사단법인 한국환경과학회 가을학술발표회 발표논문집 제17권(제1호), 282~285, 2008
Proceedings of the Korean Environmental Sciences Society Conference, Nov, 20~22, 2008

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Survival and growth of the red tide organism *Cochlodinium polykrikoides* after the addition of yellow loess

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1. Introduction

Over the last 10 years, a number of studies have examined the relationship between *Cochlodinium polykrikoides* red tides and yellow loess (Choi et al., 1998; Lee et al., 2008). However, most have focused on the efficiency of yellow loess for the removal of *C. polykrikoides* to prevent red tides; none has examined the survival or growth of *C. polykrikoides* after the addition of yellow loess. Therefore, many questions remain, such as whether the *C. polykrikoides* that has settled in the bottom layer can later move to the surface layer and cause another red tide. It is difficult to determine the precise effects of yellow loess on *C. polykrikoides* without such information.

In this study, we conducted culture experiments to observe the possibility of red tides caused by *C. polykrikoides* organisms precipitated by loess addition. We also examined the variation in algal cell density after yellow loess addition.

2. Materials and Method

The strain of *C. polykrikoides* used in this experiment was isolated from coastal seawater off Naro Island in the South Sea of Korea during the summer of 2002. Stock cultures were maintained with f/2 medium using surface seawater taken from the South Sea of Korea. Cultures were maintained at 23 ± 2°C with fluorescent illumination of 140 ± 10 μmol $m^{-2}s^{-1}$ on a 12-h light 12-h dark cycle. Yellow loess that had been used to control C. *polykrikoides* red tide in summer 2007 in Tongyong, Korea, was dried at 60°C for 2 days and mixed. Particles smaller than 63 um were used for these experiments. Lag, exponential, and stationary phases of *C. polykrikoides* were used to observe the survival rates of each growth stage after the addition of yellow loess. Growth was monitored with a PHYTO-PAM chlorophyll *a* fluorometer (Heinz Walz, Effeltrich, Germany; Schreiber et al., 2002). For survival experiments, the *C. polykrikoides* with yellow loess was stored in a culture room (23 ± 2°C) under fluorescent illumination (10 ± 2 μmol m^{-2} s^{-1}) on a 12-h light12-h dark cycle, which is

considered the same illumination as that at a depth of 10 m. Live *C. polykrikoides* cells were examined using an optical microscope (BX 50; Olympus, Tokyo, Japan). To observe whether *C. polykrikoides* that precipitated to the bottom after yellow loess addition would grow, culture experiments were conducted using precipitated *C. polykrikoides*. For this experiment, we used exponential and stationary phase *C. polykrikoides* after 7 and 8 days, respectively, with the addition of yellow loess (2000 μg/L) or without it (0 μg/L), under low fluorescent illumination (10 ± 2 μmol m^{-2} s^{-1}) because live cell densities of *C. polykrikoides* were very low after 7-8 days. The controls were cultures from the same phase that were not exposed to loess or low fluorescent illumination. Growth was monitored by cell density under a BX 50 Olympus optical microscope.

3. Results and Discussion

3.1. Survival rate of *C. polykrikoides* after the addition of yellow loess

During the lag phase, cell density without yellow loess increased to 666 cells ml^{-1} after 1 day, and 153 and 44 cells/ml were observed after 7 and 14 days, respectively. Seven days after the addition of 100, 1000, or 2000 μg llyellow loess, 123, 73, and 53 cells/ml, respectively, were observed. After 14 days, cell density was >4 cells/ml. Despite the addition of 2000 μg/L yellow loess, 15% of *C. polykrikoides* cells survived for 7 days.

During the exponential phase, cell density after 1-3 days increased slightly with or without the addition of 100 μg/L yellow loess. After 7 days, cell density was 373 or 173 cells/ml with or without the addition of 100 μg/L yellow loess, respectively. Cell density decreased from the first day onward after the addition of 1000 or 2000 μg/L of loess. After 7 days, cell density was 360 and 253 cells/ml, respectively, more than 15% of the initial cell density.

During the stationary phase, the variation in the number of surviving *C. polykrikoides* cells was similar to that during the exponential phase. In the 0-2000 μg/L loess treatment, cell density increased slightly after 1 day. After 6 days, cell density decreased, and after 8 days, 173-360 cells/ml were observed, representing about 15% of the initial cell density, as in the exponential phase. As mentioned above, most studies have focused on the precipitation rate of *C. polykrikoides* cells on adding yellow loess, while no study has examined the survival rate of these cells precipitated to the bottom layer by yellow loess. In addition, why did only about 15% of the *C. polykrikoides* cells that were precipitated to the bottom layer live for around 1 week, despite being in the same growth phase? At present, we have no answer for these questions and need further study to explain these phenomena.

3.2. Growth rate of *C. polykrikoides* precipitated to the bottom layer by yellow loess

In the exponential phase, the maximum growth of *C. polykrikoides* not exposed to loess was observed after 8 days. There was almost no variation in cell density for 11 days either with the addition of loess (2000 μg/L) or without it (0 μg/L). Cell density then increased, and at 26 days, the numbers of cells had increased to 2886 and 3366 cells/ml with or without the loess addition, respectively.

During the stationary phase, the density of *C. polykrikoides* that were not exposed to low fluorescent illumination or loess (control) began to increase after 3 days; a density of 4030 cells/ml was observed after 18 days. The density of *C. polykrikoides* that were not exposed to loess (0 μg/L) and exposed to loess (2000 μg/L) for 7 days did not increase. When the initial density is low, a culture time of at least 14 days is needed to identify whether *C. polykrikoides* has grown. In this experiment, the initial density of *C. polykrikoides* was 20 or 40 cells/ml in the 0 or 2000 μg/L loess treatments, respectively, and the cells disappeared after 18 days at the stationary phase. As these initial cell densities were >20 cells/ml, a low cell density does not appear to be the reason that the cells did not grow. Most culture experiments have used the exponential phase of *C. polykrikoides*, not the stationary phase. Therefore, the reason that the cells in the exponential phase grew while those in the stationary phase did not, despite being at the same initial cell densities, seems to be the activity of *C. polykrikoides* cells.

During the exponential and stationary phases, a stock culture of *C. polykrikoides* that was not exposed to loess but grown under light was used as the control. Except for the addition of loess and the light condition, the conditions were the same among the treatment groups. In the controls, cell growth was measured from 1 and 3 days after inoculation. However, without loess, cells in the exponential phase (exponential phase, 0 μg/L) grew after 14 days and cells in the stationary phase (stationary phase, 0 μg/L) did not grow until 18 days. Therefore, extended low light conditions at the bottom layer appear to inhibit the activity of *C. polykrikoides*.

During the exponential phase, *C. polykrikoides* growth was observed after 14 days, and cell densities over 2886 cells/ml were observed after 26 days with or without the addition of 2000 μg/L loess. However, during the stationary phase, growth was not observed either with or without the 2000 μg/L loess treatment. Also, based on microscopy, the external morphology of the cells in the two phases was similar. Therefore, loess addition seems to precipitate *C. polykrikoides*, but does not control its growth through morphological damage.

4. Summary

At least 15% of the *C. polykrikoides* cells that precipitated to the bottom layer either

by the addition of loess or no addition survived for 1 week at all growth phases, rather than disappearing immediately after precipitating. However, no live cells were observed after 20 days, regardless of phase or loess addition. In the exponential phase, the number of *C. polykrikoides* cells increased to >2886 cells/ml after loess was added. However, in the stationary phase, the number of cells did not increase until 18 days. In the exponential phase, those *C. polykrikoides* that survived precipitation caused by scattering loess on cultures did not appear to have the ability to cause red tides again because of the short red tide periods in the field, long lag time after loess addition, and low survival rate after loess addition.

References

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