Elucidation of the Triggers on Musty Odor Production by Cyanobacteria

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Abstract

Over the past few decades, eutrophication and climate change caused by excessive human activities have led to severe cyanobacterial blooms, bringing about many water quality problems including toxic compounds as well as taste and odor (T&O) in drinking water sources. Although these odor compounds have no toxic risk to human health, they could decline the water organoleptic quality and affect the aquaculture, triggering profound socio-economic impacts. T&O problems can cause consumer caution, distrust, and complaints about the drinking water supply, resulting in significant treatment costs for the drinking water industry. Geosmin and 2-methylisoborneol (2-MIB) are the most common earthy/musty odor compounds with an extremely low human odor threshold concentrations (OTCs) (< 10 ng/L), which are mainly produced by cyanobacteria in water bodies. The challenges posed by geosmin/2-MIB events in drinking water around the world have led to concerns about this issue. Many studies have elucidated the cell growth and odor production of cyanobacteria under different environmental conditions. However, the triggers of geosmin/2-MIB synthesis remain unclear due to the inconsistent results based on the previous researches. Up to now, no clear pattern between geosmin/2-MIB production and environmental factors has been concluded, which creates difficulties and challenges for effective monitoring and control of T&O issues. Thus, more knowledge and evidence are needed to develop the theories and mechanisms regarding geosmin/2-MIB production in cyanobacteria.

In this thesis, the effects of nitrate (NO_3-N), temperature and magnesium ion (Mg^{2+}) on cell growth and geosmin/2-MIB production of cyanobacteria were investigated. More specifically, expression levels of one geosmin synthase gene (*geoA*) and two 2-MIB synthase genes (*mtf* and *mtc*) under different conditions were explored, aiming to elucidate the triggering factors on geosmin/2-MIB production at molecular level.

Results showed that elevated NO_3-N concentration promoted the production of chlorophyll *a* (Chl-*a*), whereas the geosmin synthesis decreased in *Dolichospermum smithii* NIES-824 (*D. smithii*). However, higher 2-MIB production and lower Chl-*a* content were obtained under the higher NO_3-N condition in *Pseudanabaena foetida* NIES-512 (*P. foetida*). The *geoA* gene expression of *D. smithii* was decreased at the higher NO_3-N concentration during the exponential growth phase of cyanobacterial cells, while the *mtf* and *mtc* gene expression of *P. foetida* increased at the higher NO_3-N concentration. The decrease of *geoA* expression during the decline phase suggested that *geoA* transcription was

closely related to cell activity and isoprenoid productivity. The expression levels of two 2-MIB synthase genes revealed significant positive correlation.

Of the three temperatures tested (15, 25 and 35°C), the cells growth of *D. smithii* and *P. foetida* at low temperature (15°C) was obviously suppressed, indicated by the lower Chl-*a* content and growth rate than those at 25°C and 35°C. The maximum Chl-*a* content was detected at 25°C in both *D. smithii* and *P. foetida*. Besides, the maximum total geosmin concentration (19.82 μg/L) of *D. smithii* was detected at 25°C. The total 2-MIB concentration (82.5 μg/L) of *P. foetida* was the highest at 35°C. However, the gene expression levels of target genes relating to geosmin and 2-MIB synthesis at 15°C were significantly higher than those at 25°C and 35°C. Higher temperature (35°C) also upregulated gene expression levels of *mtf* and *mtc* gene in *P. foetida* compared with those at 25°C. Therefore, unfavorable temperature can increase the potential of geosmin/2-MIB synthesis from gene expression level.

Among the four concentrations of Mg^{2+} (0, 10, 24 and 50 mg/L), *D. smithii* and *P. foetida* could not grow normally at 0 mg/L Mg^{2+}. The minimum cell density and Chl-*a* content as well as total geosmin/2-MIB concentration were obtained under this Mg^{2+}-limited condition. While the maximum cell density, Chl-*a* content and total geosmin/2-MIB concentration were noticed at 10 mg/L Mg^{2+}. Excessive Mg^{2+} (50 mg/L) can also suppress the cell growth of *D. smithii* and *P. foetida*, as the cell density and Chl-*a* content were lower than those at 10 mg/L Mg^{2+}. Moreover, the expression levels of *geoA*, *mtf* and *mtc* gene were the lowest at 0 mg/L Mg^{2+}, while the excessive Mg^{2+} (50 mg/L) promoted the target gene expression levels compared with those at 10 mg/L Mg^{2+}.

The present study can contribute to a better understanding and management of T&O problems and provide fundamental knowledge of geosmin/2-MIB production.

Keywords: Geosmin; 2-methylisoborneol; Cyanobacteria; Earthy/musty odor; Chlorophyll *a*; Gene expression; Nitrate; Temperature; Magnesium