Influence of light wavelength and intensity on geosmin production of *Streptomyces coelicolor* A3(2)

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Introduction

In recent years, the occurrence of musty odor concerns originated from microorganisms in freshwater environments like as lakes, rivers, and reservoirs has been reported in all over the world (ex. Juttner and Watson 2007). Actinomycetes are known one of the causal microorganisms to produce geosmin and 2-methylisoborneol (2-MIB), both are responsible for musty odor in fresh waters. Waterworks are strongly interested in when geosmin production occurs in the reservoir, because musty odor compounds can be perceived by human being at very low concentration level (ng/L) (Young et al. 1996), and it is ineffective to remove by conventional water supply treatment processes such as coagulation, sedimentation, filtration and chlorination (Bruce et. al. 2002). Establishment of effective removal methods is essential for reservoirs in situ, but it is still unclear what environmental factors control the production of these compounds in aquatic environments.

Geosmin biosynthesis by *Streptomyces coelicolor* A3(2), a strain whose genome has been fully sequenced (Bentley et al. 2002), is studied (Cane et al. 2003). But the biosynthesis trigger of geosmin production is still unclear. Light is one of the important trigger factors for metabolic response of actinomycetes; as have reported that *S. coelicolor* A3(2) produced carotenoids when exposed under blue light (2.4 μmol $m^{-2} s^{-1}$), but didn't produce under red light (2.4 μmol $m^{-2} s^{-1}$) (Takano et al. 2005, Takano et al. 2006). Both of carotenoid and geosmin are terpenoids. Furthermore, isopentenylpyrophosphate (IPP) is common precursor of geosmin and carotenoid. Therefore, it is assumed that light influences on geosmin production by actinomycetes. Based on these backgrounds, we have studied what kind of environmental factors influence on geosmin production of actinomycetes. Here, we reported that the results of plate culture experiments under different light irradiation conditions (wavelengths and intensities) to elucidate the factors that influence on geosmin production of *S. coelicolor* A3(2).

Materials and methods

S. coelicolor A3(2) was pre-cultured at 28°C for 2 days in 100 mL YMPD medium (2.0 g yeast extract, 2.2 g meat extract, 4.0 g Bacto peptone, 2.0 g NaCl, 1.0 g $MgSO_4 \cdot 7H_2O$, 1.0 g glucose, pH 7.2, per litter) in a 300 mL baffled Erlenmeyer flask, and incubated with shaking at

120 rpm under dark condition. All medium pH were adjusted with NaOH solution before autoclaving. Cells from each 1 mL of cultured medium were harvested by centrifugation (5,000 × *g*, 5 min) and were washed twice with BS medium without carbon source (BS negative; 2.0 g $(NH_4)_2SO_4$, 2.0 g NaCl, 1.0 g $MgSO_4 \cdot 7H_2O$, 0.5 g K_2HPO_4, 0.05 g $FeSO_4 \cdot 7H_2O$, pH 8.0, per litter). After homogenized weakly by 1 mm glass beads with 1 mL of sterilized distillated water using Fast Prep system (Thermo Savent), cell suspension at a final concentration of 1×10^5 CFU/mL were spread on SFM agar plate (20 g soybean flour, 20 g mannitol, 15 g ager, pH 8.0, per litter). All plates were cultured at 28°C for 7 days under specified light conditions or dark condition. Under light condition, we used white, blue (470 nm), green (525 nm), and red (660 nm) LED light equipment for illuminating at 1, 10, 20, 30 μmol m^{-2} s^{-1} onto the plates respectively. After 7 days cultivation, 5.0 mL of methanol was directly added to each plate for geosmin extraction, and then the plates were kept at room temperature for 30 min. 1.0 mL of methanol geosmin extracts was collected in glass tube and added 2.0 mL of n-hexane. After that, these tubes were stirred for 30 min and then centrifuged at 800 × *g* for 30 min to separate the n-hexane and methanol layer. The n-hexane layer was carefully collected and filtered through in a Pasteur pipette packed by Na_2SO_4 for dehydration (David and He 2006, Komatsu et al. 2008). The extracts were analyzed by GC-MS QP2010 plus (Shimadzu Co Ltd. Japan) with AOC-20is series autosampler (Shimadzu Co Ltd. Japan) for measuring geosmin concentration. The GC-MS conditions were as follows: Capillary column, Rxi-5ms, 30 m × 0.25 mm i.d. × 0.5 μm (Restek, Japan); temperature program, isothermal for 1 min at 50°C, change from 50°C to 250°C at a rate of 15°C/min, and isothermal for 5 min at 250°C; injection volume, 1 μL; carrier gas, He; linear velocity, 5.19 cm/s; sampling rate, 1 s; MS mode, EI; detector voltage, 1.2 kV; interface temperature, 230°C; ion source temperature, 200°C.

Results and Discussion

After 7 days cultivation, all plate surface of light or dark condition were filled with *S. coelicolor* A3(2) mycelia. On the other hand, geosmin concentration in the plate was increased under light (white, blue, green, red) conditions compared with dark condition. Furthermore, geosmin concentration was gradually increased under blue and white light condition between 10 and 20 μmol m^{-2} s^{-1} light intensity. But the concentration tended to decrease above 20 μmol m^{-2} s^{-1}. These results strongly suggested that the lights played a key role in the induction of geosmin production activity by *S. coelicolor* A3(2). Especially short wavelength light such as blue influenced potently to producing geosmin by *S. coelicolor* A3(2). Also the production respondency might have threshold level of light intensity.

From the previous studies, carotenoid production of *S. coelicolor* A3(2) is induced by irradiating of blue light (2.4 μmol m^{-2} s^{-1}) but isn't induced when irradiated with red light (2.4 μmol m^{-2} s^{-1}) (Takano et al. 2005, Takano et al. 2006). In this study, on the other hand, geosmin was produced by *S. coelicolor* A3(2) under green and red light irradiation conditions. Because metabolic pathway of carotenoid and geosmin has different parts, we suggested that carotenoid and geosmin production could be affected by different factors under long wavelengths light irradiation. In conclusion, it was indicated that various wavelengths light irradiation would induce geosmin production of *S. coelicolor* A3(2).

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