CHARACTERIZATION OF MUSTY ODOR PRODUCING ACTINOMYCETES IN MALAYSIA

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SUMMARY: The presence of geosmin and 2-methylisoborneol (2-MIB) becomes an increasing concern as they are known to cause earthy or musty odor in freshwater environments. Geosmin and 2-MIB outbreaks in Malaysia are not well understood and since Malaysia has a stable temperature throughout the year, no information has been reported on effect of temperature to the odor production. In this study, 6 isolated strains were selected for study of the effect of temperature (20, 25, 30, 35, 40, 45 & 50°C) on geosmin and 2-MIB production. Preliminary results indicate that at temperature 30 °C, Strain 5 showed highest geosmin production (129.06 µg/L) and Strain 2 produced highest 2-MIB (19.89 µg/L). PCR band was obtained in a test whether these isolated strains had *geoA* gene or not.
***Keywords*—** Actinomycetes; Geosmin; 2-methylisoborneol; Musty odor; Temperature

INTRODUCTION

Musty odor compounds (Geosmin/ 2-MIB) has become a very serious problems in freshwater worldwide and give a bad impression to the water and aquaculture production. These metabolites are produced by microorganisms including several genera of cyanobacteria, fungi, and various actinomycetes [1,2]. They can be detected by human at a very low concentration level and make it difficult to remove by conventional treatment such as chlorination, sedimentation and coagulation. Clear understanding of the processes and mechanisms of the odor production is needed as a first step in order to develop a cost-effective removal method of these odor compounds. However, it is still unclear what environmental factors control these compounds productions.

Temperature is one of the most important trigger factors for the metabolic response of actinomycetes and it was found that the difference in temperature will have an impact on the biomass and geosmin/2-MIB production by actinomycetes [3,4]. Since Malaysia has a very unique climate, with stable temperature throughout the year, therefore no information about the effect of temperature on the odor production has been reported. A temperature range 20°C - 50°C were used in this study in order to determine which isolate(s) produced higher odor production. Based on this background, we have to identify potential geosmin producing actinomycete isolate from environment in Malaysia, and determine the effect of different temperatures on odor production rate by actinomycete isolates in order to identify the key factors for start production of the odorous compounds.

2. MATERIALS AND METHODS

2.1 Sampling

Water and sediment samples were collected at 2 sampling locations: Titiwangsa Lake and University of Malaya Lake located in Kuala Lumpur.

2.2 Culture conditions

Water and sediment samples were diluted and spread on the BS agar plate. Plates were incubated at 30°C for 7 days until the colonies were visible. Selected colonies with typical actinomycetes morphology (dense, leathery colony morphology) were picked up and further purified on the YMPD agar for several times.

2.3 Temperature condition experiment

Strains were pre-cultured at 30°C for 5 days under shaking conditioned at 120 rpm in 50 ml YMPD medium. All medium were adjusted with 1M NaOH solution before autoclaving. The cells were harvested by centrifugation (5000 x g, 25°C, 5 min) and were washed with dispersed BS negative medium. 150 µl of the washed pellets were spread on Dispersed-BS agar plate. All plates were cultured for 7 days under different temperature conditions.

2.4 Analysis of geosmin and 2-MIB

After 7 days of cultivation, 5 ml of methanol was directly added to the plate for geosmin extraction and left for 30 minutes at room temperature. 1 ml of the methanol geosmin extract was collected and put into glass tube and added with 2 ml of n-hexane. The mixture then incubate at room temperature for 30 minutes and with a shaking condition of 150 rpm and then centrifuged (800 x g, 30 min., 25°C) to separate the n-hexane layer and methanol layer. Carefully collected the upper n-hexane layer and dehydrated the n-hexane extract over anhydrous Na_2SO_4 in a Pasteur pipette to remove humidity [5]. The extracts were analyzed by GC-MS for the measuring of geosmin concentration.

3. RESULTS AND DISCUSSION

3.1 Isolation of odor producing actinomycetes.

After isolation and purification on YMPD agar plate, 6 actinomycete isolates (Fig. 1) were chosen based on its morphological characteristics (dense, leathery colony morphology) and proceed with further analysis.

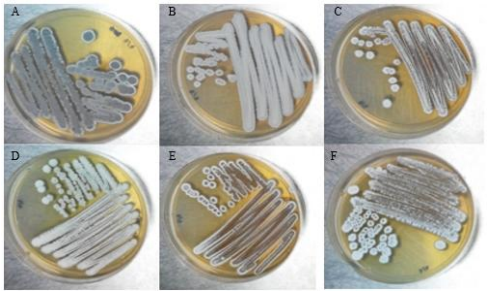

Fig. 1. Purified actinomycetes isolate (A = Strain 1; B = Strain 2; C = Strain 3; D = Strain 4; E = Strain 5; F = strain 6)

3.2 Production of geosmin and 2-MIB from isolated *Streptomyces* sp.

Fig. 2 showed the result of the effect of temperature at 30 °C on geosmin and 2-MIB production by 6 isolated strains. It can be seen that all 6 strains produced geosmin. Strain 5 produced the highest amount of geosmin with a yield of 129.06 µg/L. The next highest yield of geosmin was produced by Strain 6 with a yield of 122.99 µg/L followed by Strain 4 and 3. The least amount of geosmin production were 2.85 µg/L and 6.43 µg/L for Strain 1 and 2.

For 2-MIB production, Strain 2 showed the highest amount of 2-MIB with a yield of 19.89 µg/L followed by Strain 1 (5.99 µg/L). However, no detectable levels of 2-MIB were detected in the other four isolates. Effect of temperature at 20, 25, 35, 40, 45 and 50 °C on odor production will be conducted in future work in order to determine which isolate might be contributing to the odor problems in the lakes.

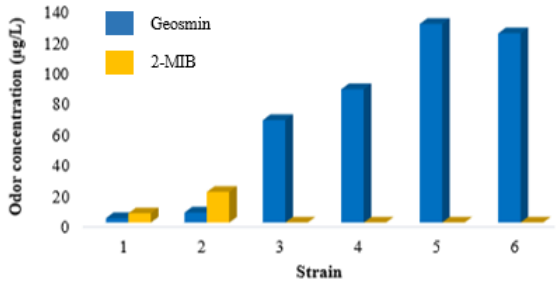

Fig. 2. Musty odor compound produced by isolated strains at 30°C.

3.3 PCR analysis.

To confirm the presence of geosmin synthase gene, DNA was extracted from the isolated cultures and amplified using geoA primers (geo249F,geo1860R). Different annealing temperatures were used to get the best amplification result. Amplification was successful with geoA primers for Strain 1 and 2 (Fig. 3) which indicate that these strains may produce geosmin. However, Strain 3,4,5 and 6 did not show any band which indicate that no geosmin production due to the absence of *geoA* gene. Based on GC/MS analysis (Fig. 2), Strain 3,4,5 and 6 produced relatively high amount of geosmin compared with Strain 1 and 2 even with the absence of the *geoA* gene. One of the possible reason is the low specificity of the primer. The primer used probably not specific enough to bind to the target sequence. PCR will be conducted again and if the result still the same it might indicate the existing of novel gene or new mechanism involved. Optimization is needed in order to get the clearer results. Detection of MIB synthase gene for each isolate will be done in future work.

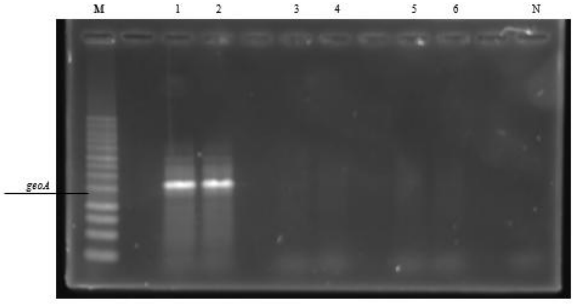

Fig. 3. Agarose gel profile of actinomycete isolates amplified by geoA primers at 60 °C of annealing temperatures.

4. CONCLUSION

All isolates produced geosmin at incubation temperature 30 °C but only 2 strains produced 2-MIB. This study is still in progress and future results will give relevant data to support the finding in this study.

ACKNOWLEDGMENTS

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REFERENCE

[1] Gerber, N.N., Lechevalier, H.A. Geosmin, an earthy-smelling substance isolated from actinomycetes. Appl. Microbiol. 1965. 13(6): 935-938.

[2] Sugiura, N., Iwami, N., Inamori, Y., Nishimura, O., Sudo, R. Significance of attached cyanobacteria relevant to the occurrence of musty odor in Lake kasumigaura. Water Res. 1998. 32(12): 3549-3554.

[3] Blevins, M.T., Schrader, K.K., Saadoun, I. Comparative physiology of geosmin production by *Streptomyces halstedii* and *Anabaena* sp. Wat Sci Technol. 1995. 31(11): 127-133.

[4] Weete, J.D., Blevins, W.T., Wilt, G.R., Durham, D. Chemical, biological and environmental factors responsible for the earthy odor in the Auburn city water supply. Bulletin of the Alabama Agricultural Experiment Station 490, Auburn University, Auburn, AL. 1977. Page 46.

[5] David, E.C., Xiaofei, He., Seiji, K., Satoshi, Ō., Haruo, I. Geosmin biosynthesis in Streptomyces avermitilis. Molecular cloning, expression, and mechanistic study of the germacradienol/geosmin synthase. J Antibiot. 2006. 59(8): 471-479.