Volatile Components of the Cyanobacterium *Oscillatoria perornata* (Skuja)

Mario R. Tellez,* Kevin K. Schrader, and Mozaina Kobaisy

Natural Products Utilization Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Box 8048, Oxford, Mississippi 38677

Volatile compounds were identified from unialgal continuous cultures of the cyanobacterium *Oscillatoria perornata*. Steam distillates of the unialgal cultures were continuously extracted with pentane, and the pentane extracts were analyzed by GC-MS. Retention indices and mass spectral data were used to identify 15 components. Relative amounts of individual components were expressed as percent peak area relative to total peak area. The main volatile components were heptadecane (57.0%), 2-methylisoborneol (29.4%), and benzaldehyde (1.2%). Together with the previously known 2-methylisoborneol, which is the major cause of the musty off-flavor problem in catfish farming operations in Mississippi, other components identified were dimethyl disulfide (1.0%), dimethyl trisulfide (0.5%), and benzothiazole (0.6%). These compounds and their organoleptic characteristics are discussed in relation to their possible contributions to cultured catfish off-flavor problems.

Keywords: *Blue-green algae; cyanobacteria; Oscillatoria perornata; catfish; Ictalurus punctatus; polysulfides; 2-methylisoborneol*

INTRODUCTION

With the exception of disease-related problems, “off-flavor” causes the largest economic losses to the channel catfish (*Ictalurus punctatus* L.) production industry in the southeastern United States. Off-flavor catfish are unpalatable and unmarketable and must be held by producers until deemed to be “on-flavor.” Most off-flavor episodes in farm-raised catfish are attributed to their absorption of earthy/musty compounds produced by certain species of cyanobacteria (blue-green algae) that grow in the production ponds. In western Mississippi, the cyanobacterium *Oscillatoria perornata* (Skuja), a producer of the musty-odor compound 2-methylisoborneol, is common and thought to be the major cause of musty off-flavor in farm-raised catfish (*5*). [*O. perornata* (Skuja) was previously identified as *Oscillatora* cf. *chalybea* (*1*), although it does not conform well to the classic description of that species (*2*). It now appears that the appropriate designation of this organism is *Oscillatoria perornata* f. *attenuata* (*3*) or, as recently reassigned, *Planktothrix perornata* f. *attenuata* (Skuja) Anagnostidis and Komárek (*4*).] Other musty compounds, such as 2,6-dimethylpyrazine, 1,8-cineole, camphor, α-campholene aldehyde, 2,2,6-trimethylcyclohexanone, octa-1,3-diene, and geosmin, have been associated with cultures of algae (*Scenedesmus subspicatus*, *Asterionella formosa*, *Synedra delicatissima*, and *Aulacoxiera granulata*) and cyanobacteria (*Anabaena variabilis* and *Anabaena oscillarioides*) (*6*, *7*). Off-flavor problems related to cyanobacteria are not limited to catfish or to Mississippi. Such problems have been reported, for instance, for other fish (*8*) and crustaceae (*9*) and in water supplies and drinking-water treatment plants in the United States [e.g., California (*10*), Pennsylvania (*11*), and Wisconsin (*12*)], Australia (*13*), France (*6*), Japan (*14*), Germany (*7*), and Canada (*15*). Musty and earthy flavors are the most common types of off-flavor found in cultured catfish and have been linked to the presence of a particular cyanobacterial species from the genera *Anabaena*, *Aphanizomenon*, *Nostoc*, *Oscillatoris*, *Lyngbya*, *Phormidium*, *Symploca*, *Schizothrix*, *Fischerella*, and *Hyella* (*16*). Additional common off-flavors in catfish attributed to the presence of unspecified cyanobacteria include “woody” and “pine” (*17*). Off-flavor descriptors in the “decay” category include sewage, rotten, and sulfury (*17*). These odors have been linked to rapid post-bloom (*Oscillatoria* sp., *Anabaena flos-aquae*, and *Microcystis aeruginosa*) decomposition in fresh water reservoirs in Australia (*13*). Little information exists concerning their origin in catfish production ponds, but the assumption has been that decaying organic matter (e.g., dead fish or vegetation such as leaves, aquatic weeds, or hay) is responsible.

Oscillatoria agardhii, *O. perornata*, and *Raphidiopsis brookii* are among the most common species of cyanobacteria found in commercial catfish production ponds in western Mississippi. Van der Ploeg and Tucker (*18*) found that *O. agardhii* and *R. brookii* were not associated with any off-flavor problems in catfish during studies to determine seasonal trends in the types and intensities of catfish flavors. With the exception of 2-methylisoborneol, little else is known about the volatiles produced by *O. perornata*. In the current study, we identified the volatiles produced by *O. perornata* to determine if it produces odorous compounds, in addition to 2-methylisoborneol, that might be responsible for nonmusty types of off-flavor in cultured catfish.

MATERIALS AND METHODS

Culture. A unialgal culture of *O. perornata* [previously isolated from the water of a catfish pond in Mississippi (*5*)] was grown in continuous culture at 29 °C under continuous light according to the method of van der Ploeg et al. (*5*), except

* Corresponding author [telephone (662) 915-5898; fax (662) 915-1035; e-mail matellez@olemiss.edu].

10.1021/jf010722p
Published on Web 10/31/2001

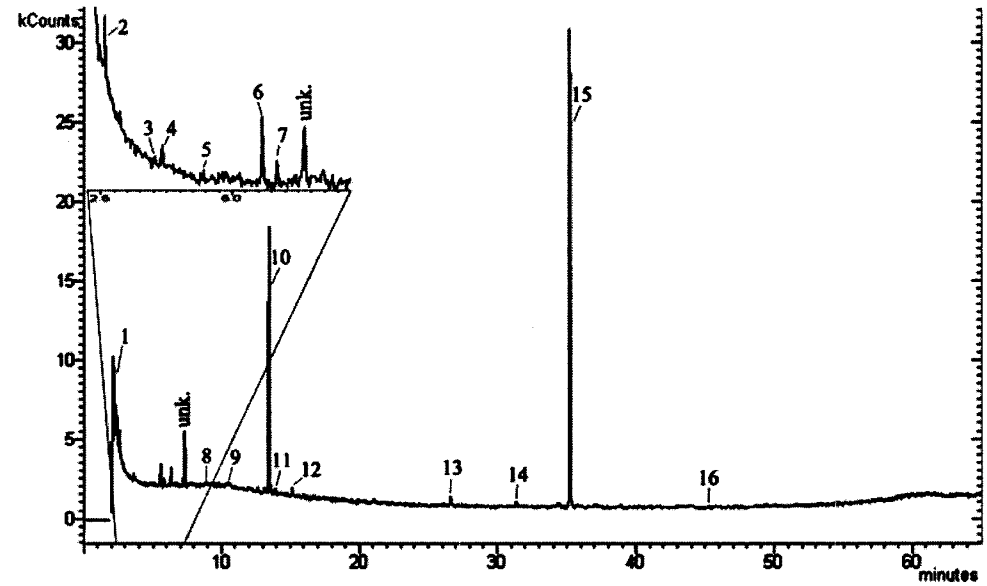

Figure 1. Reconstructed total ion chromatogram of the volatiles of *O. perornatia*: 1, dimethyl disulfide; 2, mesityl oxide; 3, ethylbenzene; 4, *p*-xylene; 5, *o*-xylene; 6, benzaldehyde; 7, dimethyl trisulfide; 8, acetophenone; 9, isophorone; 10, 2-methylisoborneol; 11, dimethyl tetrasulfide; 12, benzothiazole; 13, *E*-β-ionone; 14, hexadecane; 15, heptadecane; 16, cyclooctaatomic sulfur; unk. = unknown; inset is expanded portion of chromatogram from 2.75 to 7.25 min.

the pH of the medium was maintained at 7.7−8.8, photon flux density was 12.0−24.0 μeinstein/m^2/s, and air-flow rate was 16−33 L/h. The continuous culture contained *O. perornata* trichomes at a density that is comparable to that found in catfish ponds. Continuous culture samples were removed periodically and measured spectrophotometrically (Shimadzu model UV-3101PC scanning spectrophotometer, Shimadzu Scientific Instruments, Inc., Columbia, MD), at 750 nm to monitor cell density. Cell density was maintained at 0.20−0.34 absorbance. Dilution rate was adjusted to match the growth rate. Culture material was collected from an effluent bottle.

Steam Distillations. Distillations were carried out in a Likens−Nickerson apparatus as previously described (*19*). Distillations were carried out for 7 h with the aqueous fraction in a 10-L round-bottom flask and the distillate continuously extracted with pentane (10 mL). Distillations were performed on 4.5 L of cyanobacteria in its growth medium (738 μg of cyanobacteria cells dry weight/mL of medium) and on growth medium alone. Pentane extracts of steam distillates were dried over anhydrous magnesium sulfate and filtered, and the volume was reduced (at 0 °C under reduced pressure) 100-fold prior to analysis.

Chemicals. Pentane was obtained from Fisher Scientific, Pittsburgh, PA. All standards were purchased from Aldrich, Milwaukee, WI, with the exception of 2-methylisoborneol and geosmin, which were purchased from Wako Chemicals USA, Inc., Richmond, VA.

Analyses. Analyses were performed by GC-MS (EI, 70 eV) on a Varian CP-3800 gas chromatograph coupled with a Varian Saturn 2000 GC-MS/MS, with a DB-5 column (30 m × 0.25 i.d. mm fused silica capillary column, film thickness = 0.25 μm) using He as carrier gas (1 mL/min at 210 °C), 1 μL injection size (injector temperature = 220 °C, transfer line temperature = 240 °C). The column temperature was programmed from an initial temperature of 60 °C to a final column temperature of 240 °C at 3 °C/min (*19*, *20*). Hydrocarbon standards were used only for determination of retention times, not for quantitation. Identifications of individual components were performed by a comparison of mass spectra with literature data and by a comparison of their relative retention times with those of authentic compounds or by comparison of their retention indices with literature values (*20*). The relative amounts of individual components of the extract are expressed as percent peak area relative to total peak area.

Table 1. Steam-Distilled Components of *O. perornata* in Its Growth Medium

compound	RI[a]	organoleptic properties	relative abundance[b] (%)
dimethyl disulfide	805	foul/putrid to onion/garlic when concentrated (*13*); cooked vegetable or swampy odors (*34*); septic (*7*)	1.0
mesityl oxide	838		0.6
ethylbenzene	878	floral, sweet (*35*)	trace[c]
p-xylene	883	0.4	
o-xylene	896		trace
benzaldehyde	962		1.2
dimethyl trisulfide	972	foul/putrid to onion/garlic when concentrated (*13*); cooked vegetable or swampy odors (*34*); septic (*7*)	0.5
acetophenone	1065		trace
isophorone	1117		trace
2-methylisoborneol	1180	earthy, musty (*36*)	29.4
dimethyl tetrasulfide[d]	1210		trace
benzothiazole	1218	rubbery (*37*)	0.6
E-β-ionone	1484	violets (*7*)	1.0
hexadecane	1600		0.2
heptadecane	1700	odorless (*13*)	57.0
cyclooctaatomic sulfur[e]	2006		trace

[a] Retention index as determined on a DB-5 column using the homologous series of *n*-hydrocarbons. [b] Peak area relative to total area. [c] Trace <0.01%. [d] Tentative assignment based on MS only: *m/z* 162 $[M + 4]^+$ (5), 160 $[M + 2]^+$ (22), 158 $[M]^+$ (98), 94 (32), 79 (63); theoretical isotope ratio for $C_2H_6S_4$: *m/z* 162 $[M + 4]^+$ (1), 160 $[M + 2]^+$ (18), 158 $[M]^+$ (100). [e] Tentative assignment based on MS only: *m/z* 260 $[M + 4]^+$ (5), 258 $[M + 2]^+$ (35), 256 $[M]^+$ (100), 224 (3), 192 (45), 160 (31), 128 (40), 96 (17), 64 (66); theoretical isotope ratio for S_8: *m/z* 260 $[M + 4]^+$ (6), 258 $[M + 2]^+$ (35), 256 $[M]^+$ (100).

RESULTS AND DISCUSSION

The profile of volatiles (Figure 1) obtained from the steam distillations of *O. perornata* culture was fairly simple (Table 1). Fifteen compounds were identified from the distillate, accounting for >90% of the total composition. The major component was heptadecane (57.0%). Heptadecane has been reported to be a common major volatile component in many other cyanobacterial species (*13*, *21*−*23*). The second most abundant component was 2-methylisoborneol (29.4%), responsible for the musty odor associated with this cyanobacteria (*5*). Previously unreported in *O. perornata*, the foul-smelling compounds dimethyl disulfide (1.0%) and dimethyl trisulfide (0.5%) were also found to be components of the volatiles of *O. perornata*. Polysulfides occur in other cyanobacterial species and have been correlated with rapid post-bloom bacterial decomposition (*13*). Dimethyl disulfide and dimethyl trisulfide have been found in catfish fillets (*24*) but have not yet been linked to a specific type of off-flavor problem in catfish. Dimethyl trisulfide has been correlated with off-flavor problems in cheese, prawns, and vegetables (*13*, *25*). The presence of 2-methylisoborneol-related off-flavor in catfish fillets might "mask" the odors contributed by the presence of dimethyl disulfide and dimethyl trisulfide. Further investigations are needed to establish that dimethyl disulfide and dimethyl trisulfide accumulate in catfish during preharvest due to the presence of certain types of cyanobacterial blooms. Other compounds found in the steam-distilled volatiles of *O. perornata* culture include *p*-xylene (0.4%), ethylbenzene (<0.1%), benzaldehyde (1.2%), and benzothiazole (0.6%). The latter three compounds have also been identified in microwaved catfish fillets (*24*). Previous reports of volatiles from cultures of cyanobacteria have identified some alkylbenzenes and benzaldehyde as artifacts of the culture medium (*26*). We did not detect these compounds in the steam distillate of our growth medium. Benzothiazole can be found in certain foods after heating. Examples include cultured tiger prawn (*27*) and dairy products such as pasteurized milk (*28*) and yogurt (*29*). Other studies (*30*) indicate that benzothiazole derivatives are pyrolytic products of cyanobacteria. Although benzothiazole might accumulate in catfish during both preharvest and food preparation, because catfish fillets are usually subjected to steaming or other high-temperature procedures, it is currently not a major concern because "rubbery" is not cited as a common off-flavor encountered in catfish from commercial aquaculture ponds in the southeastern United States (*17*). Geosmin, an off-flavor compound commonly associated with other species of cyanobacteria including several *Oscillatoria* species (*31*−*33*), was not detected in *O. perornata* in our study.

In conclusion, several volatile compounds produced by *O. perornata*, aside from the previously known 2-methylisoborneol, might contribute to off-flavor problems in catfish farming operations in Mississippi, but further investigations are needed to establish that they accumulate in catfish during preharvest.

LITERATURE CITED

(1) Martin, J. F.; Izaguirre, G.; Waterstrat, P. A planktonic *Oscillatoria* species from Mississippi catfish ponds that produces the off-flavor compound 2-methylisoborneol. *Water Res.* **1991**, *25*, 1447−1451.

(2) Gomont, M. Monographie des oscillariées (Nostocacées Homocystées). *Ann. Sci. Nat. Bot. VII* **1892**, *15*, 263−368 (reprinted by Wheldon and Wesley, Hafner Publishing: New York, 1962).

(3) Skuja, H. Süsswasseralgenflora burmas. *Nova Acta Regiae Soc. Sci. Ups., Ser. 4* **1949**, *14*, 1−188.

(4) Anagnostidis, K.; Komárek, J. Modern approach to the classification system of cyanophytes. 3—Oscillatoriales. *Arch. Hydrobiol.* **1988**, *1*−*4* (Suppl. 80), 327−472.

(5) van der Ploeg, M.; Dennis, M. E.; de Regt, M. Q. Biology of *Oscillatoria* cf. *chalybea*, a 2-methylisoborneol producing blue-green alga of Mississippi catfish ponds. *Water Sci. Technol.* **1995**, *31*, 173−180.

(6) Cotsaris, E.; Bruchet, J.; Mallevialle, J.; Bursill, D. B. The identification of odorous metabolites produced from algal monocultures. *Water Sci. Technol.* **1995**, *31*, 251−258.

(7) Mohren S.; Jüttner, F. Odorous compounds of different strains of *Anabaena* and *Nostoc* (cyanobacteria). *Water Sci. Technol.* **1983**, *15*, 221−228.

(8) Whitfield, F. B.; Shaw, K. J.; Svoronos, D. Effect of the natural environment on the flavor of seafoods: the flavor of *Girella tricuspidata*. *Dev. Food Sci.* **1994**, *35*, 417−420.

(9) Whitfield, F. B.; Last, J. H.; Shaw, K. J.; Tindale, C. R. 2,6-Dibromophenol: the cause of an iodoform-like off-flavor in some Australian crustacea. *J. Sci. Food Agric.* **1988**, *46*, 29−42.

(10) Seligman, K.; Enos, A. K.; Lai, H. H. A comparison of 1988−1990 flavor profile analysis results with water conditions in two northern California reservoirs. *Water Sci. Technol.* **1992**, *25*, 19−25.

(11) Burlingame, G. A.; Muldowney, J. J.; Maddrey, R. E. Cucumber flavor in Philadelphia's drinking water. *J. Am. Water Works Assoc.* **1992**, *84*, 92−97.

(12) Young, C. C.; Suffet, I. H.; Crozes, G.; Bruchet, A. Identification of a wood-hay odor-causing compound in a drinking water supply. *Water Sci. Technol.* **1999**, *40*, 273−278.

(13) Hayes, P. K.; Burch, M. D. Odorous compounds associated with algal blooms in south Australian waters. *Water Res.* **1989**, *23*, 115−121.

(14) Hosaka, M.; Murata, K.; Likura, Y.; Oshimi, A.; Udagawa, T. Off-flavor problem in dinking water of Tokyo arising from the occurrence of musty odor in a downstream tributary. *Water Sci. Technol.* **1995**, *31*, 29−34.

(15) Slater, G. P.; Blok, V. C. Isolation and identification of odorous compounds from a lake subject to cyanobacterial blooms. *Water Sci. Technol.* **1983**, *15*, 229−240.

(16) Tucker, C. S. Off-flavor problems in aquaculture. *Rev. Fish. Sci.* **2000**, *8*, 45−88.

(17) van der Ploeg, M. *Testing Flavor Quality of Preharvest Channel Catfish*; Southern Regional Aquaculture Center Publication 431; Stoneville, MS, 1991.

(18) van der Ploeg, M.; Tucker, C. S. Seasonal trends in flavor quality of channel catfish, *Ictalurus punctatus*, from commercial ponds in Mississippi. In *Recent Developments in Catfish Aquaculture*; Tave, D., Tucker, C. S., Eds.; Haworth Press: Binghamton, NY, 1994; pp 121−140.

(19) Tellez, M. R.; Dayan, F. E.; Schrader, K. K.; Wedge, D. E.; Duke, S. O. Composition and some biological activities of the essential oil of *Callicarpa americana* (L.). *J. Agric. Food Chem.* **2000**, *48*, 3008−3012.

(20) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*; Allured Publishing: Carol Stream, IL, 1995.

(21) Tsuchiya Y.; Matsumoto, A. Identification of volatile metabolites produced by blue-green algae. *Water Sci. Technol.* **1988**, *20*, 149−155.

(22) Yasuhara, A.; Fuwa, K. Characterization of odorous compounds in rotten blue-green algae. *Agric. Biol. Chem.* **1982**, *46*, 1761−1766.

(23) Rezanca, T.; Zahradnik, J.; Podojil, M. Hydrocarbons in green and blue-green algae. *Folia Microbiol.* **1982**, *27*, 450−454.

(24) Grimm, C. C.; Lloyd, S. W.; Batista R.; Zimba, P. V. Using microwave distillation-solid-phase microextraction-gas chromatography-mass spectrometry for analyzing fish tissue. *J. Chromatogr. Sci.* **2000**, *38*, 289−296.

(25) Whitfield, F. B.; Freeman, D. J.; Banister, P. A. Dimethyl trisulphide: an important off-flavour component in the Royal Red Prawn (*Hymnopteraeus sibogae*). *Chem. Ind.* **1981**, 316−317.

(26) Jüttner, F.; Leonhardt, J.; Mohren, S. Environmental factors affecting the formation of mesityloxide, dimethylallylic alcohol and other volatile compounds excreted by *Anabaena cylindrica*. *J. Gen. Microbiol.* **1983**, *129*, 407−412.
(27) Pan, B. S.; Tsai, J. R.; Chen, L. M.; Wu, C. M. Lipoxygenase and sulfur-containing amino acids in seafood flavor formation. In *Flavor and Lipid Chemistry of Seafoods*; ACS Symposium Series 674; American Chemical Society: Washington, DC, 1997; pp 64−75.
(28) Moio, L.; Etievant, P.; Langlois, D.; Dekimpe, J.; Addeo, F. Detection of powerful odorants in heated milk by use of extract dilution sniffing analysis. *J. Dairy Res.* **1994**, *61*, 385−394.
(29) Ott, A.; Fay, L. B.; Chaintreau, A. Determination and origin of the aroma impact compounds of yogurt flavor. *J. Agric. Food Chem*. **1997**, *45*, 850−858.
(30) Henatsch, J. J.; Jüttner, F. Volatile odorous excretion products of different strains of *Synechococcus* (cyanobacteria). *Water Sci. Technol.* **1983**, *15*, 259−266.
(31) Sugiura, N.; Iwami, N.; Inamorfi, Y.; Nishimura, O.; Sudo, R. Significance of attached cyanobacteria relevant to the occurrence of musty odor in Lake Kasumigaura. *Water Res.* **1998**, *32*, 3549−3554.
(32) Utliken, H. C.; Froeshaug, M. Geosmin production and excretion in a planktonic and benthic *Oscillatoria*. *Water Sci. Technol.* **1992**, *25*, 199−206.
(33) Nakashima, S.; Yagi, M. Iron forms that influence the growth and musty odor production of selected cyanobacteria. *Water Sci. Technol.* **1992**, *25*, 207−216.
(34) Wajon, J. E.; Alexander, R.; Kagi, R. I. Dimethyl trisulphide and objectionable odours in potable water. *Chemosphere* **1985**, *14*, 85−89.
(35) Khiari, D.; Brenner, L.; Burlingame, G. A.; Suffet, I. H. Sensory gas chromatography for evaluation of taste and odor events in drinking water. *Water Sci. Technol.* **1992**, *25*, 97−104.
(36) Gerber, N. N. A volatile metabolite of actinomycetes, 2-methylisoborneol. *J. Antibiot.* **1969**, *22*, 508−509.
(37) Friedrich, E. F.; Acree, T. E. Gas chromatography olfactometry (GC/O) of dairy products. *Int. Dairy J.* **1998**, *8*, 235−241.

Received for review June 4, 2001. Revised manuscript received September 19, 2001. Accepted September 20, 2001.

JF010722P