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Microcystis aeruginosa/*Pseudomonas pseudoalcaligenes* interaction effects on off-flavors in algae/bacteria co-culture system under different temperatures

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ABSTRACT

We conducted an experiment to study the interaction effects of *Microcystis aeruginosa* and *Pseudomonas pseudoalcaligenes* on off-flavors in an algae/bacteria co-culture system at three temperatures (24, 28 and 32°C). Gas chromatography–mass spectrometry was applied to measure off-flavor compounds dimethyl sulfide (DMS), dimethyl trisulfide (DMTS), 2-methylisoborneol, geosmin (GEO) and β-cyclocitral. During the lag phase of co-cultured *M. aeruginosa* (first 15 days), *P. pseudoalcaligenes* significantly increased the production of DMS, DMTS and β-cyclocitral at all three temperatures. In the exponential phase of co-cultured *M. aeruginosa* (after 15 days), *M. aeruginosa* became the main factor on off-flavors in the co-culture system, and β-cyclocitral turned to the highest off-flavor compound. These results also indicated that DMS, DMTS and β-cyclocitral were the main off-flavor compounds in our *M. aeruginosa*/*P. pseudoalcaligenes* co-culture system. Univariate analysis was applied to investigate the effects of *M. aeruginosa* and *P. pseudoalcaligenes* on the production of off-flavors. The results demonstrated that both *M. aeruginosa* and *P. pseudoalcaligenes* could increase the production of DMS and DMTS, while β-cyclocitral was mainly determined by *M. aeruginosa*. Our results also provide some insights into understanding the relationship between cyanobacteria and heterotrophic bacteria.

Introduction

In the past decades, increasing eutrophication has led to frequent outbreaks of cyanobacterial blooms (mainly *Microcystis aeruginosa*) in many lakes around the world. Growth and decay of these blooms caused off-flavor episode, and off-flavor episodes are becoming a worldwide problem in aquatic environments, especially in eutrophic lakes and reservoirs in China (Yu et al., 2009). Since 2005, episodes of strong earthy-musty off-flavors have occurred every year, which lead to off-flavor contamination of drinking water and aquaculture systems in the Xionghe Reservoir (Hubei, China) (Zhang et al., 2010a). It also has triggered a serious drinking water pollution incident when a dense cyanobacterial bloom

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occurred in Taihu Lake during the summer in 2007 (Chen et al., 2010; Yang et al., 2008). These nuisance cases have resulted in large economic losses to the aquaculture industry, negative esthetic impacts to many tourist sites and large increases in the water treatment costs (Li et al., 2007).

Off-flavors in water supplies usually originate from volatile organic compounds (VOCs) including 2-methylisoborneol (MIB) (Rosen et al., 1968), geosmin (GEO) (Guttman and van Rijn, 2009), dimethyl sulfide (DMS) (Giger and Schaffner, 1981), methyl thiols, dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS) (Zhang et al., 2010b), β-cyclocitral (Ozaki et al., 2008), ethanethiol (Slater and Blok, 1983), 2-isopropyl-3-methoxypyrazine (Buttery and Ling, 1973) and β-ionone (Höckelmann and Jüttner, 2005). Eutrophic lakes were complicated ecosystems and studies about off-flavors should be discussed in regard to various influencing factors. In the plankton, cyanobacteria and heterotrophic bacteria are the dominant organisms, and their metabolism largely controls energy flow and nutrient cycling in aquatic ecosystems (Cole et al., 1982). Off-flavor episodes were related to physiological change of cyanobacteria and heterotrophic bacteria. Cyanobacteria blooms affect both the nature and the magnitude of their metabolite-related impacts on water quality. In more eutrophic systems, cyanobacteria are frequent sources of VOCs, such as terpenoids, thiols, and pigment derivatives (Watson et al., 2008). Meanwhile, phototrophic non-sulfur bacteria could produce methylated sulfur compounds, such as DMS and DMDS (McCarthy et al., 1993). Several works have been carried out to study the off-flavors from single aspects, such as sources and dynamics or the effects of temperature and light on the production of off-flavors (Klausen et al., 2005; Zhang et al., 2009). However, there are few studies which pay attention to the interactions between cyanobacteria and heterotrophic bacteria on the off-flavors.

Pseudomonas pseudoalcaligenes belonged to Pseudomonas species, which had been demonstrated to play a pivotal role of DMTS in cyanobacterial bloom of Taihu Lake in 2007. Detected DMTS at concentrations of 11,399 and 1768 ng/L in drinking-water intake and the water agglomerate, respectively-high enough to account for the odors (Yang et al., 2008). Thus we selected *P. pseudoalcaligenes* and *M. aeruginosa* in co-culture system to investigate the effects of *M. aeruginosa* and *P. pseudoalcaligenes* on the production of off-flavor compounds, such as DMS, DMTS, MIB, GEO and β-cyclocitral. Our results also provide new data for understanding the mutual interactions between *M. aeruginosa* and *P. pseudoalcaligenes* in co-culture systems.

1. Materials and methods

1.1. Strains, cultures and experimental design

P. pseudoalcaligenes was isolated in our laboratory from a sampling site in Gonghu Bay in Taihu Lake (Fig. 1) which was the site of the Wuxi odor accident in 2007 (Yang et al., 2008). Water samples were collected in the August of 2008. At the time of sampling, a large part of the lake surface was covered with dense cyanobacterial blooms, consisting mainly of *M. aeruginosa* (Niu et al., 2011).

The unicellular toxic *M. aeruginosa* used in the experiment was obtained from the Culture Collections of Freshwater Algae at the Institute of Hydrobiology (Wuhan, China). In order to eliminate the effects from contaminating bacteria, *M. aeruginosa* was purified using a streak plate on BG-11 agar medium (Stanier et al., 1971) before the experiment was carried out, and then grown in batch culture in BG-11 medium at (25 ± 1)°C illuminated in a 12 hr:12 hr of light:dark cycle at irradiance of 50 μE/(m^2·sec).

The axenic cultures were transferred weekly to fresh medium and maintained in exponential phase. Regular inspection using 4′,6-diamidino-2-phenylindole (DAPI) staining, in conjunction with epifluorescence microscope, showed that cultures were axenic at the beginning of the experiment and that the biomass of bacteria in the axenic cultures never exceeded 1% of *Microcystis* biomass during the experiment. Each step of the isolation procedure was carried out under sterile conditions.

A co-culture system was designed as independent axenic *M. aeruginosa* and *P. pseudoalcaligenes* (control), and co-cultured axenic *M. aeruginosa* with *P. pseudoalcaligenes* (treatment) groups. The initial concentrations of *M. aeruginosa* and *P. pseudoalcaligenes* were 1×10^5 and 1×10^7 cells/mL, respectively. Each treatment was prepared in triplicate and the experiment lasted 33 days. The batch cultures were maintained at 24, 28 and 32°C and at irradiance of approximately 20 μE/(m^2·sec). The cultures were harvested every third day to monitor changes in growth and off-flavor compounds. Samples for gas chromatography–mass spectrometry (GC–MS) analysis were filtered and stored at −20°C prior to analysis.

1.2. *Microcystis* cell count and Chlorophyll *a* determination

After staining with Lugol's solution, *Microcystis* cells were enumerated in a hemocytometer using an Olympus BX50 microscope at 600× magnification (Olympus, Tokyo, Japan). Chlorophyll *a* (Chl-*a*) concentrations in *M. aeruginosa* cells were measured after extraction overnight in 80% acetone at 4°C in the dark. The supernatant was collected by centrifugation and then analyzed at 665 nm using a spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan) with an 80% acetone blank. The concentrations of Chl-*a* were calculated according to Wellburn (MacGregor et al., 2001).

1.3. Bacterial abundance

Samples for determination of bacterial abundance were preserved with 4% (*V/V*) formaldehyde. The fixed sample of 0.5–2 mL was stained with 1 μg/mL DAPI for 10–15 min (Porter and Feig, 1980). Afterwards, the sample was gently filtered onto a 0.22 μm pore size black polycarbonate filter (Whatman, Maidstone, UK). Total bacterial cell numbers were counted using epifluorescence microscope (Zeiss Axioskop 20, Oberkochen, Germany). A minimum of 10 replicates were counted for each sample.

1.4. Determination of off-flavors

The dissolved off-flavors in water were analyzed by GC-MS according to previous studies (Chen et al., 2010). The instrumentation used for analysis the off-flavor compounds