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Isolation and killing activities of algicidal bacteria on a diatom *Skeletonema* sp. and a dinoflagellate *Noctiluca scintillans* in Thailand

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Abstract—An attempt of this study is to isolate and to investigate the algicidal bacteria that can be against the harmful algal blooms (HAB) and have killing activity by which bacteria are associated with the events and kill their target algal species. Culture of each 20 bacterial isolates from the coastal waters in Chonburi Province in the Gulf of Thailand grew with some algal bloom species such as a marine diatom, *Skeletonema* sp., and a dinoflagellate, *Noctiluca scintillans* under controlled laboratory condition. Two bacterial isolates showed antagonistic ability that they killed HAB species kept in the same culture chamber. The algicidal bacteria caused cell lysis of algal bloom species. This study is the first report of bacteria infecting and causing lysis of a dinoflagellate, *Noctiluca scintillans* and a diatom *Skeletonema* sp.

Key words: algicidal bacteria, red tide, algal bloom, dinoflagellate, *Noctiluca*, *Chaetoceros*, *Skeletonema*

Introduction

Alga bloom and red tide phenomenon was first recorded in early nineteen fifties by Charernphol (1952) and have occurred in the Gulf of Thailand in the past decades. Recently algal blooms caused by dinoflagellates, *Noctiluca* and *Ceratium*, cyanobacteria, *Trichodesmium* and diatoms, *Chaetoceros* and *Skeletonema* have occurred more frequently. In recent years, *Noctiluca* caused more frequently serious algal bloom during the long rainy season from May to October. They have bloomed along the eutrophic coastal areas in the Gulf of Thailand. They were causative for the mass mortality of fishes and shellfishes with oxygen depletion that affected to fishermen and natural resources (Suwapeepun 1995, Thongra-ar et al. 1998). And also they impacted on environments and millions of tourists and peoples who visit or live in the event areas. It has been well established that bacteria play potential roles on the ecosystem as promoting the decline termination of harmful algal blooms. Recently, Riquelme (1988) and Fukami et al. (1991) have revealed that natural bacterial assemblage play an important role on the quick change in phytoplankton communities, especially during the development and decay period of red tide. Actually, several recent papers have reported many bacterial strains such as *Cytophaga* and *Flavobacterium* obviously showing algicidal effect against various red tide species belonging to Bacillariophyceae (Mistsuitani 1992) Dinophyceae (Adachi et al. 2001, Doucette et al. 1999, Fukami et al. 1992), and Raphidophyceae (Imai et al. 1991, 1993, Fukami et al. 1996).

In this study, we first tried to isolate bacteria with algicidal activity against the diatoms such as *Chaetoceros* sp. and *Skeletonema* sp., and dinoflagellate, *Noctiluca* from natural waters in the east coast of Thailand, where the algal blooms have frequently occurred. The study was followed to estimate the initial number of inhibiting bacteria to show inhibitory effects.

Materials and Methods

Preliminary screening for isolation of algicidal bacteria

Seawater samples were collected in small glass bottles (300 ml) from the coast in Chonburi Province from May to November 2003, 2004 monthly during the period of algal blooms. Water samples were filtered through 0.8 μm Nuclepore filter. After appropriate dilution, 3 replicates of 0.5 and 1.0 ml of filtrates were inoculated into 0.5 ml culture of *Chaetoceros* sp. or *Skeletonema* sp. in each well using a 24-multi-well plate and incubated under light exposure condition at 28°C for 2 days (modified methods from Fukami et al.

1996).

Samples of 0.8 μm filtrate showing significant inhibitory effects against algal growth were spread onto modified Zobell medium and incubated at 25°C for 3 days. Several colonies were isolated from incubated plates. After being purified, each isolated bacterium was performed following the previous method. Five isolates with significant inhibitory effects on the growth of algae or algicidal properties were selected for further experiment. In addition, appropriate tenfold dilution of cell suspension of each purified active bacterium from the previous screening with effective inhibiting activity was inoculated into a 100 ml of axenic culture of *Chaetoceros* and *Skeletonema* and incubated at 25°C for 5 days. They were previous cultured with Gillard f/2 medium. Initial densities of inoculated bacteria in the culture were 10^2, 10^3, 10^4 and 10^5 cells/ml. The inhibitory effects of added bacteria on the dynamics of algal population were determined in a laboratory.

Algicidal activity on the growth of a dinoflagellate, *Noctiluca scintillans*

As the first step of investigation, each active strain was performed in the pilot study. Each bacterium in the logarithm growth phase was inoculated into the axenic culture of *N. scintillans* which was previously cultured by SWM 3. The initial density of *N. scintillans* was 2 cells/ml while the bacterial density was 2.5×10^5 cells/ml. The samples were incubated at 28°C under light at 8,000 lux. After 2 days incubation, cells morphology and numbers of algae were observed daily under the microscopy. From this preliminary laboratory culture, strains of IMS WA 1-45 and IMS WL 1-45 with significant inhibitory effect against *N. scintillans* were selected to perform the further experiment.

At the 2nd step, *Noctiluca* cells were collected from blooming areas during May to July 2005 and incubated in 2 L chamber at 28°C for 1 day. Then either culture of strain IMS WA 1-45 or IMS WL 1-45 with the initially density of 10^5 cells/ml was inoculated into the 1 L *Noctiluca* culture with concentration of 2 cells/ml.

Results and Discussion

For screening bacteria with algicidal activity, totally 20 isolates were obtained from 6 effective water samples, and they showed inhibitory activity on test algae within 48 hours. Among them, we found 2 types of colonies indicating rapid cell lysis of *Chaetoceros* sp. or *Skeletonema* sp. within 24 hours of exposure time. When the stain IMS WL 1-45 was added to culture of *Skeletonema* sp. with the density of 10^5 cells/ml, no algal cells were observed at all in a incubation well (Fig. 1). This result was similar to previous reports by Fukami et al. (1992, 1996) in which they added an isolated bacterium *Flavobacterium* sp. 5N-3 to cultures of *S. costatum* at density of 10^5 cells/ml.

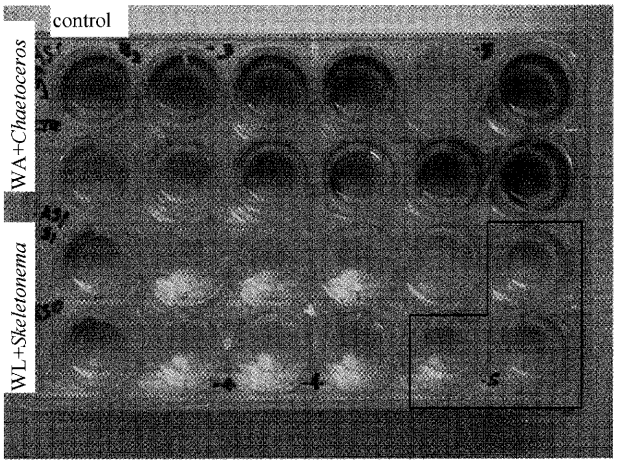

Fig. 1. Preliminary results of inhibitory effects of bacterial stainas, IMS WAS1-45 and IMS WLS1-45, on the growth of algae, *Chaetoceros* sp. and *Skeletonema* sp. after added different densities of bacterial cell suspension 10^3, 10^4, and 10^5 cells/ml. The color of *Skeletonema* culture was disappeared when bacterial cell suspension 10^5 cells/ml was added in the wells, which were shown with block line.

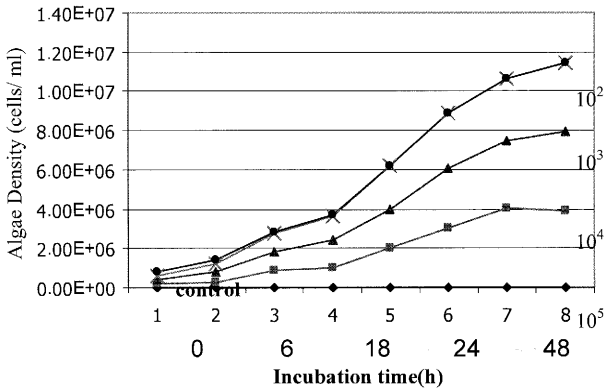

Fig. 2. Effects of isolated bacteria, IMS WAS1-45 on the growth of *Skeletonema* sp. Cell suspensions of bacteria at concentration 10^2, 10^3, 10^4, and 10^5 cells/ml were added to the culture of algae respectively then incubated at 25°C and light : dark at 12 : 12 h.

The confirmation of inhibitory effect with serial densities of inoculated bacteria revealed that the density of 10^5 cells/ml of a strain IMS WA 1-45 showed the highest effective activity on the growth of *Skeletonema* immediately after the inoculation (Fig. 2). In addition, they have obviously damaged to algal cell morphology after 18 hrs. The bacterium IMS WA 1-45 showed more significant algicidal effect than IMS WL 1-45 after 12 hrs. Most of algae were killed after 48 hrs.

These results of preliminary study showed that effect of 5 strains on the growth of laboratory culture *N. scintillans* were different. Bacterial stains that showed significant

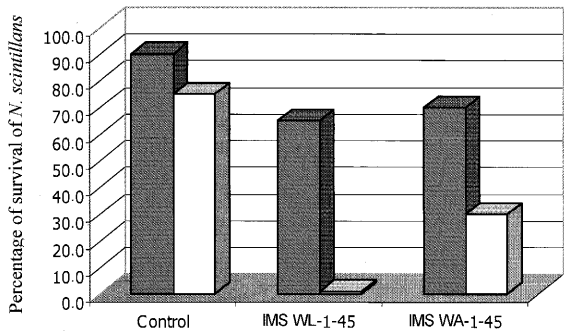

Fig. 3. Effects of isolated bacteria, IMS WAS1-45 and IMS WLS1-45, on the growth of laboratory culture dinoflagellate, *Noctiluca scintillans* during two days of experiment. Shadow columns showed result of the first day and blank column showed the second day.

growth-inhibiting effects on *N. scintillans* were IMS WA 1-45 and IMS WL 1-45. Survival rate of the test dinoflagellate *N. scintillans* in the first day was approximately 65%, and it was decreased sharply to 1% in the second day (Fig. 3). The strain IMS WA 1-45 showed higher survival rate of *N. scintillans* of approximately 70% in the first day. However it was not significantly different in the second day (Fig. 3).

Also we found the cell lysis of *N. scintillans* after 48 hrs of exposure to the strain IMS WLS1-45 while cells in control chamber were still healthy and had cell division. In addition, the strain IMS WLS1-45 at higher density showed stronger inhibitory effects against this alga. This result suggests that natural bacteria influenced significantly on red tide flagellate species, and it looks like the result of several algicidal bacteria associated with blooms of toxic dinoflagellate, *Gymnodinium catenatum* in Australian estuary (Skerratte et al. 2002)

The strain IMS WAS1-45 was initially characterized to be brown-pigment colony, gram-negative rod shape, motile and positive on DNase test, while the strain IMS WL 1-45 was characterized to be yellow-pigment colony, gram-negative rod shape, non-motile and positive on oxidase and catalase test.

Conclusion

We have shown by direct approach that algicidal bacteria were able to inhibit the growth of red tide dinoflagellate species, *N. scintillans* from laboratory culture. This supports the hypothesis that algicidal bacteria could simply control the algal growth. This is the first preliminary report of bacteria infecting and causing lysis of a red tide dinoflagellate, *N. scintillans*. We should make more experiment for deep insights at cellular level, on specific interaction between algicidal bacteria and algae from natural environment and also on the important ecological implications. Identification of active strains at genetic level should be done further, too.

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