# Growth inhibition of Prorocentrum minimum by Ulva pertusa Kjellm under different N and P nutrient limitations

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**Abstract.** The effects of different N and P nutrient limitations on the growth inhibition of Prorocentrum minimum by Ulva pertusa Kjellm fresh tissue and dry powder were studied in this paper. The results showed that U. pertusa fresh tissue and dry powder could inhibit the growth of P. minimum, and the growth inhibition was more significant with the increased contents of U. pertusa fresh tissue and dry powder. The growth inhibition of P. minimum could be changed under different N and P nutrient limitations. The growth inhibition rates of P. minimum by U. pertusa fresh tissue were N and P sufficient > P limited > N limited > N and P both limited. The growth inhibition of P. minimum by U. pertusa dry powder was decreased but not significantly ($p > 0.05$) when N and P were limited.

## Introduction

Red tides had become a serious marine environmental problem and raised the concern from international society and need to be solved urgently. In recent years, the prevention strategies of red tides had been shifted from physical and chemical methods to biological methods. The growth inhibition of red tide microalgae by macroalgae was an important research direction of red tide biological control. *Ulva pertusa* Kjellman was belonged to Chlorophyta (Phylum), Ulvales (Order), Ulvaceae (Family) which was widely distributed in the western Pacific coast and the resources were extremely rich in China. Studies had shown that *U. pertusa* could inhibit the growth of red tide microalgae. In marine ecosystems, N and P were important nutrient limiting factors. The results of these different organisms of the two different nutrient competitions would change ecosystem structure and composition [1-4]. Some researchers believe that the main reasons leading to algal blooms were the different proportion of N and P in the sea [5,6 ] . In addition, the levels of N and P nutrient would affect allelopathy between organisms [ 7-8 ] .

The effects of macroalgae- *Ulva pertusa* Kjellman on the growth inhibition of red tide microalgae- *Prorocentrum minimum* under different N and P nutrient limitations were studied in this research. The experimental results would provide foundation for the prevention strategies of red tides.

## Materials and methods

**Plant materials and treatments.** *Ulva pertusa* Kjellman was collected from Heishijiao of Dalian, China. The material was washed with natural seawater immediately after sampling then pre-culture for 7 days at room temperature with illumination of 3000 Lx.

*Prorocentrum minimum* was offered by College of Marine Technology and Environment, Dalian Ocean University.

**Growth inhibition of *P. minimum* by *U. pertusa* fresh tissue assays.** Triangle bottles (300-mL) were used as culture containers. The initial weights of *U. pertusa* fresh tissue were 0.00, 0.625, 1.25, 2.50, 5.00, 10.00g FW/L respectively. *P. minimum* ($5\times10^4$ Cells/mL) was inoculated in 100-mL f/2 culture medium [9], and aged sea water of the Dalian Current (salinity of 31) was used as the culture medium. The culture was maintained at 20°C under 3000Lx cool-white fluorescent illumination on a 12 h: 12 h light: dark cycle.

**Growth inhibition of *P. minimum* by *U. pertusa* dry powder assays.** The initial weights of *U.*

 

*pertusa* dry powder were 0.00, 0.30, 0.60, 0.90, 1.20 g FD/L respectively. *P. minimum* ($5\times10^4$ Cells/mL) was inoculated in 100-mL f/2 culture medium, and the other conditions are the same as the previous experiments.

**Growth inhibition of *P. minimum* under different N and P nutrient limitations assays.** The background of N and P in natural seawater was $NO_3^-$<9mmol/$m^3$, $NH_4^+$<1mmol/$m^3$, $PO_4^{3-}$<0.8mmol/$m^3$ respectively. The initial cell densities of *P. minimum* were $2\times10^4$ Cells/mL. The initial weights of *U. pertusa* fresh tissue and dry powder were 1.25g FW/L, 0.6g FD/L respectively. There were four different culture media types of nutrition in this trail: N and P sufficient (f/2 culture medium), N limited (Except N was not added, the other nutrients were enriched by f/2 culture medium), P limited (Except P was not added, the other nutrients were enriched by f/2 culture medium), N and P both limited (Except N and P were not added, the other nutrients were enriched by f/2 culture medium). *P. minimum* ($5\times10^4$ Cells/mL) was cultured in f / 2 medium as the control group. *P. minimum* was fixed by Lugol iodine solution and then counted using optical microscopy every two days.

**Statistics.** All experiments were set up three parallel groups**.** For all data collected SPSS 11.0J for Windows.

## Results

**Effects of *U. pertusa* fresh tissue on the growth inhibition of *P. minimum*.** Growth curves of *P. minimum* coexistence with different initial inoculation concentrations of *U. pertusa* fresh tissue were shown in Fig.1. *U. pertusa* fresh tissue could inhibit the growth of *P. minimum*, and the growth inhibition was more significant with the increased contents of *U. pertusa* fresh tissue. On the day 3, *P. minimum* was completely removed when the content of *U. pertusa* fresh tissue was 5.00g FW/L.

**Effects of *U. pertusa* dry powder on the growth inhibition of *P. minimum*.** Effects of *U. pertusa* dry powder on the growth inhibition of *P. minimum* were shown in Fig.2. *U. pertusa* dry powder could inhibit the growth of *P. minimum*, and the growth inhibition was more significant with the increased contents of *U. pertusa* dry powder. On the day 6, *P. minimum* was completely removed when the content of *U. pertusa* dry powder was 0.90g FD/L.

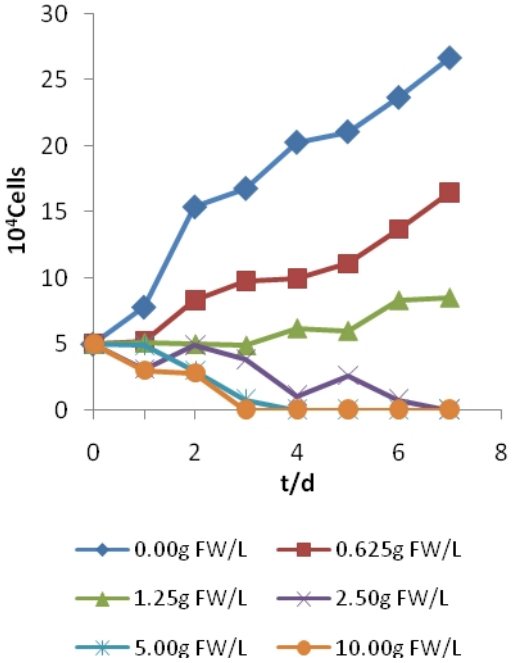


Fig.1. Growth curves of *P. minimum* coexistence with different initial inoculation concentrations of *U. pertusa* fresh tissue

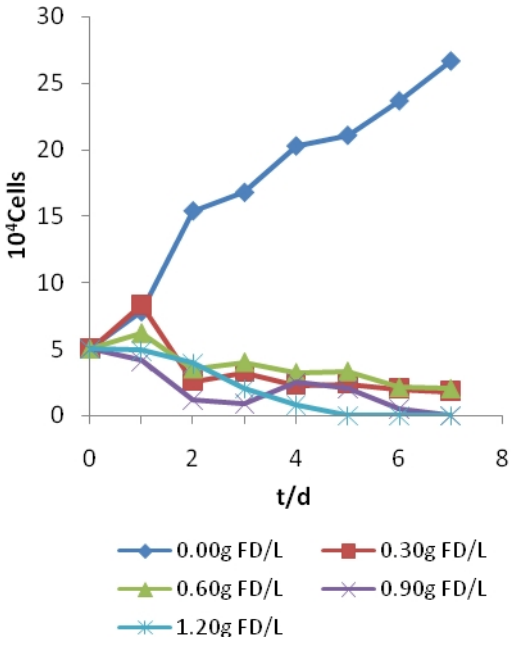


Fig.2. Growth curves of *P. minimum* coexistence with different initial inoculation concentrations of *U. pertusa* dry powder

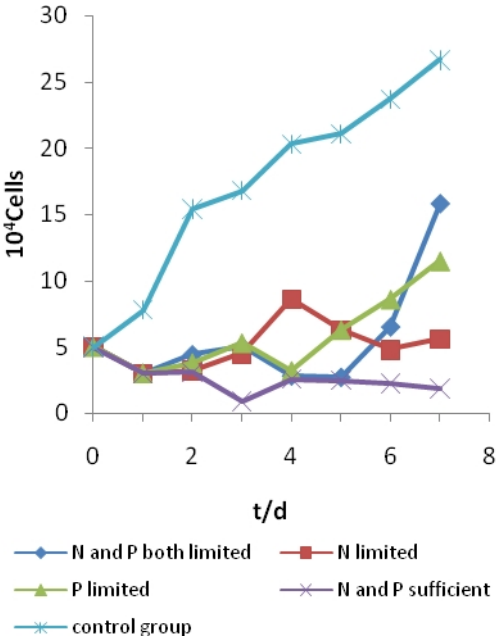


Fig.3. Growth curves of *P. minimum* coexistence with *U. pertusa* fresh tissue under different N and P nutrient limitations

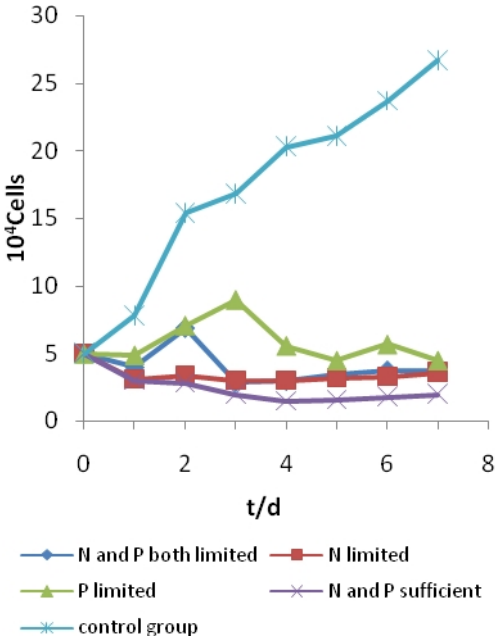


Fig.4. Growth curves of *P. minimum* coexistence with *U. pertusa* dry powder under different N and P nutrient limitations

**Effects of *U. pertusa* fresh tissue and dry powder on the growth inhibition of *P. minimum* under different N and P nutrient limitations.** Growth curves of *P. minimum* coexistence with *U. pertusa* fresh tissue under different N and P nutrient limitations were shown in Fig.3. The growth inhibition of *P. minimum* was significantly decreased ($p < 0.01$) when N and P were limited from the day 6 to 7. On the day 7, the growth inhibition rates of *P. minimum* were N and P sufficient > P limited > N limited > N and P both limited.

The effects of *U. pertusa* dry powder on the growth inhibition of *P. minimum* under different N and P nutrient limitations were shown in Fig.4. From the day 4 to 7, the growth inhibition of *P. minimum* was decreased but not significantly ($p > 0.05$) when N and P were limited.

## Discussion

Hogetsu(1960) proposed that allelopathy between macroalgae and microalgae could be utilized to control the growth of microalgae [10]. *U. pertusa* could secrete some allelopathy compounds to inhibit the growth of microalgae [11]. The experiments showed that *U. pertusa* fresh tissue and dry powder could inhibit the growth of *P. minimum*, furthermore *P. minimum* was completely killed by *U. pertusa* fresh tissue and dry powder. The red tides of *P. minimum* could be controlled by *U. pertusa*.

The allelopathy between marine biological was affected by the nutrient in aquatic environment [2-3]. Nutrient limitation might change the allelopathy secretion of macroalgae and the sensitivity of microalgae to allelopathy substances. The growth inhibition of *P. minimum* by *U. pertusa* fresh tissue was the strongest at N and P sufficient because the physiological activities of *U. pertusa* fresh tissue were exuberant in such nutritional environment. Graneli and Johansson found that the allelopathy of *P. parvum* to *P. minimum* at N limited was stronger than N and P sufficient [12]. This result was inconsistent with our experiments probably because the N and P demands of *U. pertusa* and *P. parvum* were not identical. The growth inhibition of *P. minimum* by *U. pertusa* dry powder was not sensitive to N and P changes. The reason was because N and P could not affect the release of allelopathic substances from *U. pertusa* dry powder.

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