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Research Article

The Effect of Temperature on Growth and Odour Production in Three Cyanobacteria Species

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ABSTRACT

Taste and odour episodes associated with geosmin and 2-methylisoborneol (MIB) produced by Cyanobacteria are common problems affecting drinking water supplies. However, it is difficult to find the source and species responsible for taste and odour production. The aim of this study was to investigate the effect of temperature, one of the most important environmental factors, on the growth and odour production of *Microcoleus* sp. MCAS-MC01 and compare the results with those of two other odour-producing cyanobacteria (*Oscillatoria* sp. UHCC 0332 and *Phormidium* sp. NIVA-CYA 7) isolated and kept in culture collections from geographically different areas. *Microcoleus* sp. MCAS-MC01 is a newly isolated geosmin and MIB producer from Türkiye. After complaints arose from consumers in a nearby city, samples were taken and a cyanobacterium, *Microcoleus* sp., was isolated from the samples and grown in Z8 medium. Experiments were conducted at 20°C and 25°C for 10 weeks, and the growth and MIB-geosmin concentrations of cultures were monitored weekly. The geosmin concentration reached a maximum 65.73 µg/l and the maximum MIB concentration was 626.65 µg/l in the study. Higher temperature had a significant positive effect on MIB levels in *Microcoleus* sp. MCAS-MC01 and geosmin levels in *Phormidium* sp. NIVA-CYA7 ($p<0.05$). On the other hand, the temperature did not affect the growth of all three strains ($p>0.05$). The results showed that taste and odour problems are species-specific, and in some species, they are stimulated by an increase in temperature. This study contributes to the understanding of taste and odour problems in relation to temperature.

Keywords: Cyanobacteria, Geosmin, 2-methylisoborneol, *Microcoleus*, *Oscillatoria, Phormidium*

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INTRODUCTION

Climate change and eutrophication are a growing global issue and cause an increase in the occurrence of taste- and odour-producing cyanobacteria, which are particularly significant in drinking water reservoirs (Akcaalan et al. 2022; Mantzouki et al. 2018). Even if hazardous chemicals are not always the source of aquatic taste and odour, the drinking water sector suffers adverse effects as a result (Watson 2004), since customers consider taste and odour as the main measure of the safety of drinking water and complain at levels as low as 10 ng/l geosmin and MIB (Zamyadi et al. 2015). Geosmin and MIB are produced by members of certain groups of benthic and pelagic aquatic microorganisms found in freshwater ecosystems (Watson & Jüttner 2019). Furthermore, there are a few additional biological sources that are often overlooked, especially those that originate from drinking water treatment plants and terrestrial ecosystems (Jüttner & Watson 2007).

Water utilities rarely monitor benthic cyanobacteria, although there is growing evidence that they represent a significant source of taste and odour problems (Gaget et al. 2022). Benthic cyanobacteria grow in different matrices such as sediments, biofilms, and floating mats, and they can detach and colonise in treatment plants (Gaget et al. 2020). However, a systematic understanding of how benthic cyanobacteria contribute to taste and odour episodes is still lacking (Gaget et al. 2022). To date, some benthic taste and odour-producing cyanobacteria from *Microcoleus, Oscillatoria, Phormidium, Heteroleibleinia, Leibleinia, Plectonema, Nostoc, Tychonema, Kamptonema, Jaaginema, Lyngbya, Leptolyngbya,* and *Symplocastrum* genera have been reported as geosmin or MIB producers (Watson et al. 2016). A total of 132 cyanobacterial strains from 21 genera and 72 cyanobacterial strains from 13 genera have been reported for geosmin and MIB production, respectively (Devi et al. 2021).

Consumer complaints are an important step in coping with taste and odour problems in drinking water. After increasing complaints about the taste and odour from a nearby city, samples were taken to measure geosmin and MIB in the drinking water reservoir. Some samples from benthic mats at the entrance of treatment plants were also taken, and the odour-producing cyanobacterium were isolated. The species was identified as *Microcoleus* sp. MCAS-MC01, which is the first isolate to produce MIB and geosmin in Türkiye. Furthermore, to reveal the effect of temperature on growth and taste and odour production, a study was conducted together with two different cyanobacteria strains (*Oscillatoria* sp. UHCC 0332 and *Phormidium* sp. NIVA-CYA 7) isolated from geographically different areas to understand the production of these metabolites in different cyanobacteria strains.

MATERIALS AND METHODS

Cyanobacteria strains, culture conditions, and sampling procedures

Three benthic filamentous cyanobacteria (*Microcoleus* sp., *Oscillatoria* sp. and *Phormidium* sp.) were used in this study. *Microcoleus* sp. MCAS-MC01 (Microalgae Culture Collection of Faculty of Aquatic Sciences, İstanbul University) was isolated from a drinking water treatment plant. The isolate was identified using molecular and morphological methods according to Strunecký et al. (2013). *Oscillatoria* sp. UHCC 0332 was obtained from the HAMBI Microbial Culture Collection, University of Helsinki. It is a known geosmin and MIB producer. *Phormidium* sp. NIVA-CYA 7 was obtained from the Norwegian Culture Collection of Algae, NORCCA. It is a known geosmin producer. All strains were cultured in Z8 medium at 20°C and 25°C with a 12 h light:12 h dark period for 10 weeks, and sampling was performed at weekly intervals. The lower temperature was selected as 20°C because cyanobacterial dominance generally occurs at higher (>20°C) temperatures in aquatic ecosystems (Ozbayram et al. 2022; Robarts & Zohary, 1987). The higher selected temperature was 25°C because the benthic cyanobacteria growth rate is generally optimum around 25°C in natural ecosystems and the 5°C temperature change could have a different effect on different cyanobacterial species (Mantzouki et al. 2018). To homogenise the aggregated cultures before sampling, the cultures were sonicated from the 2nd week. The growth of the three cyanobacteria strains was estimated by measuring the optical density at 750 nm (OD750) (Ernst et al. 2005).

Quantitative analysis of odour production

Geosmin and MIB analysed by Headspace-Solid Phase Microextraction (HS-SPME) coupled Gas Chromatography-Mass Spectrometry (GC-MS) according to a standard protocol (Kaloudis et al., 2016). The GC oven temperature was started at 50°C and reached 250°C (12°C/min). Helium was used as the mobile phase (1 ml/min). The commercial standards of geosmin and MIB (Sigma CRM47525) were used for qualitative and quantitative analyses. Geosmin and MIB were quantified according to their m/z ratios (95 for MIB and 112 for geosmin) using the selected ion mode. Qualitative m/z values for ions were 112 and 126 for geosmin and 95, 108, and 135 for MIB.

Statistics

The t-test in "rstatix" package (Kassambara, 2023) in R software was used to analyse the temperature effect on growth and odour (geosmin and MIB) concentration at a 95% confidence level (ver. 4.3.1) (R Core Team 2024). Spearman correlation with the level of significance as $p<0.001$, $p<0.05$, and $p<0.01$ was used to analyse the correlations between growth and odour concentration (geosmin and MIB) in Jamovi software (ver.2.3.1) (Jamovi project 2022). One-way ANOVA analysis was conducted to analyse temporal variance of geosmin and MIB concentrations at a 99% confidence level in Jamovi.

RESULTS AND DISCUSSION

Odour production was observed in all three cyanobacteria species for 10 weeks at different concentrations. The temperature had no statistically significant effect on growth ($p>0.05$) indicating these two temperatures (20°C and 25°C) did not have a dramatic effect on the growth of the three cyanobacteria strains.

It was observed that the cultures generally started to lose their colour after the 9th week of the experiment (Figure 1). *Microcoleus* sp. MCAS-MC01 and *Oscillatoria* sp. UHCC 0332 cultures started to die earlier at 20°C than at 25°C. On the other hand, *Phormidium* sp. NIVA-CYA 7 culture started to lose its colour earlier at 25°C than at 20°C (Figure 1).

Our results showed that temperature had a significant positive

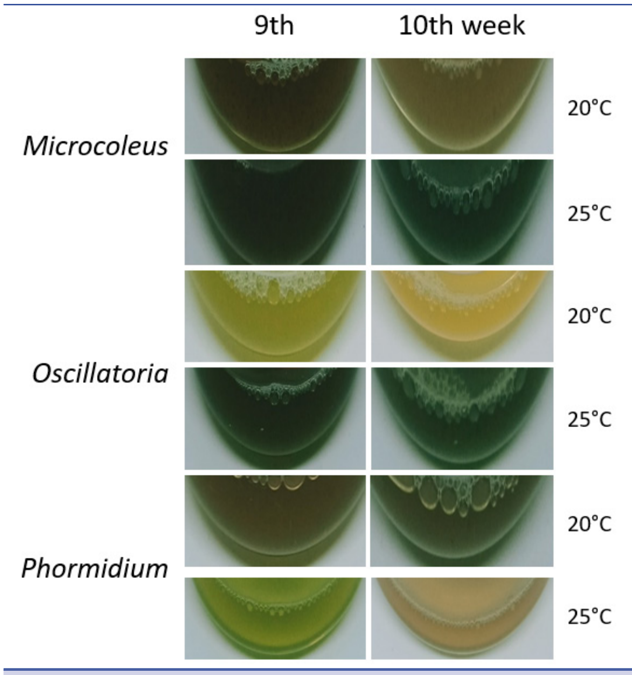

Figure 1. Colour changes in cultures.

effect on MIB production in *Microcoleus* sp. ($t_{(11.85)}$=-2.82, p<0.05) and geosmin production in *Phormidium* sp. ($t_{(10.47)}$=-2.19, p<0.05). However, there was no significant effect on geosmin production in the other two strains and MIB production in *Oscillatoria* sp. (p>0.05) (Figure 2).

The maximum geosmin concentration was detected in the 8th week at both temperatures reaching 63.11 µg/l at 20°C (Figure 2a) and 65.73 µg/l at 25°C in *Microcoleus* sp. (Figure 2b). Geosmin production followed an increasing trend until the 8th week and started to decrease afterwards (Figure 2). The optical density values increased at both temperatures during the experiment. Although geosmin production did not differ with temperature, the geosmin concentration of *Microcoleus* sp. MCAS-MC01 culture was dramatically higher than the threshold limits (10 ng/l) and reported environmental concentrations (Qiu et al. 2023). *Microcoleus* species are ubiquitous and form thick mats in different habitats, including freshwater ecosystems, a street flowerbed in an urbanised area, garden soil, and aquaculture areas (Tee et al. 2021; Churro et al. 2020; Alghanmi et al. 2018). For example, an episodic taste and odour problem related to the geosmin producer *Microcoleus* sp. was reported in a trout farming area, which was linked to the appearance of earthy/musty off-flavour and poor water quality during the water recirculation period (Robin et al. 2006). However, there are a limited number of isolated and identified geosmin/MIB-producing *Microcoleus* species: *Microcoleus pseudautumnalis* and *M. autumnalis* (formerly *Phormidium autumnale*) from Japan, *Microcoleus vaginatus* CCALA 145 (formerly *Phormidium autumnale*) from Switzerland, *Microcoleus vaginatus* from Iraq, *M. asticus* from Portugal and *Microcoleus* sp. from USA (Teneva et al. 2023; Churro et al. 2020; Niiyama & Tuji, 2019; Alghanmi et al. 2018; Oikawa et al. 2015; Izaguirre & Taylor, 1995). Therefore, our newly isolated geosmin and MIB producer strain from Türkiye makes a major contribution to research on taste and odour producers from *Microcoleus* genus, demonstrating a wide geographical distribution.

MIB levels at 25°C were significantly higher than those at 20°C in *Microcoleus* sp. ($t_{(11.85)}$=-2.82, p<0.05) reaching 626.65 µg/l at 25 °C in the 5th week (Figure 3b) and 320.48 µg/l at 20°C in the 8th week (Figure 3a). MIB production increased until the 8th and 5th weeks at 20°C and 25°C, respectively, and started to decrease in the following weeks (Figure 3). In line with our result, Alghanmi et al. (2018) also found maximum MIB concentrations at 25°C in *Microcoleus vaginatus* compared with 10°C and 33°C. Similarly, Microcoleus *autumnalis* (formerly *P.autumnale*) produces more MIB at high water temperatures above 20°C (Oikawa et al. 2015). In our study, the maximum total MIB level of our strain (626.65 µg/l) was significantly higher than the MIB concentrations measured in *M. vaginatus* (135.8 ng/l) and *Lyngbya* sp. (260 µg/l) (Izaguirre & Taylor, 1995). The results show that our isolate, *Microcoleus* sp., can respond to increasing temperatures by producing higher concentrations of the odour compound (MIB) and by surviving longer in the environment. Our strain is isolated from a water treatment plant that supplies drinking water to more than 200.000 people. These results are critical for determining water quality management strategies to be implemented in water treatment plants. Water authorities may encounter the problem intermittently in their drinking water systems, especially in summer months, and it may be necessary to implement appropriate treatment systems, such as ozonation or activated carbon, to be used when odour episodes occur (Oikawa et al. 2015).

Temperature had no significant effect on geosmin and MIB production in *Oscillatoria* sp. (p>0.05). The maximum geosmin production was 125.86 µg/l at 25°C and 90.8 µg/l at 20°C in the 9th week, followed by a significant decrease in the geosmin concentrations at both temperatures (Figure 4). Geosmin concentration increased from the 4th week at 20°C until the 9th week, and following this, the level of geosmin started to decrease (Figure 4a), whereas at 25°C, the trend over time was not as pronounced (Figure 4b).

On the contrary, maximum MIB concentrations were significantly higher than geosmin at both temperatures and measured as 1339 µg/l at 25 °C (9th week) and 768.69 µg/l at 20°C (8th week) (Figure 5). A significant MIB increase was observed at 20°C from the 4th week until the 8th week, following which the level of MIB

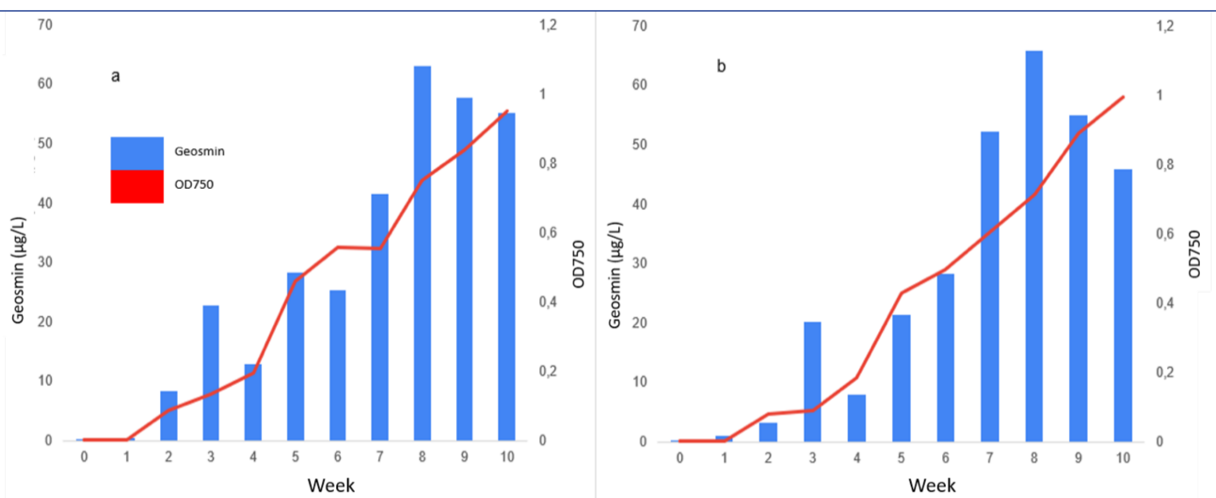

Figure 2. Comparison of geosmin production and growth in *Microcoleus* sp. at a) 20°C and b) 25°C.

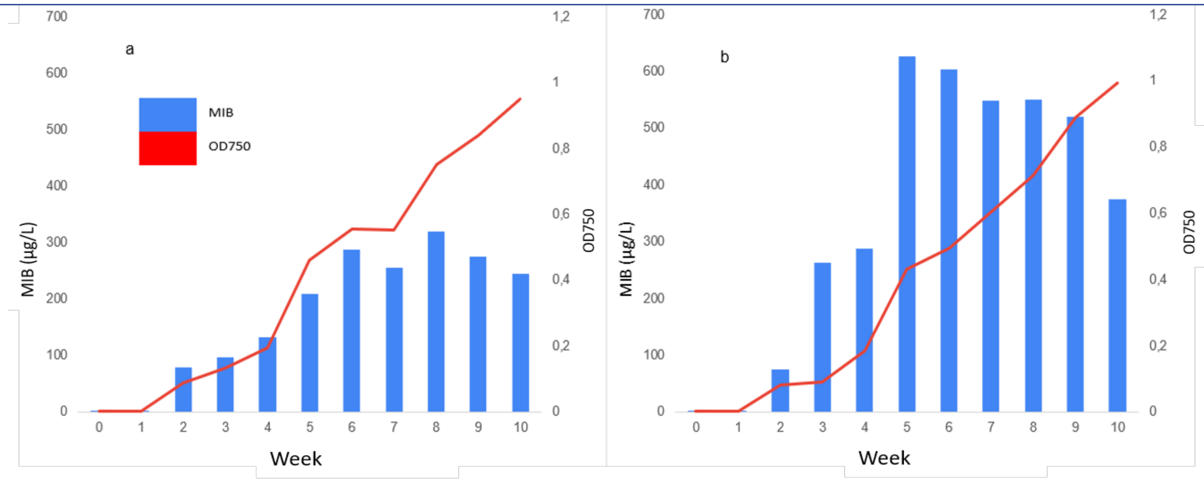

Figure 3. Comparison of MIB production and growth in *Microcoleus* sp. at a) 20°C and b) 25°C.

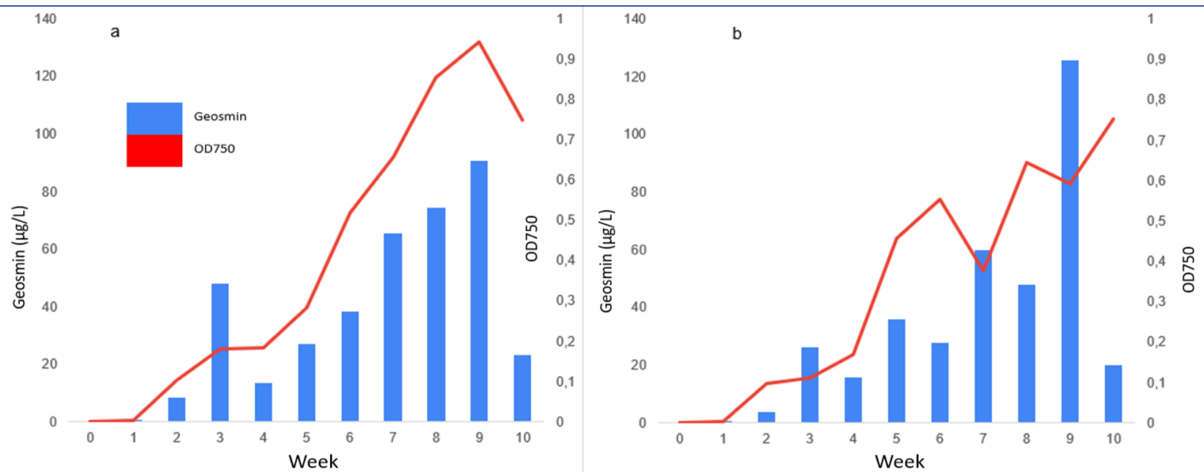

Figure 4. Comparison of geosmin production and growth in *Oscillatoria* sp. at a) 20°C and b) 25°C.

started to decrease (Figure 5a). Although there was not a distinct MIB trend at 25°C over time (Figure 5b), *Oscillatoria* sp. showed better growth at that temperature, consistent with the literature (Sivonen 1990, Cai et al. 2017). Chu et al. (2007) found that *Oscillatoria* sp. became dominant over the common cyanobacterium *Microcystis* sp. at temperatures below 25°C, whereas the contrary occurred at temperatures of 30°C and above. When there is competition between these two cyanobacteria species in natural ecosystems, temperature could be an important driving factor, and *Oscillatoria* sp., an earthy and mouldy odour producer, may have an advantage over *Microcystis* sp., which does not produce such odours, at 25°C and below. The information observed in the experimental studies also supports our understanding of the odour production dynamics in aquatic ecosystems.

The concentrations of geosmin and MIB in *Oscillatoria* sp. varied greatly at both temperatures. Although these metabolites utilise common metabolic pathways in cyanobacteria, their production levels may differ (Jüttner & Watson, 2007). However, since the removal of MIB from water is more difficult compared with geosmin, high levels of MIB can cause a very high economic loss for treatment plants (Zamyadi et al., 2015). The temperature could triggered odour production in various cyanobacteria species. Our results showed that *Oscillatoria* sp. produced both metabolites in higher concentrations at 25 °C than at 20 °C. In line with our results, Cai et al. (2017) also found that the maximum geosmin production in *Oscillatoria limosa* occurred at 25 °C.

Higher temperatures resulted in higher geosmin production in *Phormidium* sp. (Figure 6). The maximum geosmin production was measured at 25°C as 62.06 µg/l in the 7th week (Figure 6b). Our results are in line with *Phormidium amoenum* isolate from South Australia, which had a maximum geosmin concentration measured as 49 µg/l at 25°C (Li et al. 2012). Additionally, *Dolichospermum smithii* could also produce a maximum total geosmin concentration of 19.82 µg/l at 25°C (Shen et al. 2022). In our study, the maximum geosmin production at 25°C was three times higher than that at 20°C (19.02 µg/l at 20°C, 62.06 µg/l at 25°C) in *Phormidium* sp. The geosmin concentration started to increase from the 5th week until the 7th week and started to decrease the following weeks at 25°C (Figure 6b). On the other hand, there was no significant change over time, with maximum production

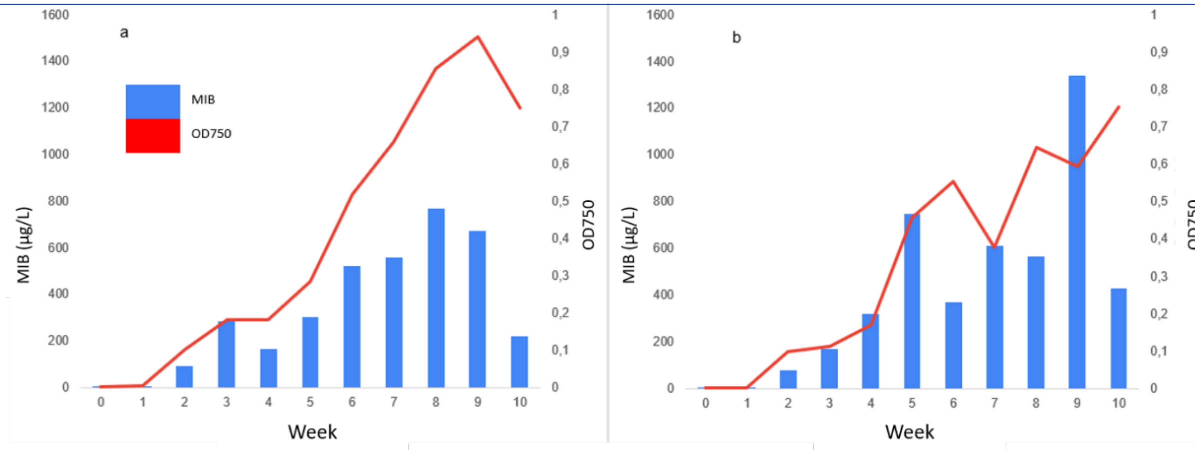

Figure 5. Comparison of MIB production and growth in *Oscillatoria* sp. at a) 20°C and b) 25°C.

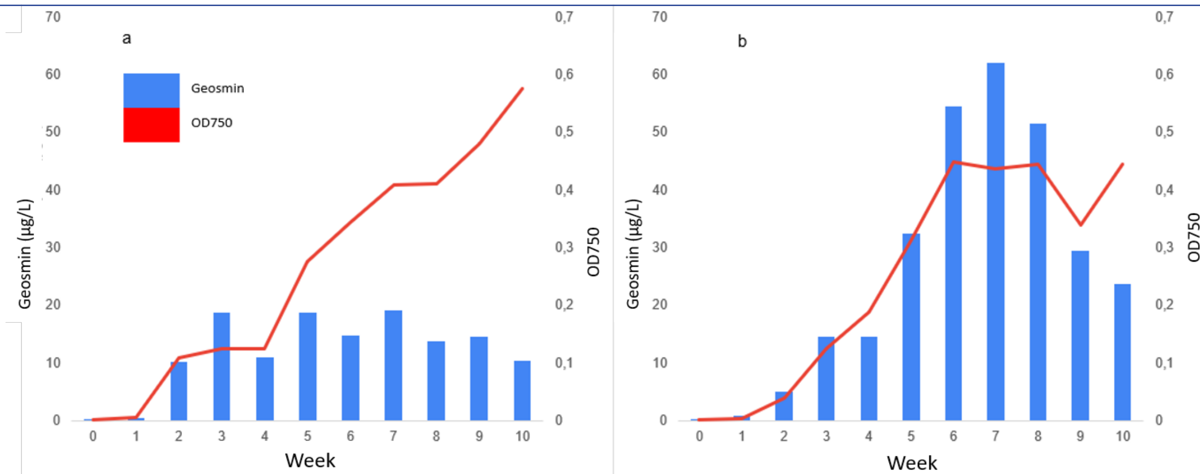

Figure 6. Comparison of geosmin production and growth in *Phormidium* sp. at a) 20°C and b) 25°C.

at week 7 being slightly higher than in other weeks at 20°C (Figure 6a). However, the growth curve of the strain maintained at 20°C continued to increase throughout the experiment. Fujimoto et al. (1997) found that *Phormidium* sp. could become dominant over *Microcystis* sp. at 20°C and a high N:P ratio, whereas the advantage shifted to *Microcystis* sp. at 25°C. Therefore, the competitive advantage of *Phormidium* sp. and associated odour production at relatively lower temperatures in aquatic environments should be considered in water quality management.

These three odour-producing cyanobacteria, *Microcoleus* sp. MCAS-MC01, *Oscillatoria* sp. UHCC 0332 and *Phormidium* sp. NIVA-CYA 7, showed similar thermotolerance profiles at 20°C and 25°C. Comparing the total geosmin production capacity, *Oscillatoria* sp. UHCC 0332 showed highest capacity reaching up 125.86 µg/l, *Microcoleus* sp. MCAS-MC01 reaching up 63.11 µg/l, and *Phormidium* sp. NIVA-CYA 7 to 62.06 µg/l (Figure 7). Regarding MIB production capacity, *Oscillatoria* sp. UHCC 0332 showed higher capacity by reaching up 1339 µg/l in comparison to the highest concentration measured as 626.65 µg/l in *Microcoleus* sp. (Figure 8).

Geosmin variation was significant over time in *Microcoleus* sp., *Oscillatoria* sp., *and Phormidium* sp. at both 20°C and 25°C (Figure 7). Comparing the temporal variation of geosmin, the concentration was significantly different for each week at both 20°C and 25°C in all strains except for *Phormidium* sp. (Figure 7).

Comparing temporal variation of MIB concentration, each week was significantly different in *Microcoleus* sp. and *Oscillatoria* sp. at both 20°C and 25°C (Figure 8).

Geosmin had a strong positive correlation with growth in *Microcoleus* sp. at both temperatures ($r = 0.903$ for 20°C and $r=0.842$ for 25°C respectively, $p < 0.001$), while MIB had only a strong correlation with growth at 20°C ($r=0.840$ $p<0.001$). At 25°C, a moderate positive correlation between MIB and growth ($r = 0.588$ $p<0.05$) was detected . Similarly, both geosmin and MIB had a strong positive correlation with growth in *Oscillatoria* sp. at both temperatures ($r = 0.794$, $p < 0.01$ for geosmin at 20°C and $r=0.612$, $p<0.05$ at 25°C; $r = 0.842$, $p < 0.01$ for MIB at 20°C and $r=0.721$, $p<0.05$ at 25°C) (Figure 4 and Figure 5), which was consistent with the results of previous studies (Shen et al. 2022, Jüttner & Watson 2007). Shen et al. (2022) highlighted that tem-

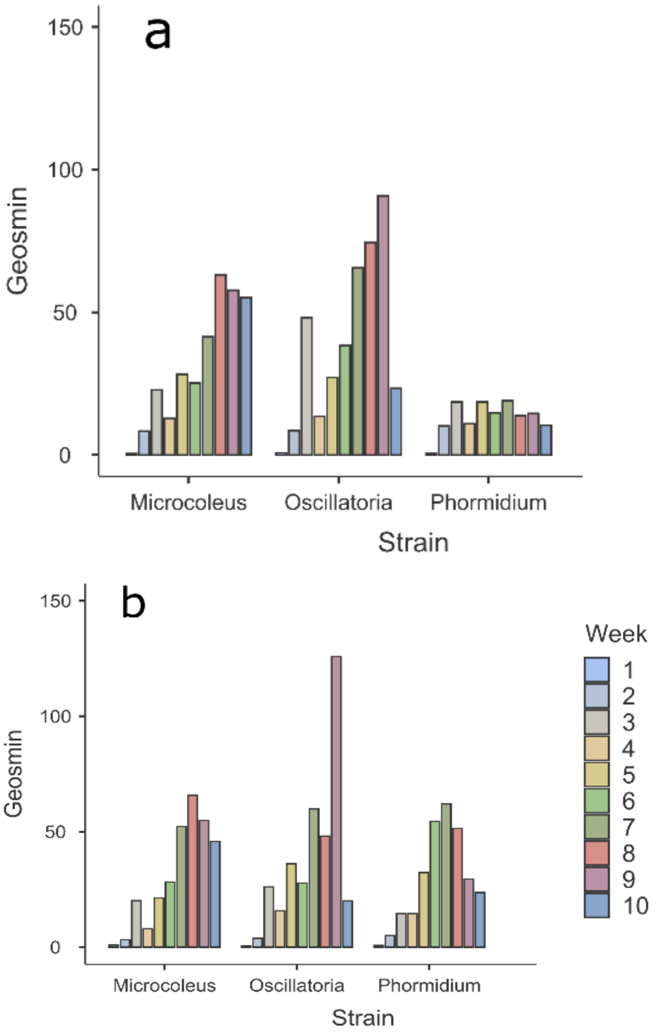

Figure 7. Comparison of total geosmin concentration in three strains (a. 20°C, b. 25°C).

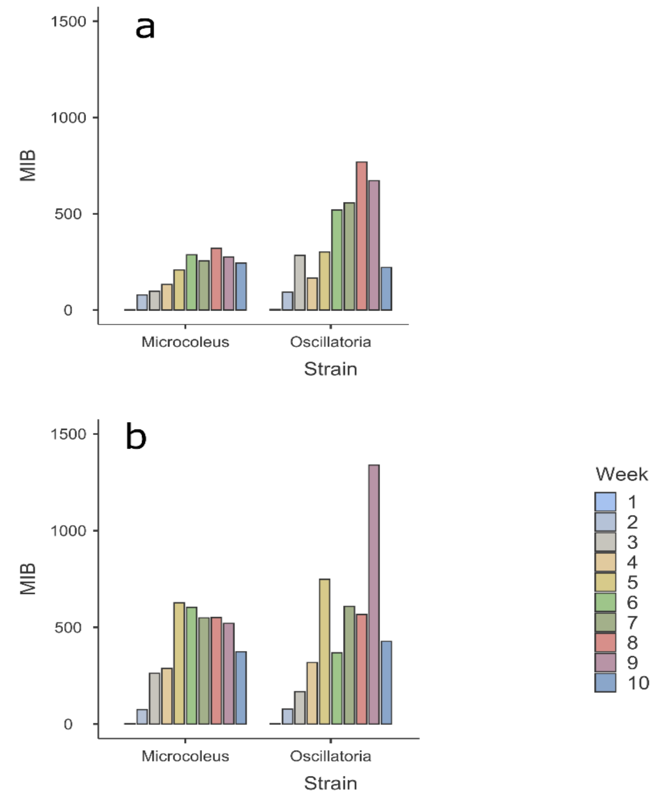

Figure 8. Comparison of total MIB concentration in three strains (a. 20°C, b. 25°C).

perature can increase the potential of geosmin/MIB synthesis. We also found 20°C - 25°C temperature range is generally favourable for growth and odour production in cyanobacteria. Geosmin had no correlation with growth in *Phormidium* sp. at 20°C ($r = 0.274$ $p > 0.05$) (Figure 6a), whereas it had a strong correlation at 25°C ($r = 0.851$ $p < 0.001$) (Figure 6b). We can conclude that geosmin production in *Phormidium* sp. at 20°C may be suppressed because of a possible competition for the essential common substrates with Chl-*a* synthesis, as we found growth continued while geosmin concentration had a generally stationary trend at 20°C (Shen et al. 2021; Wang & Li, 2015).

CONCLUSION

Our results highlight the important role of the strain-specific response to temperature changes. Furthermore, the production of geosmin and MIB compounds differed under different temperatures in our strain *Microcoleus* sp. MCAS-MC01 and *Phormidium* sp. NIVA CYA7. We found that a 5°C temperature difference had a dramatic effect on the odour production of different cyanobacterial strains. The total odour concentrations without comparing intra- and extracellular components were measured in this study. Further studies are needed to address whether the temperature had a mechanism to increase intracellular odour production or to affect the release of odour compounds outside the cell. In addition to *in vitro* studies, to predict the effects of changing environmental conditions on odour production in natural ecosystems, it is also important to determine the levels of odour compounds using *in situ* studies, such as mesocosm experiments to analyse other biotic and abiotic triggers.

Conflict of Interest: The author has no conflicts of interest to declare.

Ethics committee approval: Ethics committee approval is not required.

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REFERENCES

Akcaalan, R., Devesa-Garriga, R., Dietrich, A., Steinhaus, M., Dunkel, A., Mall, V., ... & Kaloudis, T. (2022). Water taste and odor (T&O): Challenges, gaps and solutions from a perspective of the WaterTOP network. *Chemical Engineering Journal Advances, 12*, 100409. https://doi.org/10.1016/j.ceja.2022.100409

Alghanmi, H. A., Fo'ad, M. A., & Al-Taee, M. M. (2018). Effect of light and temperature on new cyanobacteria producers for geosmin and 2-methylisoborneol. *Journal of Applied Phycology, 30*(1), 319-328. https://doi.org/10.1007/s10811-017-1233-0

Cai, F., Yu, G., Zhang, K., Chen, Y., Li, Q., Yang, Y., Xie, J., Wang, Y., & Li, R. (2017). Geosmin production and polyphasic characterization of *Oscillatoria limosa* Agardh ex Gomont isolated from the open canal of a large drinking water system in Tianjin City, China. *Harmful Algae, 69,* 28–37. https://doi.org/10.1016/J.HAL.2017.09.006

Churro, C., Semedo-Aguiar, A. P., Silva, A. D., Pereira-Leal, J. B., & Leite, R. B. (2020). A novel cyanobacterial geosmin producer, revising Geo A distribution and dispersion patterns in Bacteria. *Scientific Reports, 10*(1), 8679. https://doi.org/10.1038/s41598-020-64774-y

Devi, A., Chiu, Y. T., Hsueh, H. T., & Lin, T. F. (2021). Quantitative PCR based detection system for cyanobacterial geosmin/2-methylisoborneol (2-MIB) events in drinking water sources: Current status and challenges. *Water research, 188*, 116478. https://doi.org/10.1016/j.watres.2020.116478

Ernst, A., Deicher, M., Herman, P. M., & Wollenzien, U. I. (2005). Nitrate and phosphate affect cultivability of cyanobacteria from environments with low nutrient levels. *Applied and environmental microbiology, 71*(6), 3379-3383. https://doi.org/10.1128/AEM.71.6.3379-3383.2005.

Fujimoto, N., Sudo, R., Sugiura, N., & Inamori, Y. (1997). Nutrient-limited growth of Microcystis aeruginosa and Phormidium tenue and competition under various N: P supply ratios and temperatures. *Limnology and Oceanography*, *42*(2), 250-256. https://doi.org/10.4319/lo.1997.42.2.0250

Gaget, V., Hobson, P., Keulen, A., Newton, K., Monis, P., Humpage, A. R., ... & Brookes, J. D. (2020). Toolbox for the sampling and monitoring of benthic cyanobacteria. *Water research, 169,* 115222. https://doi.org/10.1016/j.watres.2019.115222

Gaget, V., Almuhtaram, H., Kibuye, F., Hobson, P., Zamyadi, A., Wert, E., & Brookes, J. D. (2022). Benthic cyanobacteria: A utility-centred field study. *Harmful Algae, 113*, 102185. https://doi.org/10.1016/j.hal.2022.102185

Izaguirre, G., & Taylor, W. D. (1995). Geosmin and 2-methylisoborneol production in a major aqueduct system. Water science and technology, 31(11), 41-48. https://doi.org/10.1016/0273-1223(95)00454-U

The jamovi project (2022). jamovi. (Version 2.3) [Computer Software] (https://www.jamovi.org)

Jüttner, F., & Watson, S. B. (2007). Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters. *Applied and environmental microbiology, 73*(14), 4395-4406. https://doi.org/10.1128/AEM.02250-06

Kaloudis, T., Triantis, T. M. , & Hiskia, A. (2016). Determination of Geosmin and 2-Methylisoborneol in Water by HS-SPME-GC/MS. In J. Meriluoto, L. Spoof, & G. A. Codd (Eds.), *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis (1st ed.)*. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119068761

Kassambara (2023) Pipe-friendly Framework for Basic Statistical Tests in R (https://rpkgs.datanovia.com/rstatix/)

Li, Z., Hobson, P., An, W., Burch, M. D., House, J., & Yang, M. (2012). Earthy odor compounds production and loss in three cyanobacterial cultures. *Water research, 46*(16), 5165-5173. https://doi.org/10.1016/j.watres.2012.06.008

Mantzouki, E., Lürling, M., Fastner, J., de Senerpont Domis, L., Wilk-Woźniak, E., Koreivienė, J., ... & Warming, T. P. (2018). Temperature effects explain continental scale distribution of cyanobacterial toxins. *Toxins, 10*(4), 156. https://doi.org/10.3390/toxins10040156

Niiyama, Y., & Tuji, A. (2019). *Microcoleus pseudautumnalis* sp. nov. (Cyanobacteria, Oscillatoriales) producing 2-methylisoborneol. *Bulletin of the National Museum of Nature and Science, 45,* 93–101, https://cir.nii.ac.jp/crid/1523388080253362688

Oikawa, T., Tsunoda, T., Nakahigashi, H., Shimoriku, M., Kanami, T., & Kimura, S. (2015). Musty odor producing benthic cyanobacteria in the Tama River (Japan) and identification of species by genetic analysis. *Journal of Water Supply: Research and Technology—AQUA, 64*(7), 839-846. https://doi.org/10.2166/aqua.2015.068

Ozbayram, E.G., Camur, D., Koker, L., Oguz Cam, A., Akcaalan, R., Albay, M. (2022). The effects of climate change on aquatic ecosystems in relation to human health. *Aquatic Sciences and Engineering, 37*(3), 123-128. https://doi.org/10.26650/ASE20221057160

Qiu, P., Zhang, Y., Mi, W., Song, G., & Bi, Y. (2023). Producers and drivers of odor compounds in a large drinking-water source. *Frontiers in Ecology and Evolution, 11*, 1216567. https://doi.org/10.3389/fevo.2023.1216567

R Core Team (2024) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria (https://www.R-project.org)

Robarts, R. D., & Zohary, T. (1987). Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *New Zealand journal of marine and freshwater research, 21*(3), 391-399. https://doi.org/10.1080/00288330.1987.9516235

Robin, J., Cravedi, J. P., Hillenweck, A., Deshayes, C., & Vallod, D. (2006). Off flavor characterization and origin in French trout farming. *Aquaculture*, *260*(1-4), 128-138. https://doi.org/10.1016/j.aquaculture.2006.05.058

Shen, Q., Wang, Q., Miao, H., Shimada, M., Utsumi, M., Lei, Z., ... & Shimizu, K. (2022). Temperature affects growth, geosmin/2-methylisoborneol production, and gene expression in two cyanobacterial species. *Environmental Science and Pollution Research, 29,* 12017–12026. https://doi.org/10.1007/s11356-021-16593-5

Shen, Q., Shimizu, K., Miao, H., Tsukino, S., Utsumi, M., Lei, Z., ... & Akiba, M. (2021). Effects of elevated nitrogen on the growth and geosmin productivity of *Dolichospermum smithii*. *Environmental Science and Pollution Research, 28*, 177-184. https://doi.org/10.1007/s11356-020-10429-4

Sivonen, K. (1990). Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth of and hepatotoxin production by *Oscillatoria agardhii* strains. *Applied and environmental microbiology, 56*(9), 2658. https://doi.org/10.1128/aem.56.9.2658-2666.1990

Strunecký, O., Komárek, J., Johansen, J., Lukešová, A., & Elster, J. (2013). Molecular and morphological criteria for revision of the genus *Microcoleus* (Oscillatoriales, C yanobacteria). *Journal of phycology, 49*(6), 1167-1180. https://doi.org/10.1111/jpy.12128

Tee, H. S., Wood, S. A., Bouma-Gregson, K., Lear, G., & Handley, K. M. (2021). Genome streamlining, plasticity, and metabolic versatility distinguish co-occurring toxic and nontoxic cyanobacterial strains of Microcoleus. *Mbio*, *12*(5), e02235-21 https://doi.org/10.1128/mbio.02235-21

Teneva, I., Belkinova, D., Paunova-Krasteva, T., Bardarov, K., Moten, D., Mladenov, R., & Dzhambazov, B. (2023). Polyphasic characterisation of *Microcoleus autumnalis* (Gomont, 1892) Strunecky, Komárek & JR Johansen, 2013 (Oscillatoriales, Cyanobacteria) using a metabolomic approach as a complementary tool. *Biodiversity Data Journal, 11*. https://doi.org/10.3897/BDJ.11.e100525

Wang, Z., & Li, R. (2015). Effects of light and temperature on the odor production of 2-methylisoborneol-producing *Pseudanabaena* sp. and geosmin-producing *Anabaena ucrainica* (cyanobacteria). *Biochemical Systematics and Ecology, 58*, 219-226. https://doi.org/10.1016/j.bse.2014.12.013

Watson, S., & Jüttner, F. (2019). Biological production of taste and odour compounds. Taste and Odour in Source and Drinking Water: Causes, Controls, and Consequences. London, UK, IWApublishing, 63-112. https://doi.org/10.2166/9781780406664_0063

Watson, S. B., Monis, P., Baker, P., & Giglio, S. (2016). Biochemistry and genetics of taste-and odor-producing cyanobacteria. *Harmful Algae, 54*, 112-127. https://doi.org/10.1016/j.hal.2015.11.008

Watson, S. B. (2004). Aquatic taste and odor: a primary signal of drinking-water integrity. *Journal of Toxicology and Environmental Health, 67*(20-22), 1779-1795. https://doi.org/10.1080/15287390490492377

Zamyadi, A., Henderson, R., Stuetz, R., Hofmann, R., Ho, L., & Newcombe, G. (2015). Fate of geosmin and 2-methylisoborneol in full-scale water treatment plants. *Water research, 83,* 171-183. https://doi.org/10.1016/j.watres.2015.06.038