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Relation of algae to earthy odors of fish in Taiwan

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Abstract. The occurrence of earthy odors in fish ponds of Taiwan was found to meet well with the presence of three species of blue-green algae. They were *Anabaena macrospora* var. *crassa*, *A. viguieri* and *Oscillatoria tenuis*. The axenic cultures of the isolates of these three species exhibited the characteristic earthy and/or musty odors. The closed-loop stripping technique was applied to concentrate the odorous compounds produced by algae. The subsequent gas chromatographic analysis showed that all these three species were capable of producing geosmin. *O. tenuis* produced, in addition to geosmin, also 2-methylisoborneol simultaneously. The quantitative study of the extra- and intracellular distributions of odorous compounds in the cultures was performed. The production of odorous compounds by the above three species was compared.

Key words: *Anabaena*; Earthy odor; Fish pond; Geosmin; 2-Methylisoborneol; *Oscillatoria*.

Introduction

Earthy odors are common problem for drinking water and aquaculture. This problem has been established to be due to the odorous compounds such as geosmin, 2-methylisoborneol and, in rare case, mucidone (Lovell, 1976; Tsuchiya *et al.*, 1979; Yurkowski and Tabachek, 1980; Mashni and Safferman, 1982; Persson, 1983; Burlingame *et al.*, 1986). These compounds are produced either by algae (Safferman *et al.*, 1967; Kikuchi *et al.*, 1973; Tabachek and Yurkowski, 1976; Izaguirre *et al.*, 1982) or actinomycetes (Gerber and Lechevalier, 1965; Medsker *et al.*, 1968). Also the chemical structure of these compounds has been determined by Gerber (1968) and Medsker *et al.* (1969).

The occurrence of earthy odors in aquaculture of Taiwan has been a serious problem. Some of the main cultured freshwater fishes such as tilapia (*Oreochromis* sp.), grass carp (*Ctenopharyngodon idella* Valenciensees) and Japanese eel (*Anguilla japonica* Temminck et Schlegel) have been found to suffer from such problem. A preliminary investigation showed that it seemed to exist a correlation between the presence of some algal species and the occurrence of earthy odors. In order to further establish this relation, the study in this paper was approached by investigating the species composition of phytoplankton in fish pond, by cultivating the suspected species isolated from fish pond and consequently by chemically determining the odorous compounds produced by algal isolates. The production and extra- and intracellular distributions of odorous com-

pounds in the cultures were also studied.

Materials and Methods

Phytoplankton samples from 10 fish ponds situated at north and south of Taiwan were collected every month from July 1985 to June 1986. The algal species were identified under microscope and their frequency of presence was calculated based on 1,000 counts. The diversity of algal communities was calculated according to Shannon and Weaver (1949). The extent of odor in fish was evaluated by means of sensory taste. Fishes were cooked in a microwave oven immediately after they were captured from fish pond. The evaluation of odor was performed by The Taiwan Fishery Research Institute in Tzupei and Tainan.

The algal species, which were suspected to be accounting for the odorous problem, were isolated from the fish ponds and purified to axenic condition. The isolates were then cultivated at 28°C with illumination of 4 kLux and aeration of air containing 0.3% CO_2. The procedures for the desorption of odorus compounds from the cells and the subsequent stripping technique were the same as described previously (Jüttner, 1988; Wu and Jüttner, 1988). The odorous compounds were absorbed by Tenax and then analyzed by gas chromatography. The kind of odorous compounds was determined according to the retention time on chromatograms and the comparison with that of authentic compounds of geosmin and 2-methylisoborneol (MIB). Borneol was added to samples as the internal standard. The protein content of the cells was quantified by the modified method of Lowry (Schacterle and Pollack, 1973). Chlorophyll *a* was calculated by using the absorption coefficient given by Ogawa and Vernon (1971), after it had been extracted from the cells with methanol and read at 665 nm.

Results

In the 10 fish ponds studied, the occurrence of earthy odors in fish did not meet with the presence of the dominant species such as *Microcystis, Spirulina, Phormidium, Chlamydomonas, Chlorella, Euglena, Trachelomonas, Pediastrum* or *Oscillatoria* other than *O. tenuis*. Figs. 1 to 3 show that earthy odors was occurred concomitantly with the presence of three blue-green algae species instead. They were *Anabaena macrospora* var. *crassa, A. viguieri* and *O. tenuis*. In fish ponds T4 (Fig. 2) and T5 (Fig. 3), *A. macrospora* var. *crassa* and *O. tenuis* were found to present simultaneously in June and January 1986 respectively. The concomitance of the presence of these three algal species with the earthy odors

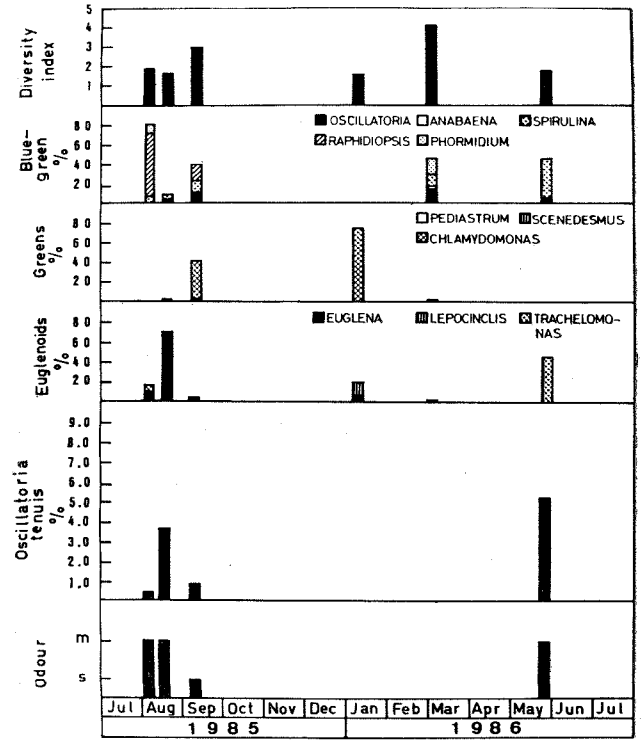

Fig. 1. Changes of percentage composition and speceis diversity index of phytoplankton in fish pond N1 from August 1985 to May 1986, showing that *Oscillatoria tenuis* presented concomitantly with the occurrence of earthy odor in fish. Extent of odor: m=moderate; s=slight.

in fish was not valid in every case. In fish pond T4, there was no suspected algal species that might be accounting for the occurrence of earthy odors in fish in February 1986. It is noteworthy that it exists no correlation between the occurrence of earthy odors in fish and the diversity index of algal communities in fish ponds. The algal species that were considered to be accounting for the earthy odors of fish are illustrated in Table 1.

The axenic cultures of the isolates of the

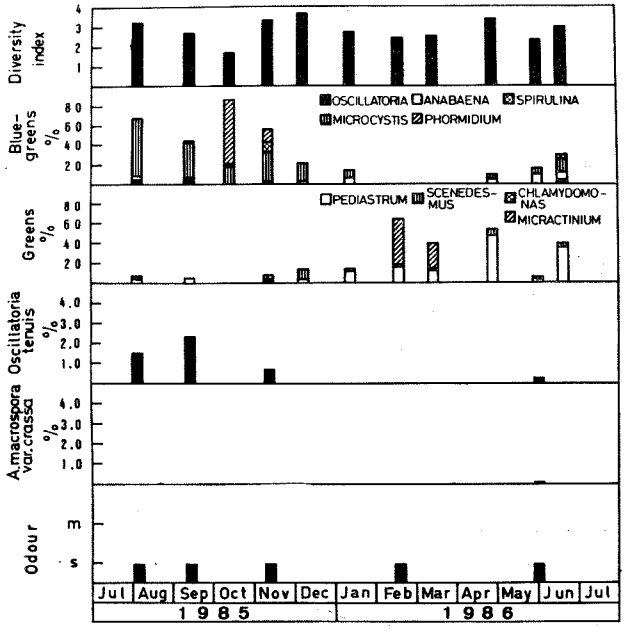

Fig. 2. Changes of percentage composition and species diversity index of phytoplankton in fish pond T4 from August 1985 to June 1986, showing that *Oscillatoria tenuis* and *Anabaena macrospora* var. *crassa* presented concomitantly with the occurrence of earthy odor in fish. Extent of odor: m=moderate; s=slight.

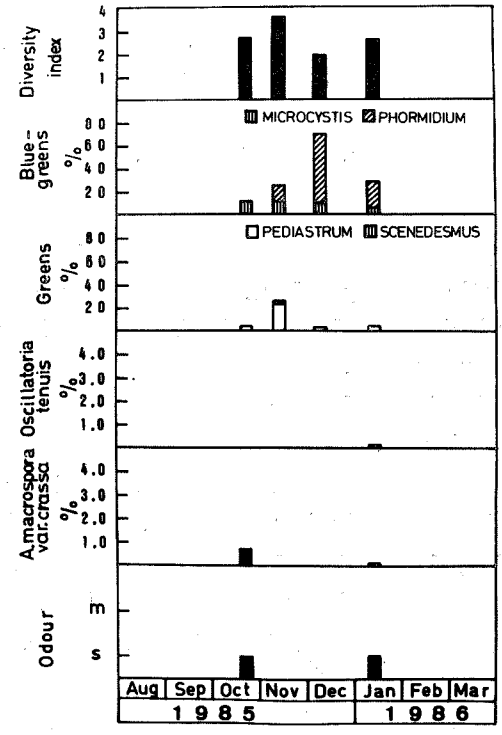

Fig. 3. Changes of percentage composition and species diversity index of phytoplankton in fish pond T5 from October 1985 to January 1986, showing that *Anabaena macrospora* var. *crassa* and *Oscillatoria tenuis* presented concomitantly with the occurrence of earthy odor in fish. Extent of odor: m=moderate; s=slight.

Table 1. *Location of the fish ponds investigated and the algal species suspected to be accounting for the earthy odor in fish.*

Fish pond	Location	Algal species[a]	Fish pond	Location	Algal species
T3	Hsintzu	1+3	N 1	Tainan	3
T4	Hsintzu	1+3	N 2	Tainan	3
T5	Hsintzu	1+3	N 3	Tainan	2
K1	Kaoshiung	3	N 5	Tainan	3
K2	Kaoshiung	3	N 7	Tainan	3

[a] Algal species 1=*Anabaena macrospora* var. *crassa*; 2=*A. viguieri*; 3=*Oscillatoria tenuis*.

Table 2. *Kind and concentrations in terms of protein and chlorophyll a contents of the odorous compounds produced by* ***Anabaena macrospora*** *var.* ***crassa, A. viguieri*** *and* ***Oscillatoria tenuis*** *grown at 28°C*

Algal species	Geosmin		2-Methylisoborneol	
	ng/μg protein	ng/μg chloro.	ng/μg protein	ng/μg chloro.
A. macrospora var. *crassa*	4.0±0.4	304±24	n.d.*	n.d.
A. viguieri	4.2±0.6	319±29	n.d.	n.d.
O. tenuis	9.2±2.3	501±48	4.8±0.8	267±28

* n.d.: not detectable.

above two *Anabaena* speceis exhibited the characteristic earthy odors. The cultures of *O. tenuis* isolates had the mixed odors of musty and earthy one. The gas chromatographic analysis of the odorous compounds produced by these cultures, as shown in Table 2, indicated that the isolates of all the three species were capable of producing geosmin. *O. tenuis*, in addition, also produced 2-methylisoborneol. The concentrations of odorous compounds produced by algae were various in different species. The isolates of two *Anabaena* species nearly produced the same amount of geosmin in regard to protein content as well as chlorophyll *a* concentration. *O. tenuis* produced geosmin about twice as much as that done by *Anabaena* isolates. The ratio of geosmin to 2-methylisoborneol produced by *O. tenuis* was about 1.8.

In the cultures of *O. tenuis*, the majority of the both odorous compounds, geosmin and 2-methylisoborneol, had been revealed to be retained in the cells rather than released to the extracellular medium and the air (Wu and Jüttner, in preparation). The same experiment done by cultivating the cells in the fermentation flask and measuring the distribution of odorous compounds in the culture was made with *A. viguieri*. The results showed that also the majority, about 98%, of geosmin produced by this alga was retained in the cells.

Discussion

Based on the investigation of phytoplankton in fish ponds it was pointed out that it existed an ecological concomitance of the presence of algal species and the occurrence of earthy odor in fish. The cultivation of algal isolates and the gas chromatographic analysis of odorous compounds produced by isolates further provide the evidences that *A. macrospora* var. *crassa, A. viguieri* and *O. tenuis* are capable of producing the earthy and/or musty odorous substances: geosmin and MIB. It is therefore established that these three species are probably the algae accounting for the earthy odor in fish.

The ecological investigation has showed that *O. tenuis* is more widely distributed in Taiwan than *Anabaena* species. In addition, the gas chromatographic analysis of odorous compounds indicated that *O. tenuis* produced geosmin and 2-methylisoborneol simultaneously. The quantitation of the production of these odorous compounds showed furthermore that this species produced much more odorous compounds than *Anabaena* species. From the quantitation of odorous compound production or the ecological point of view, *O. tenuis* is the main species accounting for the earthy odors of fish in Taiwan.

Both the results from the experiment with

O. tenuis (Wu and Jüttner, in preparation) and that with *A. viguieri* pointed out that the majority of geosmin as well as 2-methylisoborneol produced by these algae was retained in the cells. The same fact has also been revealed in the cultures of *Fischerella muscicola* (Wu and Jüttner, 1988). Lovell (1979) had mentioned that the odorous problem in fish was resulted either from the direct digestion of algal cells by fish or from the absorption of odorous compounds dissolved in water by the fish through the gills. Our results indicate that the majority of the odorous compounds produced by algae is retained in the cells rather than dissolved in water. It is therefore more likely that earthy odors in fish are resulted majorily from the digestion of algal cells by fish.

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藻類與臺灣養殖魚體泥土味之關係

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從養殖魚池之藻類調查中發現，本地10個養殖池之魚體帶泥土味，其泥土味出現之時間和三種藍綠藻之存在一致。此三種爲 *Anabaena macrospora* var. *crassa*, *A. viguieri* 和 *Oscillatoria tenuis*。經分離純化之藻種培養，確會產生泥土異味。以氣相層析法作異味成份之定性定量時，發現三藻種均會產生土味素 (geosmin)。*O. tenuis* 並會產生 2-methyl-isoborneol. 此種分佈最廣，是造成本地泥土味之主要藻種。本文對異味成份在細胞內外之分佈作探討，並對不同藻種所產生之異味成份予定量比較。