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Allelopathic Effects of *Alexandium* spp. on Growth of *Prorocentrum donghaiense* Lu: Is It Toxin Dependent?

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Abstract

The allelopathic properties of three *Alexandrium* species: *A tamarense* (ADHK), *A. minimus* (AM-1) and *A. catenella* (ACDH), on one species of dinoflagellate *Prorocentrum donghaiense* were determined using bioassays of allelopathic test. Their culture filtrates were prepared at different growth phases of exponential (EP) and stationary one (SP), and were applied for the cultivation of *P. donghaiense* in the co-culture. Results revealed that all the culture filtrates presented growth inhibition on the co-cultured *P. donghaiense*, among which ADHK presented the most severe inhibition. Culture filtrates at SP showed stronger inhibition as compard to that in EP, and the dilution of the culture filtrates could alleviate their negative effects. Analysis on hemolytic activity presented that all of the *Alexandrum* species had hemolytic activity, which was consistent with their reported PSP toxins. It was thus speculated that the toxin-independent allelochemical(s) were involved in the noxious effects of *Alexandrum* spp. on *P. donghaiense*.

Keywords: *Alexandrium* spp., *Prorocentrum donghaiense*, Allelopathy, Haemolytic activity, Toxin-independent

1. Introduction

Alga-released allelochemicals and algal toxins are suggested to be the main reasons responding for the phytoplankton succession in aquatic ecosystem by affecting some species more than others (Fistarol et al., 2005; Gross, 2003). However, few reports focused on discriminating the different roles of toxin-mediated allelopathy between toxic phytoplankton (TPP) and non-toxic phytoplankton (NTP) during the succession process. Granèli and Johansson (2003) found *P. parvum* exerted negative effects on the co-cultured microalgae by producing toxins, which was suggested to play an allelopathic role. It was reported that toxin-allelopathy might lead to the overturn of competitive exclusion among phytoplankton species. In addition, the toxin-allelopathy was favorable for the stable coexistence of non-toxic phytoplankton that otherwise not coexisted Roy and Chattopadhyay (2006).

Dinoflagellates of the genus *Alexandrium* are well known for their feature of the occurrence of paralytic shellfishpoisoning (PSP). Moreover, the potentiallytoxic allelopathic substances were suspected to have detrimental effects on surrounding organisms. The effect of toxins or allelopathic substances produced by *Alexandrum* in its ecological success had received great attention. Tillman and John (2002) had reported that the toxic effects of *Alexandrium* spp. on the co-cultured dinoflagellates were not due to PSP toxins, but were caused by the released extracellular substances. *A. taylori* was found to produce a kind of proteinacelous hemolytic exotoxin against mammalian erythrocytes, and this toxin did not belong to PSP toxins (Emura et al., 2003). Dinoflagellates species of *Prorocentrum donghaiense* and *Alexandrium tamarense* were identified as the causative species of the bloom occurred annuyally in East China Sea (Lu and Goebel, 2001). We thus chose *P. donghaiense* as the target organism to examine the effects of different *Alexandrum* species with or without PSP toxin on its growth. The purpose of the present study aimed at elucidating the role of toxins and allelochemical(s) independt of toxins in the succession of the bloom.

2. Materials and Methods

2.1 Microalgal Cultivation

Three *Alexandrium* species*: Alexandrium tamarense, Alexandrium minutum, Alexandrium catenella* and *Prorocentrum donghaiense* were obtained from the Algal Center of the Institute of Oceanography, Chinese Academy of Sciences (IOCAS) and kept in Ecological laboratory of Ocean University of China. *P. donghaiense* was isolated from a bloom in East China Sea and was provided by IOCAS. *A. tamarense* strain ADHK was isolated in 2003 from a bloom occurred in South China Sea in Hongkong, China. *A. minutum* strain AM-1 was isolated from coastal area of Taiwan, China. *A. catenella* strain ACDH was isolated from Yangzi River estuary in East China Sea. The toxic properties of ADHK, ACDH and AM-1 were 2.60, 1.61 and 0.36 pg eq .STX.cell^{-1}, respectively according to the previous reports (Chen et al., 2007). They were designated as strong, medium and weak toxicity respectively in the present study.

All the microalgae were cultured in modified f/2 medium (Guillard, 1975) at 18±0.5 °C in flasks under illumination condition of 70-μmol m^{-2} s^{-1} and 12-h light: dark cycle, initial pH of 8.0, and salinity of 30. All flasks were shaken manually twice a day at set times. The microalga in exponential growth phases were used in the experiments. During the experiment, the microalga were inoculated into 250-mL flasks containing fresh f/2-enriched seawater and the total experimental volume was 150 mL.

2.2 Allelopathic Test

The allelopathic activity of the *Alexandrium* sp. cultures towards *P. donghaiense* was tested by the method developed by Gentien and Arzul (Gentien and Arzul, 1990).

2.2.1 Culture Filtrate Preparation

In order to verify the effects of the extracellular products, the cell-free culture filtrates of *Alexantrium* spp. were studied. The culture filtrates were prepared from *A. tamarense, A. minutum* and *A. catenella* at their expoential phase (EP) at an initial cell density of 1.0×10^4 cells mL^{-1}. The culture media were firstly gentlely filtered through 0.45-μm and then 0.20-μm Millipore membrane with the vacuum value below 30 Pa for the sterilizing process to eliminate the bacteria. This sterilize filtrate was replete with f/2 stock medium (Guillard, 1975) and used for *P. donghaiense* cultivation. The initially inoculating cell density of *P. donghaiense* was set at 1.0×10^4 mL^{-1} and the correspondingly fluorenscence (fsu) was 3.6. Its growth was determined by measuring fluorenscence using a Turner Designs Model 10-700R fluorometer (Wang and Tang, 2008) every 24 h. All other conditions and procedures in this experiment were the same as those described in 2.1, and the experiment lasted for 18 days.

2.2.2 Experimental Set-Up

The culture filtrates above prepared were divided into two groups: one was directly used for *P. donghaiense* cultivation and the other was diluted for 3 times and then applied for microalgal cultivation. All the other conditions and procedures in this experiment were the same as those described in 2.2.1. Another experiment was performed to determine if there's any difference of the extracellular products released by the microalge at different growth phases, and *A. tamarense* was used as an example. It was firstly cultivated to their late stationary phase (SP) (about 20 days according to the preliminary experiment) and then diluted to a cell density of 1.0×10^4 mL^{-1} enriched with f/2 medium to eliminate the possible nutrient limitation. This filtrate was used for *P. donghaiense* cultivation with an initial cell density of 1.0×10^4 mL^{-1}. The experiment lasted for 18 days. The growth rate was determined and the results were expressed as μ according to the method of Guillard (1975):

$$\mu = \frac{\ln N - \ln N_0}{t - t_0}$$

t and t_0 denoted the times when there were N and N_0 cells L^{-1} respectively, t_0 and N_0 corresponding to the initial situation.

2.3 Hemolytic Analysis

The haemolytic property of the seawater in *Alexandrium* cultures was assayed according to the method of Arzul et al. (1999). The assays were performed on rabbit red blood cells, which were obtained from Beijing Solarbio Science & Technology Company. The rabbit blood cells was stored in the dark at 4 °C to avoid photolysis. The results of haemolysis were expressed in Haemolytic Units per litre (HU L^{-1}).

2.4 Statistics Assay

Results were analyzed with SPSS 20.0 and Sigmaplot 12.0. The significance between experimental groups was analyzed by one-way ANOVA with significance set at $P < 0.05$.

3. Results

3.1 Allelopathic Test

The cellular behaviours of *P. donghaiense* in the culture filtrates obtianed different strains of *Alexandrium* spp. were quite different (Fig 1). Little difference was observed between the group of control and that in the culture filtrate of *A. catenella* ($P>0.05$), but obvious growth inhibition was observed in groups of cultivated in culture filtrates of *A. tamarense* and *A. minutum* ($P<0.01$) (Fig.1A). However, great changes were occurred in groups cultivated in the diluted culture filtrates (Fig.1B). The growth of *P. donghaiense* grown in filtrate of *A. catenella* was greatly increased and the arbitrary value of fluorescence was increased by about 35% as compared to the control ($P<0.01$). The growth inhibition in groups of culture filtrates of *A. minutum* and *A. tamarense* were not as severe as that in the groups without dilution. It seemed that the dilution could alleviate the growth inhibition. On possible explanation might be that the dilution decreease the concentraion of the substances existing in the culture filtrate, which might be allelochemical(s).

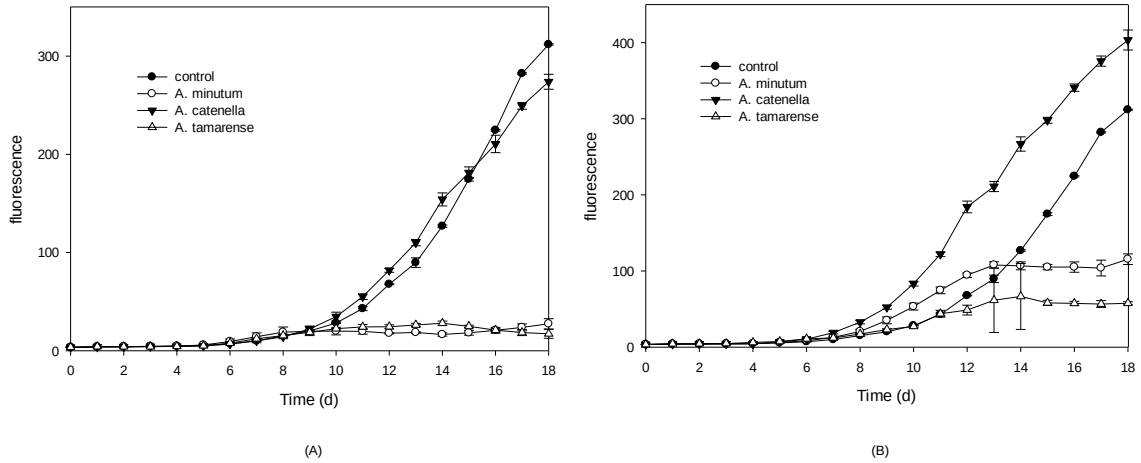

Figure 1. Growth of *P. donghaiense* in culture filtrate of three *Alexandium* species with and without dilution (expressed as arbitrary fluorescence units)

Note: A. growth in culture filtrates without dilution; B. growth in diluted culture filtrates.

In order to further evaluate the possible effects of allelochemcial (s) produced in different growth phase, the culture filtrates prepared from exponential growth phase (EP) and stationary growth phase (SP) were applied for *P. donghaiense* cultivation. Little difference was observed during the first 9 days after inoculation between the treated goups and the control with and without dilution in either EP or SP groups. However, great difference was observed thereafter, and significant growth inhibition was found in group of SP. in this rgards, we speculated that more allelochemical(s) produced more that in EP. Dilution was also perfomed on culture filtrates at different growth phase.

When the culture filtrates prepared from different growth phases were diluted for 3 times for the microalgal cultivation, the microalgal growth differed obviously. Little difference was found in the first 8 days after inoculation ($P>0.05$) and thereafer, growth in Effects of culture filtrates obtained from different and great significance was observed thereafter. The microalgal growth in EP group was much higher than that in the SP group, inferring the existence of the potential allelochemical(s).

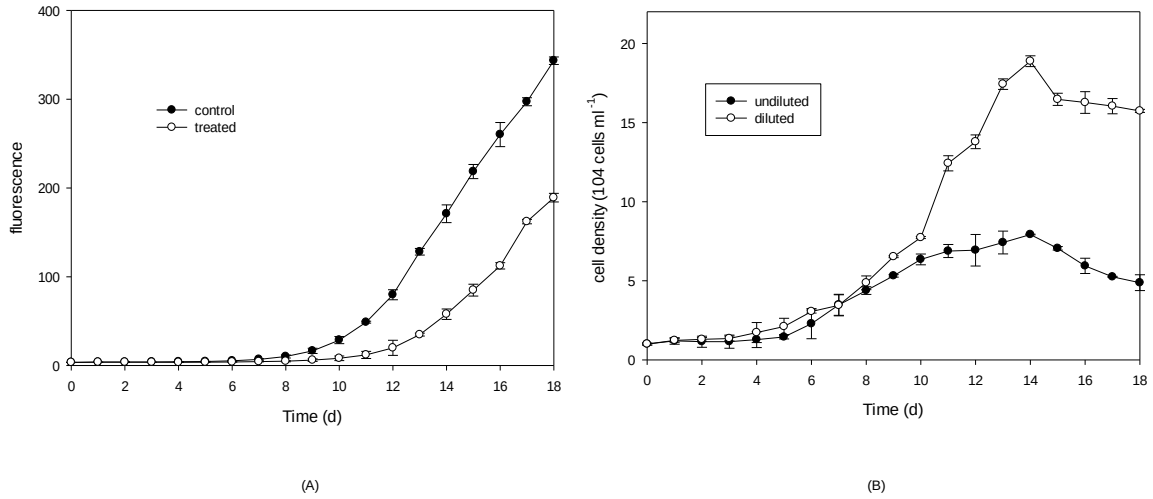

Figure 2. Effects of culture filtrates obtained from different growth phases on growth of *P. donghaiense*

Notes: A. Growth of *P. donghaiense* grown in culture filtrates of *A. tamarense* at different growth phases; B. Growth of *P. donghaiense* grown in culture filtrates of *A. tamarense* at SP with and without dilution

3.2 Hemolytic Activity of Different Alexandrium Strains

Figure 3 shows the hemolytic activities of filtrate of different Alexandrium strains cultures. ADHK presented the highest hemotytic activity, followed by ACDH and AM-1. This sequence was consistent with that of contents of STX reported by Chen et al. (2007).

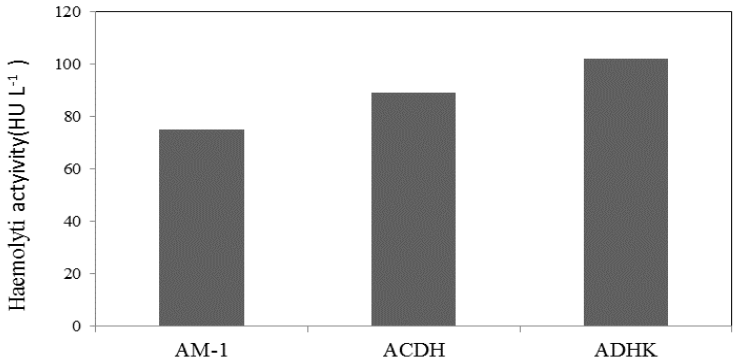

Figure 3. Relative hemolytic activities of filtrates of different *Alexandrium* strain cultures

4. Discussion

Allelopathy in *Alexandrium* spp. was first described by Hansen (1989) and so far, it has been demonstrated by many reports. However, little was known about the action of allelochemical involved in this process. Tillmann and John (2002) investigated the effects of *A. tamarense* on the other dinoflagellates and found that the observed toxicity did not correlate with PSP toxins in cells. Some *Alexandrium* strains that did not contain PSP toxins were allelopathic. Therefore, the allelopathy in *Alexandrium* spp. was attributed to unknown toxins. In addition

to PSP toxins, dinoflagellates can produce hemolytic substances which might be helpful in their competition with other organism in natural envionments. In the present study, the allelopathic test was applied to determine the toxic effects of different *Alexantrum* species on growth of *P. donghaiense.* We found that the culture filtrates presented growth inhibition on *P. donghaiesne*, inferring the existence of allelochemical(s). When the culture filtrates were duluted, the growth inhibition was alleviated and even growth increment was observed on group of *A. minimum.* In fact, low levels of alleochemicals may stimulate the growth of target organisms, which was known as hormesis (Stebbing, 1982). The growth inhibtition or growth stimulation with different treatment further evidenced that the toxin-independent allelochemical(s) produced by *Alexandrum* spp. was involved in the interaction between them. On the other hand, the allelochemical(s) was found to perform differently in different growth phases, and those produced in stationary phase (SP) seemed more toxic as compared to those in exponential phase (EP).

Moreover, we also observed the existence of hemolytic activity in the three strains. The extracellular components produced by ADHK showed similar effect on *P. donghaiense* as ACDH, while AM-1 had less inhibiting effect. These observations paralleled the measured activity of hemolytic components, as well as the toxins (Chen et al., 2007) present in the exudates of the strains. Hemolytic activity is a common biochemical feature of *Alexandrium* species, but its relationship between the PSP toxins was controversial. For instance, Simonsen and Moestrup (1997) found that one *A. tamarense* strain contained low level of PSP toxins had strong hemolytic activity; and thus attributed the hemolytic activity to still unknown compounds. Hemolytic activity of *Alexandrium* species was also reported by Eschbach et al (2001). However, no one definitely bridged the gap between the allelopathy and hemolytic toxins in *Alexandrium* species. Results suggested an important role of hemolytic components in the interaction between *Alexantrum* spp. and *P. donghaiense*.

When combined considered results in the present study, we speculated the PSP-independent allelochemical(s) was involved in the toxicity exerted on *P. donghaiense*. However, since the consistence of the PSP toxins and hemolytic activity of different Alexantrum spp., we wondered if it was the combination of PSP toxins and allelochimcal(s) that was responsible for the overall phenomenon observed in the population dymanics? Further idenrification might be focus on this problem.

References

Arzul, G., Segue, M. I., Guzman, L., & Denn, E. L.1999. Comparison of allelopathic properties in three toxic *Alexandrium* species. *J. Exp. Mar. Biol. Ecol.*, *232*(2), 285-295. https://doi.org/10.1016/S0022-0981(98)00120-8

Chen, Y., Yan, T., Tan, Z. J., Wang, L. P., & Zhou, M. J. 2007. Toxicity of dinoflagellate *Alexandrium* species. *Oceanol. et Limnol. Sinica*, *38*(1), 55-61.

Eschbach, E., Scharsack, J. P., & John, U. 2001. Improved erythrocyte lysis assay in microtitre plates for sensitive detection and efficient measurement of haemolytic compounds from ichthyotoxic algae. *J. Appl. Toxicol. Jat.*, *21*(6), 513. https://doi.org/10.1002/jat.797

Fistarol, G. O., Legrand, C., & Granéli, E. 2005. Allelopathic effect on a nutrient-limited phytoplankton species. *Aquat. Microbial Ecol.*, *41*, 153-161. https://doi.org/10.3354/ame041153

Gentie, P., & Arzul, G. 1990. Exotoxin production by *Gyrodinium* cf. *aureolum* (Dinophyceae). *J. Mar. Biol. Ass. U. K.*, *70*, 571-581.

https://doi.org/10.1017/S0025315400036596

Grane´li, E., & Johansson, N. 2003. Increase on the production of allelopathic substances by *Prymnesium parvum* cells grown under N- or P-deficient conditions. *Harmful Algae, 2*, 135–145. https://doi.org/10.1016/S1568-9883(03)00006-4

Gross, E. M. 2003. Allelopathy of aquatic autotrophs. *Crit. Rev. Plant Sci.*, *22*, 313–339. https://doi.org/10.1080/713610859

Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates, In: Smith W. L., Chanley, M. H. (eds.), *Culture of marine animals*. Plenum Press, New York, pp. 26-60. https://doi.org/10.1007/978-1-4615-8714-9_3

Hansen, P. J. 1989. The red tide dinoflagellate *Alexandrum tamarense* on behaviour and growth of a *tintinnid ciliate*. *Mar. Ecol. Prog. Ser.*, *53*, 105-116. https://doi.org/10.3354/meps053105

Jin, Q., & Dong, S. L. 2003. Comparative studies on the allelopathic effects of two different strains of U. pertusa on *Heterosigma akashiwo* and *Alexandrium tamarense*. *J. Exp. Mar. Biol. Ecol*., *293*, 41–55. https://doi.org/10.1016/S0022-0981(03)00214-4

Lu, D., & Goebel, J. 2001. Five red tide species in genus Prorocentrum including the Scrippsiellaion of *Prorocentrum donghaiense* Lu sp. nov. from the East China Sea. *Chin. J. Oceanogr. Limnol*., *19*(4), 334–337.

Rice, E. L. (Ed.). 1984. Allelopathy (2nd ed). Academic Press, London, p. 422.

Roy, S., & Chattopadhyay, J. 2006. Toxin-allelopathy among phytoplankton species prevents competitive exclusion. *Journal of Biological Systems*, *15*(1), 73-93. https://doi.org/10.1142/S021833900700209X

Simonsen, S., & Moestrup, Ø. 1997. Toxicity tests in eight species of Chrysochromulina (Haptophyta). *Canadian J. Bot.*, *75*(1):129-136. https://doi.org/10.1139/b97-015

Stebbing, A. R. D. 1982. Hormesis-the stimulation of growth by low levels of inhibitors. *Sci Total Environ*, *22*, 213-234. https://doi.org/10.1016/0048-9697(82)90066-3

Tillmann, U., & John, U. 2002. Toxic effects of Alexandrium spp. on heterotrophic dinoflagellates: an allelochemical defence mechanism independent of PSP-toxin content. *Mar. Ecol. Prog. Ser*., *230*, 47–58. https://doi.org/10.3354/meps230047

Wang, Y., & Tang X. X. 2008. Interactions between *Prorocentrum donghaiense* Lu and *Scrippsiella trochoidea* (Stein) Loeblich III under laboratory culture. *Harmful Algae*, *7*, 64-75. https://doi.org/10.1016/j.hal.2007.05.005