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Activities of Algicidal Bacteria and Their Influences on Microbial Communities

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It is said that algicidal bacteria can be used as biological control agents for algal blooms. We examined influences of algicidal bacteria on microbial communities in a laboratory experiment as a first step of ecological risk assessment. A microcosm, which consists of wild protozoa, bacterioplankton, and cultured *Microcystis aeruginosa* NIES 99, was constructed as a model microbial ecosystem of eutrophic lakes with algal blooms. *Lysobacter enzymogenes* subsp. *enzymogenes* AL-1 (DSM 1895) was added to the microcosm as an algicidal bacterium. Ciliates, flagellates, fungi, and *M. aeruginosa* NIES 99 were observed in control runs. In the runs with *L. enzymogenes* AL-1, although *M. aeruginosa* NIES 99 was not lysed, the other microbes disappeared. We address that applications of algicidal bacteria to actual lakes are considered to be ecologically risky contrary to the previous ideas.

Toxic cyanobacterial blooms have been widespread in lakes, reservoirs, and rivers throughout the world. The blooms cause livestock death and threaten human health and aquatic ecosystems[1)]. Although several approaches have been proposed to eliminate the blooms, those approaches were hardly successful. There is no complete way to remove them. Therefore, we need various approaches to devise multiple strategies.

There are algicidal bacteria that kill algae and can be used as biological control agents. However, applications of algicidal bacteria for actual lakes or rivers may destroy the ecosystems. Since they can lyse several other microbes [2, 3, 4)]. Nevertheless, there are no study for ecological risks of algicidal bacteria. We have microscopically observed the influences of algicidal bacteria on the microbial community using microcosms as a first step of ecological risk assessment.

MATERIALS AND METHODS

Influences of algicidal bacteria on microbial community. *Microcystis aeruginosa* NIES 99 (obtained from microbial culture collection at national institute for environmental studies) was used as an algal bloom forming alga, and *Lysobacter enzymogenes* subsp. *enzymogenes* AL-1 DSM 1895 (obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, abbreviate to AL-1) was used as an algicidal bacterium[3)]. *M. aeruginosa* NIES 99 were routinely cultured in MA medium at 25℃ under 12 hours : 12 hours light-dark cycle. AL-1 was routinely cultured on trypticase soy agar.

Lake water was obtained from Lake Sagami (Kanagawa prefecture, Japan), and river water was obtained form Katsuragawa River, the upstream of Lake Sagami. The total nitrogen (TN) and total phosphorus (TP) were 3.78 ppm and 0.2 ppm respectively. The TN and TP of river water were adjusted to 1.6 ppm and 0.16 ppm by sodium nitrate and dipotassium hydrogen phosphate respectively.

M. aeruginosa NIES 99 was mixed with 1,200 ml of lake water, which was filtered through a sterile 5 μm filter, in a 2,000 ml Erlenmeyer flask. This microcosm was incubated over night. The microcosm was dispensed into four 500 ml Erlenmeyer flasks. The volumes were 270 ml for each.

Thirty milliliter of AL-1 stored in sterile distilled water (DW) for 3 days was added to two microcosms, and river water was added to another two microcosms as controls. Final concentrations of AL-1 were approximately 10^{10} cells/ml.

The compositions of microbes were observed by a phase-contrast microscope.

Testing conditions where algicidal bacteria are activated. AL-1 did not kill *M. aeruginosa* NIES 99 in the above experiment. Thus, we also examined the conditions where AL-1 kills *M. aeruginosa* NIES 99.

Seven condition were tested (Fig. 1). Shortly, 3 culture media (Fig.1 (i-a), (i-b), and (i-c)), 2 suspending solutions (Fig. 1 (ii-a) and (ii-b)), and 2 durations of starvation (Fig. 1 (iii-a) and (iii-b)) were tested.

Axenic *M. aeruginosa* NIES 99 was used to test the algicidal activities. Cultured *M. aeruginosa* NIES 99 was centrifuged at 5000 rpm (approx. 5000 × g) for 15 min. The supernatant was discarded and fresh MA medium was added onto the pellet. The pellet was broken down by pipetting. The concentration of *M. aeruginosa* NIES 99 was

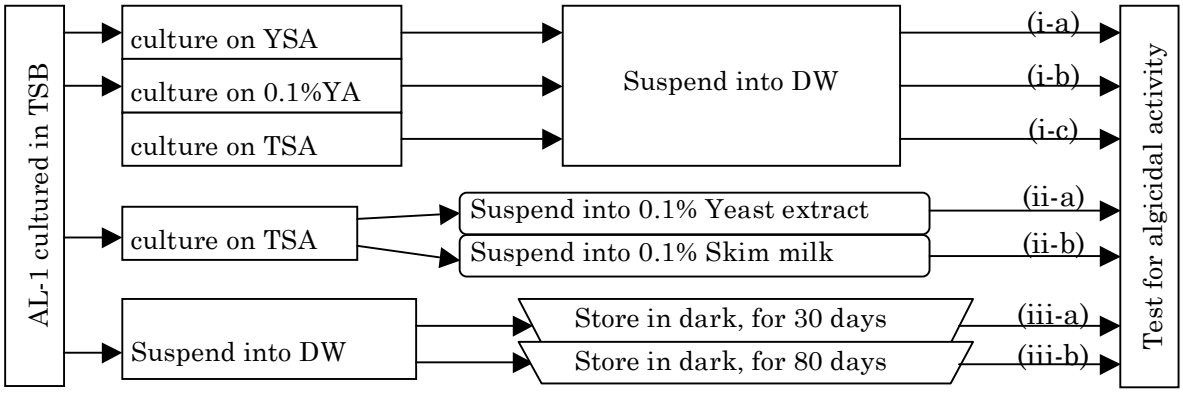


Fig.1 Seven conditions were tested. AL-1 was precultured in TSB (Torypticase Soy Broth). AL-1 was cultured (i-a) on YSA (Yeast extract Skim milk Agar) and suspended in to DW, or (i-b) on 0.1% YA (0.1% Yeast extract Agar) and suspended in to DW, or (i-c) on TSA (Trypticase Soy Agar) and suspended in to DW, then tested the algicidal abilities. AL-1 was cultured on TSA and (ii-a) suspended into 0.1% Yeast extract, or (ii-b) suspended into 0.1% Skim milk, then tested the algicidal abilities. AL-1 was cultured on TSA, suspended into DW, and stored in the dark at room temperature (iii-a) for 30 days or (iii-b) for 80 days.

adjusted to 1.5×10^5 cells/ml by adding fresh MA medium. Ten ml of it was dispensed into 30 ml test tubes. One ml of AL-1 under each conditions were added to the test tubes. The concentration of *M. aeruginosa* NIES 99 were measured for 3 days. Sterile DW was added to it instead of AL-1 as a control.

RESULTS

Influences of algicidal bacteria on microbial community. Flagellates, ciliates, yeasts, filamentous organisms, bacteria, and *M. aeruginosa* NIES 99 were observed in the control runs at the third day. Some of the microbes were showed in Fig. 1.

However, only small bacteria-like particles and some fine filaments, which might be some parts of filamentous organisms, were observed in runs with AL-1 (Fig. 2). Furthermore, *M. aeruginosa* NIES 99 increased from 1.75×10^5 cells/ml to 2.82 cells/ml (control : to 1.65 cells/ml).

Testing conditions where algicidal bacteria are activated. *M. aeruginosa* NIES 99 were increased after additions of AL-1 shortly after cultivation on any culture media (Fig. 4 (i-a), (i-b), and (i-c)).

Mixtures of AL-1 and 0.1% yeast extract decreased *M. aeruginosa* NIES 99 to 21% of control at 3rd day (Fig. 4 (ii-a)). mixtures of AL-1 and skim milk inhibited *M. aeruginosa* NIES 99 (Fig. 4 (ii-b)). The concentrations were 75% of control at 3rd day.

In runs with AL-1 stored for 30 days in DW, *M. aeruginosa* NIES 99 were not observed at the 3rd day (Fig. 4 (iii-a). AL-1 stored in DW for 80 days inhibited the growth of *M. aeruginosa* NIES 99 (iii-b). The concentrations of *M. aeruginosa* NIES 99 were 73% of control at 3rd day.

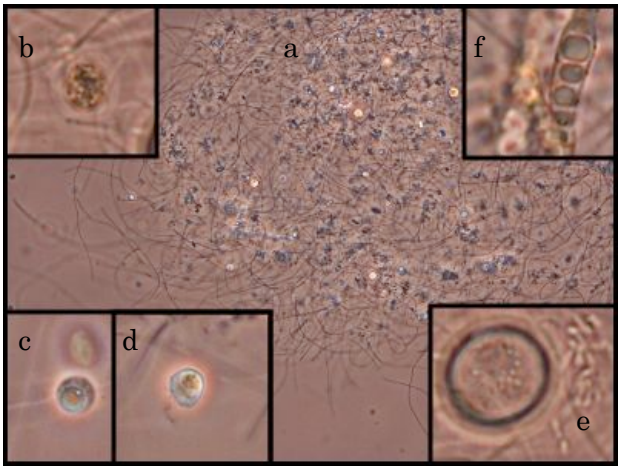


Fig. 2 The pictures of microbes observed in the control runs. a: biofilm (×400), b: *M. aeruginosa* NIES 99 (×1000), c and d: *Monas* spp. (×1000), e: yeast and some bacteria (×1000), f: fungi (×1000). The organisms showed in c to f were observed in or around the biofilms (a).

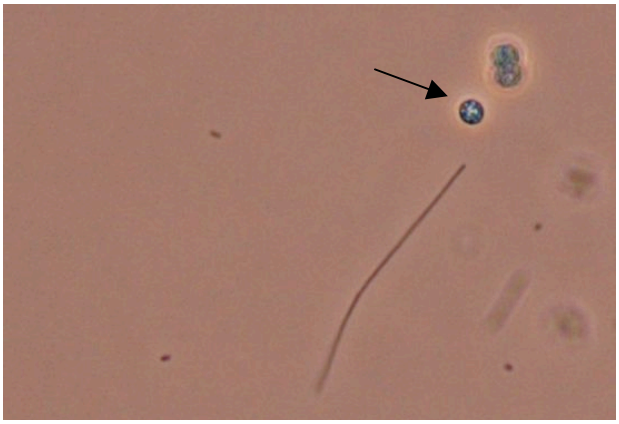


Fig. 3 The picture of microbes observed in the runs with AL-1. Only some filaments, bacteria-like particles, and *M. aeruginosa* NIES 99 (indicated by an arrow) were observed in runs with AL-1 (×400). Nonetheless, the other organisms were not observed at all.

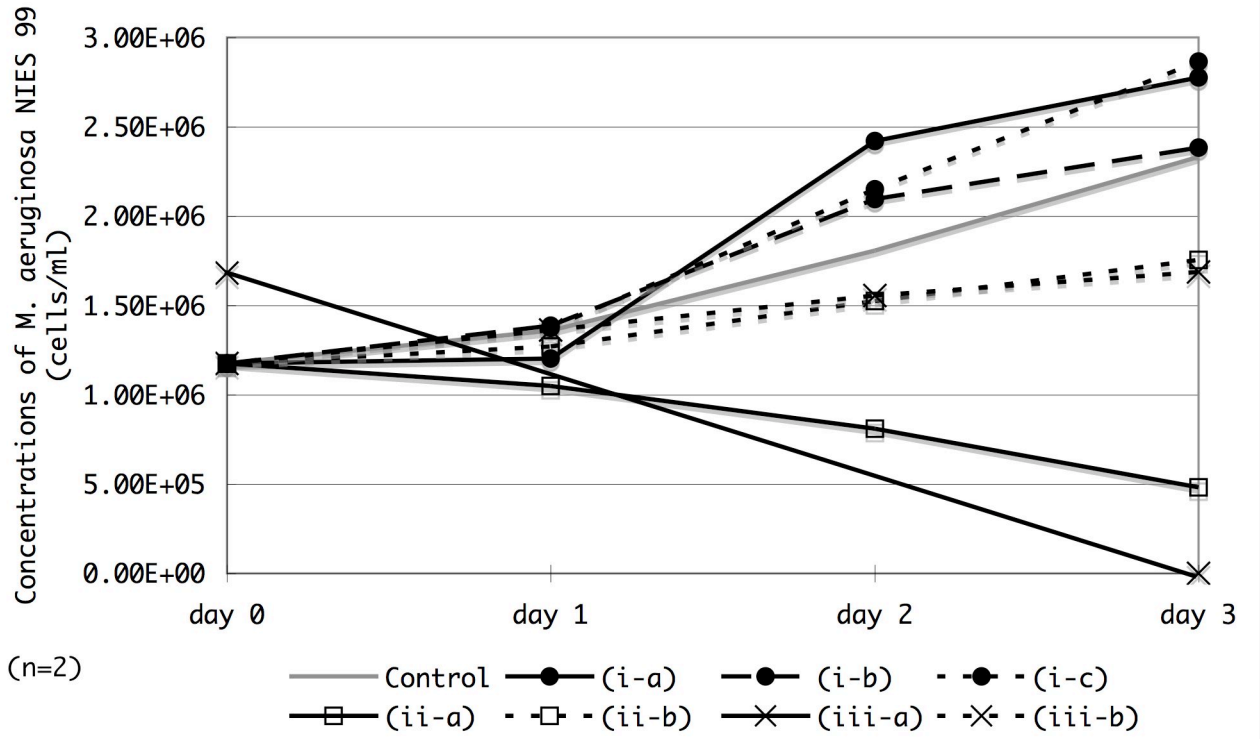


Fig.4 The changes of concentrations of *M. aeruginosa* NIES 99. (i-a), (i-b), (i-c), (ii-a), (ii-b), (iii-a), and (iii-b) are same as Fig.1. DW was added instead of AL-1 in control.

DISCUSSION

Influences of algicidal bacteria on microbial community. Although AL-1 did not lysed *M. aeruginosa* NIES 99; ciliates, flagellates, yeasts, and fungi were lysed. These microbes play important roles on nutrient cycle. Consequently, this would result stagnations of nutrient cycle and large amount of detritus. Moreover, some of the microbes are known as predators of phytoplankton. For instance, *Monas* spp. (Fig. 2 c and d) are predators of *Microcystis*[6]. Therefore, addition of algicidal bacteria might cause increase of algal bloom. Furthermore, extinction of these microbes provides chances of mass propagations for surviving phytoplankton, and results only a shift of its algal composition without eliminating it.

The influences of algicidal bacteria on microbial communities are clearly hazardous. We need to address the influences, and assess the risks.

Testing conditions where algicidal bacteria are activated. AL-1 did not decreased *M. aeruginosa* NIES 99 shortly after cultivation on any agar media. They need to be under specific conditions to decrease *Microcystis* spp.

They decreased *M. aeruginosa* NIES 99 with dilute yeast extract. This was reported that the AL-1 lyses various bacteria that cultured in 1% yeast extract broth[2].

Some of algicidal bacteria related with *Lysobacter* or *Myxobacter* secrete proteolytic enzyme[2]. Those enzyme are known as one of algicidal substances. Since skim milk is protein, they secrete proteolytic enzyme to digest it. However, AL-1 with 0.1% skim milk slightly inhibited *M. aeruginosa* NIES 99, but did not decrease them. AL-1 would need other substances that helps proteolytic enzyme to lyse *M. aeruginosa* cells, or have other mechanisms to kill them.

AL-1 drastically lysed *M. aeruginosa* NIES 99 in 3 days after 30 days storage in DW. Starvations are effective stimulating method of algicidal ability. However, AL-1 stored for 80 days only inhibited and could not decrease them. Excessive starvations deactivate the algicidal ability. There must be an optimal duration of starvation.

Eventually, adding yeast extract and 30 days starvation were effective. However, additions of yeast extract causes further eutrophication of the lakes. Thus, storage in DW is thought to be useful activating method. Nonetheless, the minimal concentrations of AL-1 to lyse *M. aeruginosa* NIES 99 are too high to apply actual lakes. We need to isolate effective algicidal bacteria. These activating methods would roughly apply to the other algicidal bacteria.

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