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# Isolation and Properties of a Bacterium Inhibiting the Growth of *Gymnodinium nagasakiense*

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A bacterium 5N-3 possessing a remarkable inhibitory effect on the growth of *Gymnodinium nagasakiense* was isolated from Uranouchi Inlet, Kochi. This bacterium was tentatively identified as *Flavobacterium* sp. The growth inhibiting effect of 5N-3 on *G. nagasakiense* was drastic in particular when the alga was in the logarithmic growth phase, and cell density decreased to less than 1% of the initial concentration within 4 days after inoculating 5N-3, indicating that the effect was algicidal. The effect was obtained when the density of the bacterium was more than $10^6$ cells/m*l*. However, they grew very rapidly up to $10^8$ cells/m*l* by using extracellular released organic carbon from various phytoplankton species. On the other hand, the algicidal effect of 5N-3 was only observed on *G. nagasakiense* but not on *Chattonella antiqua*, *Heterosigma akashiwo*, or *Skeletonema costatum*. These results indicate that the effect of 5N-3 was *G. nagasakiense*-specific and suggest that it grows to a level of cell density effective in inhibiting the alga in the field by using naturally occurring organic carbon from phytoplankton.

Since a couple of decades ago, heavy algal blooming has often broken out in coastal areas of Japan, and red tides of Raphidophyceae and Dinophyceae frequently cause serious damage to mariculture. Today the prediction or prevention of the red tide is one of the most important and urgent subjects in Japanese fisheries.

It is now widely appreciated that bacteria are one of the most effective organisms on the physiology of microalgae,[1-5] and they would appear to play important roles on the development and decay of phytoplankton bloom. Based on recent knowledge of these, a project for prevention of the red tide from the bacteriological aspect was started at the initiative of Fisheries Agency of the Japanese government, and many participating microbiologists have conducted intensive studies. Imai *et al.*[6] reported that a bacterium, *Cytophaga* sp., which killed *Chattonella antiqua*, had been isolated. Some strains of algicidal *Saprospira* spp. were also isolated by Sakata[7] and his group.[8] Fukami *et al.*[9] have recently demonstrated that, during the period when one species of phytoplankton decreased and was replaced by another one, it coincided with the distribution of a bacterial assemblage suppressive against the growth of the former but stimulative for the latter. They[9] suggested that natural bacteria may influence the succession of phytoplankton communities which predominated.

Using the process of a previous investigation,[9] we isolated a bacterium which showed significant growth-inhibitory, or rather algicidal, effects on *Gymnodinium nagasakiense*. This bacterium possessed the *G. nagasakiense*-specific inhibiting effect and showed no effects on several other species of red tide phytoplankton.

In this paper, we describe the isolation and some properties of a bacterium which inhibits the growth of *G. nagasakiense*, and discuss the possibility of its availability for the prevention of *G. nagasakiense* red tide.

## Materials and Methods

Seawater samples were collected from several stations in Uranouchi Inlet, Kochi-ken, in 1989 and 1990. Water samples were filtered through either 0.2 μm (Millipore) or 0.8 μm (Nuclepore) filters, and the algal growth potential (AGP) of the two filtrates for an axenic culture of *Gymnodinium nagasakiense* were determined. The

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detailed procedure has been described previously.[9] The axenic culture of *G. nagasakiense* ax-2 was provided by Dr. M. Yamaguchi of Nansei National Fisheries Research Institute.

Samples of 0.8 $\mu$m-filtrate showing a significantly lower AGP for *G. nagasakiense* compared with that of the 0.2 $\mu$m-filtrate were spread onto peptone-agar plates and incubated for 2 weeks at 20°C. Several tens of colonies were isolated from incubated plates. After being purified, each isolated bacterium in the logarithmic growth phase was inoculated into the axenic culture of *G. nagasakiense* which was previously cultured by either SWM-3 or modified Ishimaru medium.

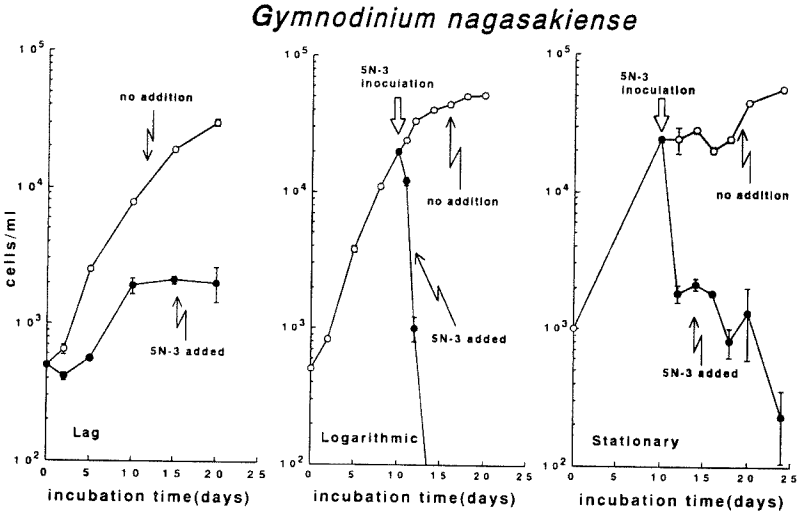


**Fig. 1.** Effects of bacterium 5N-3 on the growth of *Gymnodinium nagasakiense* in different growth stages. Time of bacterial inoculation is indicated by the open arrow.

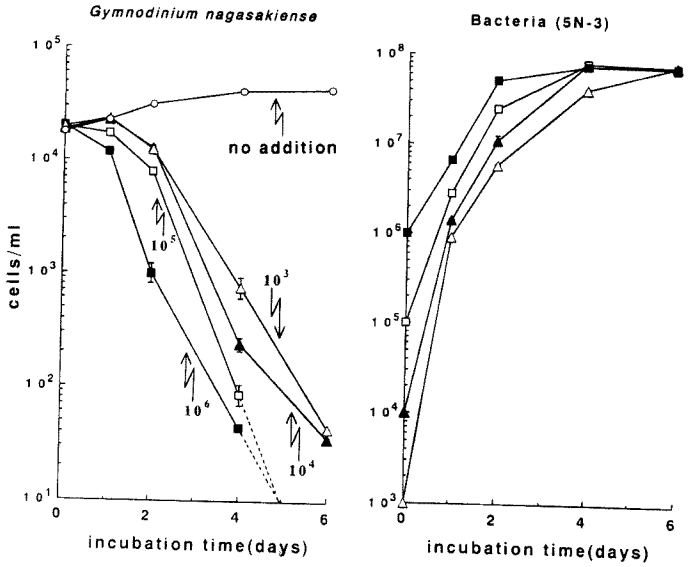


**Fig. 2.** Changes in cell densities of *Gymnodinium nagasakiense* (left) and bacterium 5N-3 (right) after inoculating of 5N-3 with different initial densities. △, $10^3$ cells/m*l*; ▲, $10^4$ cells/m*l*; □, $10^5$ cells/m*l*; ■, $10^6$ cells/m*l*.

The initial density of the bacteria was $1 \times 10^6$ cells/m*l*, unless specifically mentioned.

Consequently, one strain (5N-3) with a significant inhibitory effect against *G. nagasakiense* was able to be screened from the sample of October 23, 1989. The effects of the 5N-3 strain on the growth of *G. nagasakiense* in different growth stages besides on those of three other red tide phytoplankton species were investigated. Axenic cultures of two Raphidophyceae, *Heterosigma akashiwo* NIES-6 and *Chattonella antiqua* NIES-1, and a Bacillariophyceae, *Skeletonema costatum* NIES-324, were obtained from the National Institute of Environmental Studies. *H. akashiwo* and *C. antiqua* previously incubated in f/2 and *S. costatum* in $ASP_6$ media were inoculated by 5N-3 cell suspension.

It was also checked whether the filtrate of 5N-3 culture had an inhibitory effect on *G. nagasakiense* or not. The cell suspension of 5N-3 previously cultivated in a peptone medium to a level of $10^8$ cells/m*l* was centrifuged at 12,000 rpm and the supernatant was filtered twice through a 0.2 $\mu$m-filter. Cell-free filtrates of different volumes (10 to 40 m*l*) were added to the culture of *G. nagasakiense* in SWM-3 medium, and were made up to 80 m*l* in total. The initial density of *G. nagasakiense* was prepared to $5 \times 10^2$ cells/m*l*.

For all experiments, the same volume of peptone medium without bacterial cells as 5N-3 inoculation was added to the culture as a control, unless specifically mentioned.

The taxonomical characteristics of 5N-3 were studied according to Ezura's scheme (personal communication).

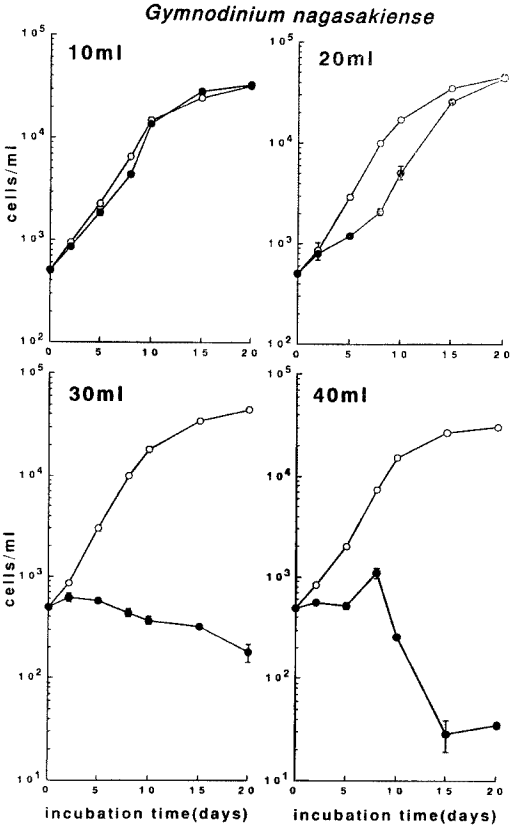


**Fig. 3.** Effects of the culture filtrate of bacterium 5N-3 at different volumes (10, 20, 30, and 40 m*l*) in 80 m*l* of incubation system on the growth of *Gymnodinium nagasakiense*. ○, No addition; ●, culture filtrate added.

## Results

The effects of 5N-3 on the growth of *Gymnodinium nagasakiense* at different growth stages are illustrated in Fig. 1. No matter what growth stage the alga was in, the bacterium showed significant growth-inhibiting effects on *G. nagasakiense*. When the alga was in the logarithmic growth phase, the effect was particularly drastic. The effects of the initial density of 5N-3 on *G. nagasakiense* were investigated. Cell suspension of 5N-3 in the logarithmic growth phase was diluted with peptone medium, and that with densities of $10^3$ to $10^6$ cells/m*l* was prepared. These suspensions were inoculated into *G. nagasakiense* cultures of the late log phase. The results (Fig. 2) showed that the density of *G. nagasakiense* decreased to less than 1% of the initial level within 1 week at any inoculum size of 5N-3, although the initial 5N-3 density of less than $10^5$ cells/m*l* introduced a slightly longer lag period before the decrease in algal cell numbers. Changes in the number of 5N-3 bacterium during the experiment are also shown in Fig. 2. Although the initial density of 5N-3 was as low as $10^3$ cells/m*l*, the bacterium grew rapidly to the $10^6$ cells/m*l* level within one day, as a result of which the decrease in the algal number was initiated. Because the 5N-3 could not grow in inorganic SWM-3 or Ishimaru media, they efficiently consume the extracellular released organic matter from *G. nagasakiense* and grow on this.

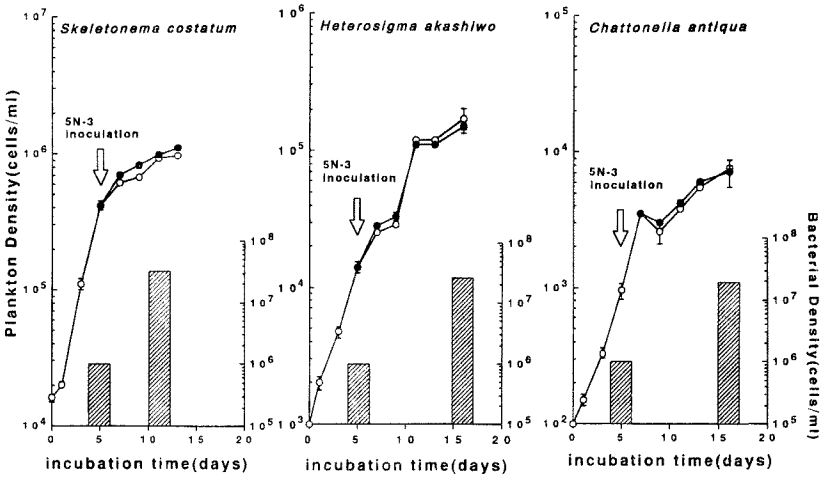


Fig. 4. Effects of bacterium 5N-3 on the growth of a Bacillariophyceae, *Skeletonema costatum* (left), and two Raphidophyceae, *Heterosigma akashiwo* (middle), and *Chattonella antiqua* (right). Cells of 5N-3 were added to each culture on the 5th day (open arrows). The 5N-3 densities at the initiation and the end of experiments are also shown by shadow columns. ○: no addition, ●: 5N-3 added.

**Table 1.** Taxonomical properties of bacterium 5N-3

| Isolate | 5N-3 |
|---|---|
| Morphology | Rods |
| Pigment | Yellow |
| Gram Reaction | — |
| OF test | None |
| Oxidase | — |
| Catalase | — |
| Motility | — |
| Gliding | — |

The inhibitory effect of 5N-3 strain on the growth of *G. nagasakiense* was detected in the filtrate of the bacterium (Fig. 3). A slight inhibitory effect was observed in the 20 m*l* addition and *G. nagasakiense* was completely suppressed by 30 m*l* or more of bacterial culture fluid. These results indicate that the growth-inhibiting effect of 5N-3 was due to some chemical material(s) released from the bacterial cells.

Although the 5N-3 strain exerted a drastic inhibitory effect on *G. nagasakiense*, it showed almost no influence on the growth of the other three species of red tide phytoplankton (Fig. 4). These results strongly suggest that the 5N-3 strain showed a relatively strict species-specific inhibitory effect on the growth of *G. nagasakiense*.

The taxonomical characteristics of 5N-3 were investigated (Table 1). This bacterium was a gram negative rod with faint yellow pigment. It had no motility or gliding movement, suggesting that it would tentatively be identified as *Flavobacterium* sp.

## Discussion

In the present study, a bacterium (5N-3) which had a significant growth-inhibiting effect on *G. nagasakiense* was isolated. The effect of 5N-3 was so remarkable that it decreased the algal density to less than 1% of the initial in 4 days (Fig. 1), indicating that the effect of 5N-3 on *G. nagasakiense* could rather be called "algicidal". One of the biggest advantages of this strain is that this bacterium was ready to grow on the released organic matter of phytoplankton, including *G. nagasakiense* itself. The effective algicidal activity of 5N-3 was obtained when its cell density was more than $10^6$ cells/m*l* (Fig. 2). In considering the practical utilization of this bacterium for the prevention of *G. nagasakiense* red tide, it is almost impossible to inoculate such a high initial amount of the bacterium in the field. However, 5N-3 grew very rapidly to more than $10^7$ cells/m*l* in the mixed culture with any phytoplankton of four species used in the present study (Fig. 4). This result suggests that it is possible to expect that 5N-3 grows and increases

cell density to a significant level in the field by using natural organic carbon from phytoplankton.

Another noteworthy advantage of 5N-3 was its strict species-specific effects on *G. nagasakiense* alone. Most bacteria previously reported as algicidal were inhibitive against multi-species. Ishio *et al.*[10] reported a bacterium which killed several species of dinoflagellates. This bacterium, moreover, also inhibited the growth of *Chattonella antiqua* and *C. marina*. A strain of *Cytophaga* sp. reported by Imai *et al.*[6] also had a very wide hostile range against phytoplankton. As a result of their recent study, this *Cytophaga* bacterium killed 5 species of Raphidophyceae, one Dinophyceae and 4 Bacillariophyceae species (personal communication). Such bacteria with a broad range of algicidal effect is apparently "a must" for the prevention of red tide. However, it must on the other hand be rather unfavorable since even the normal assemblage of phytoplankton in natural seawater would surely affected by the bacteria. This means that such bacteria would act more or less like a "poison" and the bacteria themselves will certainly be another factor changing the balance of the ecosystem. Algal blooming and consequent red tide can be considered to be an explosive growth of a few selective species of phytoplankton due to the turbulence of a well-balanced ecosystem. We should suppress only the additional and undesirable growth of such algal species. From such a point of view, the 5N-3 isolated in this study is a bacterium which promises actual utilization in the prevention of the red tide of *G. nagasakiense* in the field.

## Acknowledgements

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