Note

Production of Characteristic Odors
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We reported that *Neurospora* sp. ATCC 46892 produced a fruity odor, for which ethyl caproate may be responsible,¹⁾ but few studies have been done on odor production by other *Neurospora* strains. This study was aimed at examining the production of odors by several *Neurospora* strains.

We examined 12 *Neurospora* strains, including the ATCC 46892 described previously.¹⁾ *Neurospora* sp. T, which was newly isolated from *beiju*, a fermented food in Brazil, had been obtained from Dr. Satoh (Universidade Estadual de Campinas, Brazil) and was classified by us from its morphological characteristics. Other strains were purchased from the Institute for Fermentation, Osaka, Japan.

These *Neurospora* strains were inoculated into 5% malt broth and grown aerobically at 30°C for several days in a 500-ml shaking flask. Then each culture was filtered through Miracloth (Calbiochem Corp.) or a microfilter (0.45 μ m).

Volatiles were extracted and analyzed. Two hundred and fifty ml of the filtrate obtained was saturated with NaCl and then 125 ml of ethyl acetate containing an internal standard, methyl caproate, was added to extract volatiles. After the mixture was shaken vigorously for one minute by a Mini-Beadbeater (Biospec Products), four microliters of the extract was analyzed by GC or GC-MS.

GC was done with a Perkin-Elmer 8320B capillary gas chromatograph. The conditions were the same as described elsewhere³⁾ with some modifications: column, a bonded fused silica capillary column, 15 m, 0.53 mm i.d., film thickness = 1.0 μ m; stationary phase, DB-WAX from J and W Scientific Inc.; carrier gas, helium at 4.6 ml/min (split ratio = 1/5); column temperature, kept at 70°C for 3 min, raised from 70°C to 100°C at the rate of 10°C per min, from 100°C to 230°C at the rate of 20°C per min and then kept at 230°C for 7.5 min; injection port and detector temperature 250°C.

GC-MS was done with a Hewlett Packard 5970B GC-MS System. The conditions of gas chromatography were

Table I. ODOR FORMATION BY VARIOUS
Neurospora STRAINS

The experimental details are given in the text.

Strain	Odor
<i>Neurospora</i> sp. ATCC 46892	Fruity
<i>Neurospora</i> sp. T	Fruity
<i>Neurospora africana</i> IFO 31378	ND ^a
<i>Neurospora crassa</i> IFO 6067	ND
6068	ND
6178	ND
6966	ND
6977	ND
<i>Neurospora sitophila</i> IFO 4596	Mushroom-like, fruity
6069	ND
6070	ND
<i>Neurospora tetrasperma</i> IFO 6982	ND

^a Not detectable.

Fig. 1. Gas Chromatograms of Volatiles Produced by *Neurospora* Strains.

A, *Neurospora* sp. ATCC 46892; B, *Neurospora* sp. T; C, *Neurospora sitophila* IFO 4596.

1, methyl caproate as an internal standard; 2, isoamyl alcohol; 3, ethyl caproate; 4, acetoin; 5, unknown substance.

Table II. VOLATILES PRODUCED BY THREE *Neurospora* STRAINS
The experimental details are given in the text.

	Isoamyl alcohol (ppm)	Ethyl caproate (ppm)	Acetoin (ppm)	1-Octen- 3-ol (ppm)
<i>Neurospora</i> sp. ATCC 46892	32.2	29.6	19.1	ND ^a
<i>Neurospora</i> sp. T	57.2	17.2	12.0	1.65
<i>N. sitophila</i> IFO 4596	ND	ND	ND	20.1

^a Not detectable.

the same as described above.

All strains had significant growth in malt broth and as shown in Table I, *Neurospora* sp. ATCC 46892, *Neurospora* sp. T, and *N. sitophila* IFO 4596 produced characteristic fruity or mushroom-like aromas in their culture broth, but the other strains produced no remarkable odors under these conditions. Figure 1 shows the gas chromatograms of the volatiles produced in cultures by the 3 odoriferous *Neurospora* strains and Table II shows the results of quantitative analyses of them. Strain ATCC 46892 produced about 30 ppm of ethyl caproate, which caused the fruity odor and strain T produced half. They also produced significant amounts of isoamyl alcohol and acetoin. However strain IFO 4596 did not produce them; instead an unknown substance appeared at the retention time of 8 min. This unknown substance was seen in the culture of strain T but not in that of strain ATCC 46892. Because this substance was thought to be the body of the mushroom odor, we analyzed it by GC-MS. The result coincided with authentic 1-octen-3-ol (Fig. 2). GC analysis also supported this coincidence. Therefore, the unknown substance was identified as 1-octen-3-ol. GC analysis showed that it was produced in quantities of approximately 20 ppm in the culture broth and might cause the mushroom-like odor (Table II). 1-Octen-3-ol had been discovered in an extract of *Armillaria Matsutake* Ito et Imai by Murahashi *et al.*⁴⁾ which was called "Matsutake alcohol." Isoamyl alcohol or acetoin might contribute a little to these odors.

Thus the sensory and analytical evaluations showed that these aromas were mainly caused by ethyl caproate or 1-octen-3-ol which was not produced by *Neurospora* sp. ATCC 46892. Then, as judged by the production of these volatiles having characteristic odors in the malt broth, there were at least three kinds of odoriferous *Neurospora* strains. That is, those with the production of only ethyl caproate, both ethyl caproate and 1-octen-3-ol, and mainly 1-octen-3-ol respectively. However, strain IFO 4596

Fig. 2. GC-MS Spectra of the Unknown and 1-Octen-3-ol.

A, authentic 1-octen-3-ol; B, the unknown.

sometimes produces a little ethyl caproate and *N. sitophila* IFO 6070 and *N. tetrasperma* IFO 6982 rarely produce weak aromas.³⁾

It was reported that 1-octen-3-ol was contained in some kinds of mushrooms⁵⁾ and was produced by various fungi in solid agar media.⁶⁾ To our knowledge, no reports have dealt with the production in liquid cultures by microorganisms like *Neurospora*. The considerable production of 1-octen-3-ol in liquid cultures by these *Neurospora* strains is noteworthy.

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