a characteristic impact compound which emits soy sauce-like odor in traditional Korean soy sauce (7) and is known to be presence in Japan shoyu (17, 18), and to prevent from thrombosis.

DMTS is known to be produced by the action of microbes such as *Pseudomonas putrepaciens* and *Pseudomonas perolens* (12), and *Bacillus* sp. is related to producing the aroma of traditional Korean soy sauce (10). However, it is still unknown what sort of microorganisms produce this component in fermented foods.

The results of this study show that DMTS is produced by *Bacillus* sp. in cooked soybean.

Soybean medium was prepared as follows: soybeans were soaked in water at room temperature for 16 hrs, boiled at 106℃ for 3 hrs and crudely crushed. They weighed 300 g in an Erlenmeyer flask. They were then autoclaved at 121℃ for 30 min..

Bacillus licheniformis SSA3 (10) and *Bacillus subtilis* PM3 (6) isolated from the traditional Korean soy sauce and the soybean paste, respectively, were inoculated at soybean medium with 0.1% (preincubated culture at same medium for 7 days) of the total culture volume. They were incubated at 30℃ for 55 days.

The volatile flavor components produced by these strains in soybean medium (700 g) were extracted by steam distillation-solvent extraction apparatus (14). Dimethyl ether (100 ml) was used as an extracting solvent.. Steam distillating-extraction was continued for 2 hrs.

The neutral fraction was isolated from whole volatile flavor components after extracting with 5% HCl first and with 5% NaOH followed, as Fujimaki *et al.* (4)'s method, and concentrated by the use of a rotary evaporator up to 150 μl, and identified according to GC/MS and Kovats retention index (5, 13).

GC/MS analysis was carried out with a Finnigan MAT 4510B as follows: column; CARBOWAX-20M-25M, injection port temp.; 230℃, detector port temp.; 250℃, carrier gas; He (5 ml/min.), electron voltage; 70 eV, split ratio; 30:1, programed oven temp.; 45℃ for 2min., from 45℃ to 220℃ at the rate of 15℃/min. and then at 220℃ for 11.4 min..

DMTS was identified in the neutral fraction of volatile flavor components produced by *Bacillus licheniformis*

*Corresponding author
Key words: Dimethyl trisulfide, *Bacillus licheniformis*, *Bacillus subtilis*.

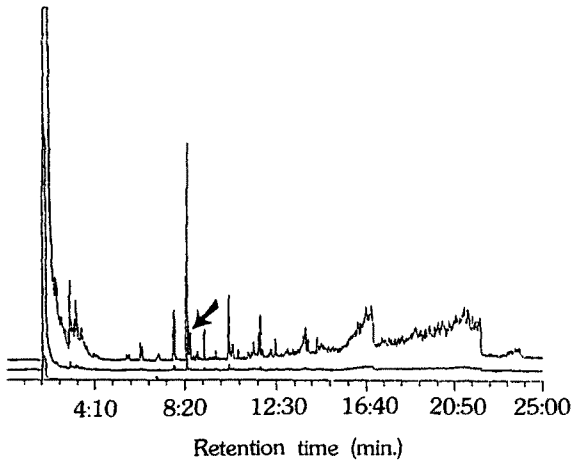

Fig. 1. Gas chromatogram of the neutral fraction fractionated from whole volatile components produced by *Bacillus licheniformis* SSA3.
➔: DMTS indicated by an arrow.

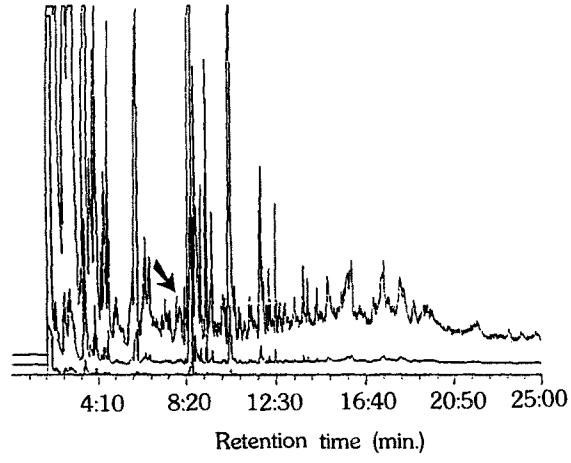

Fig. 2. Gas chromatogram of the neutral fraction fractionated from whole volatile components produced by *Bacillus subtilis* PM3.
➔: The arrow indicates DMTS.

SSA3 and *Bacillus subtilis* PM3 after being incubated in cooked soybean at 30℃ for 55days. Fig. 1 and 2 show gas chromatograms of the neutral fraction of volatile flavor components produced by these strains. Fig. 3 shows mass spectrum of DMTS.

DMTS was identified in traditional Korean soy sauce (7) and Japan Shoyu (17, 18). Generally, *Bacillus* sp. does not only produce the aroma of traditional Korean soy sauce, but also participates in the manufacturing of fermented foods such as traditional Korean soy sauce

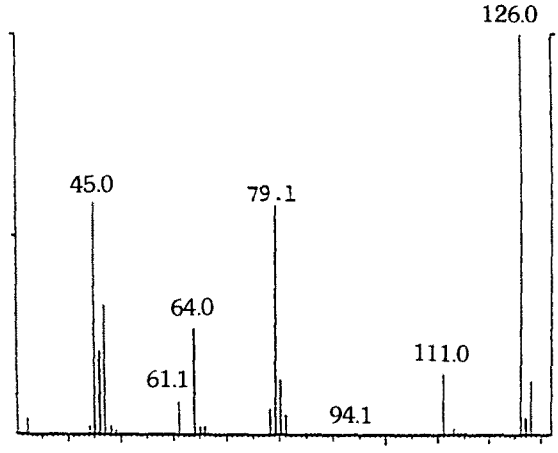

Fig. 3. Mass spectrum of dimethyl trisulfide obtained from indicated peaks of Fig. 1 and Fig. 2.

(8), soybean paste (9) and Japan Shoyu (1).

These results strongly suggest that DMTS may be produced by *Bacillus* sp.

Chen *et al.* (3) identified this volatile flavor component in Shitake mushroom and proposed the following mechanism. Cyclic S compounds in Shitake mushroom originate from lentinic acid which is a derivative of γ-glutamylcystein sulpoxide. There are two enzymes responsible for the conversion of lentinic acid into cyclic S compounds: they are, γ-glutamyl transpeptidase and cystein sulpoxide lyase (C-S lyase). These cyclic S compounds may be undergo methyl disulfide which indicates an important intermediate for the polymerization of S compouns such as DMTS.

The other formation mechanism of DMTS was suggested by Miller III *et al.* (7) as follows: methanethiol, which can be derived from cystine, cystein, methionine or possibly glutathione, may be undergo a direct oxidation to DMDS, involving sulfenic acid intermediates. The formation of DMTS may be resulted in the reaction of hydrogen sulfide with the unstable sulfenic acid.

REFERENCES

1. **Atzui, H., T. Saitou, S. Tou and M. Rinmoku.** 1981. Shoyu. Shyugo Shyokuryo Kogyo (*Comprehensive Food Engineering*). p.516-538.
2. **Buttery, R.G., D.G. Guadagni, L.C. Ling, R.M. Seifert and W. Lipton.** 1976. Additional volatile components of Cabbage, Broccoli, and Cauliflawer. *J. Agric. Food Chem.* **24**: 829-832.
3. **Chen, C.C. and C.T. Ho.** 1986. Identification of sulfurous compounds of Shitake mushroom (*Lentinus edodes* sing.). *J. Agric. Food Chem.* **34**: 830-833.

4. **Fujimaki, M., T. Tsugita and T. Kubata.** 1977. Fractionation and identification of volatile acids and phenols in the steam distillate of rice brane. *Agric. Biol. Chem.* **41**: 1721-1725.
5. **Jennings, W. and T. Shibamoto.** 1980. Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography. Academic press Inc., London.
6. **Ji, W.D.** 1989. Volatile components producing by *Bacillus subtilis* PM3. MS Thesis,Yeungnam University, Korea.
7. **Ji, W.D., E.J. Lee, S.Y. Kim and J.K. Kim.** 1992. Characteristic volatile componens of traditional Korean soy sauce*J. Korean Agric. Chem. Soc.* **35**: 346-350.
8. **Kim, J.K.** 1992. Microorganisms producing a novel pigments and the utilization for the production of soy sauce by using the same. Patent Specifications 92-5751, Korea.
9. **Kim, J.K.** 1992. Microorganisms for preparing traditional Korean soybean paste and the method for the production of soybean paste by using the same. Patent Specifications 92-5750, Korea.
10. **Kwon, O.J., J.K. Kim, and Y.G. Chung.** 1986. The characteristics of bacteria isolated from ordinary Korean soy sauce and soybean paste. *J. Korean Agric. Chem. Soc.* **29**: 422-428.
11. **Maruyama, F.T.** 1970. Identification of dimethyl trisulfide as a major aroma component of cooked Brassicaceous vegetables. *J. Food Science*, **35**: 540-543.
12. **Miller III, A., R.A. Scanlan, J.S. Lee and L.M. Libbey.** 1973. Volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas putrefaciens, Pseudomonas fluoresaens,* and an *Achromobacter* species. *Appl. Microbiology,* **26**: 18-21.
13. **Sadtler.** 1986. The sadler standard gas chromatography retention index library. Sadtler research labotories. Division of biolad labotories Inc., U.S.A.
14. **Schults, J.H., R.A. Flath, T.R. Mon, S.H. Eggland and R. Teranish.** 1977. Isolation of volatile components from a model system. *J. Agric. Food Chem.* **25**: 446-449.
15. **Wajon, J.E., R. Alexander and R.I. Kagi.** 1985. Dimethyl trisulfide and objectionable odours in potable water. *Chemosphere* **14**: 85-89.
16. **Whitfield, F.B., D.J. Freeman and P.A. Banister.** 1981. Dimethyl tri sulfide:an important off-flavor component in the royal red prawn (*Hymenopenaens sibogae*). *Chemistry and Industry,* **3**: 692-693.
17. **Yokotsuka, T., M. Sasaki, N. Nunomura and Y. Asao.** 1980. Shoyu no Kaori(1)[The flavor of Shoyu(1)]. *Nippon Zycuzou Kyoukai Zasshi.* **75**: 516-522.
18. **Yokotsuka, T., M. Sasaki, N. Nunomura, and Y. Asao.** 1980. Shoyu no Kaori(2)[The flavor of Shoyu(2)]. *Nippon Zyouzou Kyoukai Zasshi.* **75**: 717-728.

(Received 4 May, 1993)