Note

Production of *S*-Methyl Thioacetate from Methyl Mercaptan by *Saccharomyces cerevisiae*†

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The involvement of volatile sulfur compounds in brewing has been studied by many workers, as reviewed by Garza-Ulloa.[1] Among these compounds, hydrogen sulfide, dimethyl sulfide and thiols have been already known to significantly contribute to the occurrence of off-flavors in beer. Recently, *S*-methyl thioacetate (MeSAc) was newly identified in beer as an another possible source of sulfury flavor.[2,3] This compound was also identified in smear coated cheeses,[4] and it is postulated that MeSAc in the cheese is produced by an associated activity of *Micrococcus* cheese strains and *Brevibacterium linens*.[5]

MeSAc in beer was assumed as a product by brewer's yeast from methyl mercaptan (MeSH)[2] or methionine[3] during the fermentation of wort, but the biosynthetic pathways for its formation are not yet clarified in full detail. In this consideration, we examined the production of MeSAc from MeSH and L-methionine by *Saccharomyces cerevisiae*, using synthetic medium.

In the present experiments, *S. cerevisiae* IFO 1234 was used as a material strain.

MeSH (*ca.* 30% in methanol) was obtained from Nakarai Chemicals, Ltd., Kyoto, Japan, and it was further diluted with methanol immediately before use. MeSH concentration in the reagent was determined by the gas chromatography with a flame photometric detector (FPD-GC). For the calibration, MeSH standard solution (1 μg/μl in benzene), which was obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan, was used.

The material yeast strain was pre-cultured in MYGP medium (0.3% malt extract, 0.3% yeast extract, 5% glucose and 0.5% peptone) at 25°C for two weeks, and the growing yeast cells were employed for the experiment on the MeSAc production from MeSH in the basal medium, which was composed of 5% glucose, 0.5% peptone, 0.05% yeast extract, 0.2% $(NH_4)_2SO_4$, 0.2% $Na_2HPO_4 \cdot 12H_2O$, 0.1% KH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 0.001% $CaCl_2 \cdot 2H_2O$, and 0.0005% $FeSO_4 \cdot 7H_2O$. After autoclaving the basal medium at 120°C for 15 min, MeSH was added at the concentrations of 10 and 30 mg/liter to 100 ml portion of the cooled medium (3°C) in a 500 ml-conical flask fitted with a stopper and a U-tube containing water. Thereafter, the pre-cultured yeast cells were inoculated into the medium (10^4/ml) in a flask, which was horizontally shaken (45 rev/min, 4 cm amplitude) at 25°C. After 72 hr, MeSAc content in the culture broth was measured by the headspace method using a FPD-GC. Yeast cell growth was determined by measuring the turbidity of broth at 660 nm.

As shown in Table I, noticeable amount of MeSAc was detected in culture broth, and it has increased with the added MeSH amounting from 10 to 30 mg/liter into the medium. On the other hand, the medium (a) with MeSH and heat treated yeast cells, or (b) without MeSH or yeast cells was incubated for the control experiment. In these cases, MeSAc production was not observed. These results indicate that approximately 5% of MeSH added to the medium is converted to MeSAc by *S. cerevisiae* IFO 1234. This finding is the first confirmation as far as *Saccharomyces* yeast is concerned.

Cuer *et al.*[6] demonstrated that *B. linens* isolated from cheese was the first microorganism that had the ability to produce MeSAc. They[5] also reported that two *Micrococcus* strains could accumulate 40 and over 50 μg/liter of MeSAc in the dairy medium added with 2 g/liter of MeSH (as CH_3SNa). In considering this report, yeast was incubated in our basal medium containing 2 g/liter of MeSH, but yeast could not grow at all and no MeSAc production was observed. On the other hand, yeast grew up in the basal medium containing 10 and 30 mg/liter of MeSH at similar rate as in the control culture. Furthermore, it was evident that at these low MeSH levels yeast could metabolize MeSH into MeSAc with much higher yield than that obtained with *Micrococcus* strains[5] grown in the culture broth enriched with MeSH.

The pH values of yeast growth media were around 3.2, being much lower than those of *Micrococcus* growth media (5.25 and 6.10).[5] It was reported that chemical hydrolysis of MeSAc proceeded faster as pH value increases[7] and was retarded only slightly even at pH 6.85.[6] This fact suggests that greater portion of MeSAc produced by yeast in our experiment did not hydrolyze chemically. Meanwhile, enzymatic hydrolysis of MeSAc by some strains of *B. linens* was also reported.[6] From this fact, two assumptions are considered: (1) enzyme responsible for the hydrolysis of MeSAc may not be produced in yeast, and (2) if enzyme is produced, it may not be so active as to bring about a significant amount of MeSAc hydrolysis. To ascertain the above mentioned points, further investigations are required.

Methionine has also been thought as an origin of beer MeSAc produced by yeast.[3] *S. cerevisiae* IFO 1234 was cultivated in the basal medium into which various amounts of L-methionine had been added, and the culture broth was

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TABLE I. AMOUNTS OF *S*-METHYL THIOACETATE PRODUCED FROM METHYL MERCAPTAN BY *S. cerevisiae* IFO 1234

Addition of MeSH (mg/ml)	pH value of culture medium	Yeast growth ($OD^{660\ nm}$)	MeSAc (mg/liter)	Yield (%)
0.01	3.1	1.68	0.5	2.7
0.03	3.2	1.66	3.8	6.8

TABLE II. AMOUNTS OF *S*-METHYL THIOACETATE PRODUCED FROM L-METHIONINE BY *S. cerevisiae* IFO 1234

L-Methionine was added to 100 ml of sterilized basal medium in a range from 0.03 to 10 mg/ml. Yeast was inoculated at a concentration of 10^4 cells/ml, and cultivated at 25°C for 72 hr with shaking.

Addition of L-methionine (mg/ml)	pH value of culture medium	Yeast growth ($OD^{660\ nm}$)	MeSAc (mg/liter)	Yield (%)
0	3.2	1.68	n.d.[a]	—
0.03	3.2	1.66	n.d.	—
0.1	3.2	1.66	n.d.	—
1.0	3.2	1.68	0.16	0.026
5.0	3.3	1.67	0.34	0.011
10.0	3.5	1.66	0.28	0.005

[a] Not detected.

assayed for MeSAc concentration. Table II indicates that MeSAc was produced by yeast in the L-methionine-containing medium as described by Leppänen *et al.*[3] However, the yields of MeSAc obtained in the L-methionine enriched media were extremely lower than those in the media containing MeSH. Furthermore, small amounts of MeSH were also detected in the L-methionine added media. Accordingly, it might be expected that a part of L-methionine in wort is first metabolized to MeSH, which is then acetylated to MeSAc by brewer's yeast during the fermentation of wort in the brewery. The detailed studies on MeSAc production by brewer's yeast from MeSH and L-methionine will be reported in the next paper.

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