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Lysis of cyanobacteria with volatile organic compounds

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Abstract

One of bacteria collected from Lake Sagami, Japan, *Brevibacillus* sp., was found to have a lytic activity of cyanobacteria, but did not produce active compounds. Instead, the co-culturing of *Microcystis* with the *Brevibacillus* sp. enhanced the production of two volatile compounds, β-cyclocitral and 3-methyl-1-butanol, and the former had a characteristic lytic activity. It was confirmed that these volatile compounds were derived from the cyanobacteria themselves. β-Ionone, geosmin and 2-methylisoborneol derived from cyanobacteria and similar volatile compounds, terpenoids, produced by plants also had a lytic activity. The minimum inhibitory concentration values of the cyanobacterial metabolites were estimated to be higher than those of compounds from plants except for a few compounds. Among them, β-cyclocitral only produced a characteristic color change of culture broth from green to blue. This color change is similar to the phenomenon observed when a sudden decline in growth of cyanobacteria begins in a natural environment.

Keywords: Cyanobacteria; Lysis; Microorganism; Volatile compound; β-Cyclocitral

1. Introduction

Blooms and scums of cyanobacteria (blue-green algae) commonly occur in lakes, reservoirs, slow-flowing rivers and brackish water environments resulting in the water having a musty odor and production of potent toxins. Microcystins are cyclic heptapeptide toxins produced by cyanobacteria, such as *Microcystis* (Harada et al., 1999; Ortea et al., 2004), and show potent hepatotoxicity and tumor-promoting activity by inhibition of protein phosphatases 1 and 2A (Kuiper-Goodman et al., 1999; Sivonen and Jones, 1999). Animal and human health problems associated with the ingestion of or contact with cyanobacterial scums have long been recognized and an incident involving the death of 50 people occurred in Brazil in 1996 due to the contamination of microcystins in the water used for hemodialysis (Jochimsen et al., 1998; Pouria et al., 1998). To avoid these tragic incidents, there is need to develop effective methods to regulate the occurrence of cyanobacteria and their toxins.

Although many methods, such as biomanipulation and algicides, have been used for the elimination of cyanobacteria in water environments, no suitable method has been developed, indicating the difficulty to regulate the occurrence of cyanobacteria by conventional methods (Fallon and Brock, 1979; Chorus and Mur, 1999; Shi et al., 2005; Mazur-Marzec et al., 2006). Furthermore, no effective method has been proposed to destroy microcystins in the natural environment. We have tried to develop a biological control system using microorganisms coexisting in the same ecosystem to decrease the outbreak of cyanobacteria (Sigee et al., 1999) and to decompose the microcystins (Bourne et al., 1996). To establish such system, many

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suitable microorganisms have been isolated from the natural environment, and more active microorganisms were selected for this purpose.

Upon the screening of bacteria living in the natural environment using the soft-agar overlayer (SAO) method (Uchida et al., 1998), 37 out of 2594 marine bacteria were found to produce anticyanobacterial compounds against *Oscillatoria amphibia* NIES-361. One of the strains, C-979, classified as a *Vibrio* sp., was found to secrete a bioactive compound, β-cyano-L-alanine (Yoshikawa et al., 2000). In addition, 83 actinomycete strains were isolated from lake sediments in Lake Suwa, Nagano, Japan, and about half of the isolates were found to lyse cyanobacteria. One of them (strain S-9), classified as *Streptomyces phaeofaciens*, grew well on the lawns of living cyanobacteria and rapidly lysed the cyanobacterial cells. This lytic activity was caused by the secretion of an amino acid, L-lysine (Yamamoto et al., 1998).

We collected waters, sediments and soils from Lakes Sagami and Tsukui in Kanagawa near Tokyo, Japan and isolated many microorganisms (Tsuji et al., 2006). Several of the bacteria caused a lysis of the cyanobacteria and two of the potent strains were characterized as *Bacillus* sp. (termed B-6) and *Brevibacillus* sp. (termed B-1). However, no definite active compounds were found from B-6 strain (Harada et al., 2007). Although no anticyanobacterial compounds were also found, an interesting phenomenon was observed in the B-1 strain. In this paper, we describe some of volatile compounds having potent anticyanobacterial activities.

2. Materials and methods

2.1. Chemicals

Volatile compounds, β-cyclocitral, β-ionone, thymol, carvacrol, cinnamaldehyde, citral, geraniol, linalool, menthol, menthone, perillaldehyde and vanillin were obtained from Sigma–Aldrich (St. Louis, Mo, USA) and 2-decanone, 3-methyl-1-butanol, colistin, geosmin and 2-methylisoborneol (2-MIB) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Methanol, ethyl acetate, acetone, acetonitrile and isopropanol were distilled at atmospheric pressure before use.

2.2. Cyanobacteria cultures

Axenic strains NIES-102, NIES-103, NIES-112 and NIES-298 belonged to *Microcystis* and NIES-73 was *Anabaena* sp., and they were obtained from the National Institute for Environmental Studies (NIES), Tsukuba, Japan. These strains were cultured in 1 l Erlenmeyer flasks containing each of modified MA medium (300 ml) at 25 °C for 8 days under 2000 lx continuous illumination. The MA medium consisted of a mixture of bicine (500 mg); $Ca(NO_3)_2 \cdot 4H_2O$ (50 mg); KNO_3 (100 mg); $NaNO_3$ (50 mg); Na_2SO_4 (40 mg); $MgCl_2 \cdot 6H_2O$ (50 mg); β-Na_2glycerophosphate (100 mg); a metal mixture solution (1 ml; composed of 1 mg of Na_2EDTA, 0.1 mg of $FeCl_3 \cdot 6H_2O$, 1 mg of $MnCl_3 \cdot 4H_2O$, 0.1 mg of $ZnCl_2$, 1 mg of $CoCl_2 \cdot 6H_2O$, 0.16 mg of $Na_2MoO_4 \cdot 2H_2O$ and 4 mg of H_3BO_3 in 200 ml of distilled water) and the resulting solution was adjusted to pH 8.6.

2.3. Cultivation of the B-1

A bacterial strain B-1 belonging to a *Brevibacillus* sp. was used in the present study. The collection, isolation and characterization were presented in a previous paper by Tsuji et al. (2006). The inoculated bacterium B-1 in flasks was cultured at 27 °C for 2 days. Continuous shaking at 200 rpm using an MIR-220 R (SANYO, Osaka, Japan) in 100 ml of 0.05% glucose, 0.1% yeast extract, and 0.2% peptone (Sakurai medium) was applied. The OD value at 660 nm was used as index to the viable bacteria count of B-1.

2.4. Isolation of anticyanobacterial compounds from B-1

The B-1 strain cultured for 2 days (approximately 6×10^6 cells) was centrifuged at 3000 rpm for 20 min, supernatant (100 ml) was collected and filtrated using a GF/A filter (Whatman International, Ltd., Maidstone, England). The filtrate was applied on methanol activated ODS column (Chromatorex ODS 24 g, 30.0 × 3.0 cm ID, Fuji Silysia Chemical, Ltd., Aichi, Japan), followed by elution with water, 20% methanol and 100% methanol (250 ml each). These fractions were used in subsequent experiment.

2.5. Measurement of anticyanobacterial activity

Two different methods were used to determine the anticyanobacterial activity. The activity was determined using the soft-agar overlayer (SAO) method (Uchida et al., 1998). Approximately 5 ml of cyanobacterial cells (10^7 cells ml^{-1}) were mixed with warm 5 ml of 0.9% (w v^{-1}) soft agar and over-layered on a 10 ml of 1.2% (w v^{-1}) agar layer solidified in a plate. After the cyanobacterium-containing layer was solidified, B-1 cells, antibiotic (colistin), or methanol (soaked in a paper disc or agar disc) were placed on top of the cyanobacterium-containing layer. The plates were incubated at 25 °C for 5 days. Anticyanobacterial activity of the samples was determined by measuring the diameter of the plaque. A colistin-containing (100 μg per disc) or methanol-containing-agar disc was used as positive and negative controls, respectively.

The anticyanobacterial activity was also determined by measuring absorbance of chlorophyll-*a* (Uchida et al., 1998). Briefly, 5 ml of the cultured B-1 cells (2 days) or several final concentrations (2.0, 6.5, 65.0 mM) of the interesting compounds were added to 100 ml of the cultured cyanobacteria (8 days), incubated at 25 °C with shaking for 1 and 7 days under a 2000 lx continuous illumination. 2-Decanone was used instead of 8-methyl-2-nonanone found in the B-1 culture broth, because it was not commercially available. Because geosmin and 2-MIB are commercially not readily available, the experiments had to be performed at a concentration of 2 mM. Methanol or water