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Original Research Paper

Growth Characteristics of *Oxyrrhis marina* and *Chattonella marina* in their Co-culture Systems

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ABSTRACT

This study was aimed to investigate the growth characteristics of *Oxyrrhis marina* and *Chattonella marina* in co-culture to provide experimental evidences for discussing successions of harmful algal blooms (HABs) and coastal biological communities. The colour changes of culture media of *C. marina* and growth characteristics of *O. marina* and *C. marina* in co-culture were analysed by the combined methods of macro-observation, microscopic examination and counting. In co-culture, the colours of culture media of *C. marina* had changed and their transparencies had increased with increasing elapsed incubation time after inoculated by *O. marina* under different initial cell densities. With the increase of the initial density of *O. marina* (0.17×10^4 cells/mL, 0.50×10^4 cells/mL and 0.64×10^4 cells/mL in *C. marina* culture media), the time required, that the populations of *O. marina* reached the stationary phases, was shorter i.e. 6d, 5d and 3d after inoculated by *O. marina*, respectively, and the death time of all cells of *C. marina* became shorter, i.e. 7d, 6d and 4d after inoculated by *O. marina*, respectively. During the 15 days culture period, all *C. marina* populations were evolved to *O. marina* populations. Residues of *C. marina* adhering to precipitates and chromatophores scattering in the culture media could strengthen the colour of culture media, *C. marina* populations were evolved to *O. marina* populations respectively within the concentrations designed in co-culture in this experiment. Disturbance feeding was one of the reasons for successions, and the results provide experimental evidences for discussing successions of red tides and coastal biological communities.

INTRODUCTION

Oxyrrhis marina Dujardin, a common heterotrophic dinoflagellate in many intertidal and coastal habitats, is typically regarded as cosmopolitan or globally distributed (Watts et al. 2011), exhibits a high degree of genetic and functional diversity, and has been used as a model marine protist in laboratory experiments (An et al. 2012a, An et al. 2012b, An et al. 2013, Hartz et al. 2011, Lowe et al. 2010, Jeong et al. 2010). On the basis of published data, the *O. marina* morphospecies is best described as broadly distributed, inhabiting areas of the Atlantic and Pacific coasts of the USA, the Gulf of Mexico, the Atlantic coasts of Europe, the Mediterranean and Baltic Seas, Persian Gulf, the Indian Ocean and the Western Pacific (Watts et al. 2011). In China, *O. marina* is widely distributed in coastal environments of Qingdao, Qinhuangdao, Shenzhen and Shanghai (An et al. 2011a, An et al. 2011b, Ke et al. 2011, Zhang et al. 2010). Owing to easy cultivation, *O. marina* has been used as a model organism to examine the feeding responses of heterotrophic protists to many marine microalgae, such as *Heterosigma akashiwo* (Jeong et al. 2003), *Thalassiosira pseudonana* (Saló et al. 2009), *Isochrysis galbana* (Martel et al. 2006), *Rhodomonas salina* (Florian et al. 2010), *Dunaliella tertiolecta* (Hartz et al. 2008, Hartz et al. 2011), and so forth.

Chattonella marina is regarded as one of the most noxious harmful algal blooms (HABs) species and has been of special concern because of great economic losses due to the serious damage to fisheries of Japan (Okaichi et al. 1987, Liu et al. 2007), Norway (Elbraechter 1999), China (Song et al. 2009), and so on. *O. marina* cannot engulf the complete cells of *C. marina*, they deformed cells of *C. marina* to broken ones and then engulfed the debris (An et al. 2014). But the growth characteristics of *O. marina* and *C. marina* in their co-culture systems have not been reported.

The goal of this study is to investigate the growth characteristics of *O. marina* and *C .marina* in co-culture to provide experimental evidences for discussing successions of HABs and coastal biological communities.

MATERIALS AND METHODS

The Source of samples: Wild populations of *O. marina* and *C. marina* were collected from the coastal waters near Qinhuangdao in the Bohai Sea in 2010 (39°48' N; 119°42' E) and in 2005 (39°46' N; 119°38' E), China, respectively.

Isolating, identification and culture of *O. marina* and *C. marina*: *O. marina* and *C. marina* were identified based on the external morphology by light microscopy, respectively. Then the population of *O. marina* was cultured in seawater (the salinity of seawater was 32) on a diet of a natural bac-

terial assemblage grown in starch-enriched seawater in a 1000 mL conical flask (Lowe et al. 2005), with culture temperature 20°C and light intensity 60 μE m^{-2} s^{-1}. The population of *C. marina* was cultured in f/2 medium with culture conditions as above.

Co-culture of *O. marina* and *C. marina*: For the experiment, initial concentrations of *O. marina* and *C. marina* were established using an autopipette to deliver predetermined volumes of known cell concentrations to the flasks. Triplicate 250 mL conical flasks (mixtures of *O. marina* and *C. marina*), and triplicate control flasks (*C. marina* only) were set up for each predator-prey combination. The flasks were filled to capacity with filtered seawater, placed on rotating wheels at 0.9 rpm for 5 min in the morning, in the afternoon and in the evening, respectively, and incubated at 20°C under illumination of 60 μE m^{-2} s^{-1} in a 12:12h light:dark cycle. To determine actual predator and prey densities at the beginning of the experiment, a 10 mL aliquot was removed from each flask, fixed with 5% acid Lugol's solution and examined with a compound microscope to determine predator and prey abundance by enumerating cells in three 1 mL Sedgwick-Rafter counting chambers (SRCs). The flasks were filled again to capacity with freshly filtered seawater, capped, and placed on rotating wheels with the environmental conditions described above. After counting, the initial concentrations of *O. marina* were 0.17×10^4 cells/mL (in No.1 flasks), 0.50×10^4 cells/mL (in No.2 flasks) and 0.64×10^4 cells/mL (in No.3 flasks), respectively, and initial concentration of *C. marina* was 0.11×10^5 cells/mL.

Taking pictures and microscopic counting: All experimental flasks were incubated for 15days, took photos before and after rotating, counted microscopically after rotating every day.

RESULTS AND DISCUSSION

Colour changes in *Chattonella marina* culture media after inoculated by *Oxyrrhis marina*: Photos of colour changes in *C. marina* culture media inoculated by *O. marina* were taken before rotating every day (Fig. 1). The culture media were yellowish-brown before inoculated by *O. marina* (Fig. 1A), colour of *C. marina* culture media changed at different extent after inoculated by *O. marina*, and colour of culture media of control flasks changed inconspicuously during 15 days (Fig. 1). Colours and transparencies of culture media of all experimental flasks changed inconspicuously after inoculated by *O. marina* for 1 day, but a little precipitates on the bottoms of all experimental flasks appeared (Fig. 1B), the precipitates including dead cells of *C. marina* and their debris (Fig. 2). On the 6th day, colours of culture media of all experimental flasks became lighter,

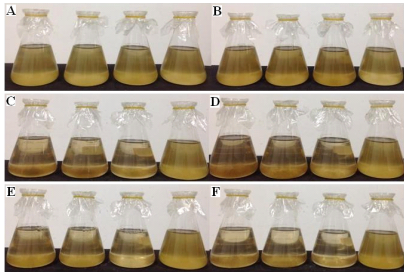

Fig. 1: Colour changes of culture media of *Chattonella marina* before rotated. Notes: A: culture media of *C. marina*, B: culture media inoculated with *O. marina* for 1 day, C: culture media inoculated with *O. marina* for 6 days, D: culture media inoculated with *O. marina* for 7 days, E: culture media inoculated with *O. marina* for 12 days, F: culture media inoculated with *O. marina* for 14 days.

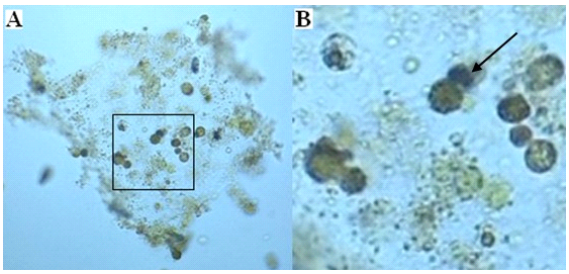

Fig. 2: Precipitates in culture media of *Chattonella marina.* Notes: A: Precipitates in culture media of *C. marina*, B: enlarged view of the box in Fig. 2A (the arrow is pointing at dead *C. marina*)

transparencies and precipitates increased (Fig. 1C). After 7 days, colours of culture media of all experimental flasks became lighter further, transparencies increased and the precipitates increased obviously (Fig. 1D-1F).

Photos of colour changes of *C. marina* culture media inoculated with *O. marina* were taken after rotating every day (Fig. 3). Colour of *C. marina* culture media changed at different extent after inoculated with *O. marina*, and colour of culture media of control flasks changed inconspicuously during 15 days. Colour and transparencies of culture media of all the experimental flasks changed inconspicuously after inoculated with *O. marina* for 1 day (Fig. 3A-3B), since then, colours of culture media became lighter and transparencies increased (Fig. 3C-3F). But, compared with colour before rotated, the colour was deeper because of dead cells, debris and scattered chromatophores of broken *C. marina* (Fig. 2).

The changes in transparencies and colour of *C. marina* culture media inoculated with *O. marina* were consistent with that of *Platymonas subcordiformis* and *Karenia mikimotoi* (An et al. 2012c). These changes were indicative of contamination with *O. marina* to *C. marina*, and were important guiding significance to batch culture of marine microalgae. On the other hand, the chromatophores scat-

tered from broken cells of *C. marina* had a great contribution to the colour of *C. marina* culture media.

Growth characteristics of *Oxyrrhis marina* and *Chattonella marina* in co-culture: With increasing elapsed incubation time, the concentrations of *C. marina* in the experimental flasks continuously decreased and down to 0 cells/ mL at 7thd in No.1 flasks (Fig. 4A), 6thd in No.2 flasks (Fig. 4B) and 4th d in No.3 flasks (Fig. 4C), respectively. The concentrations of *O. marina* in the experimental flasks increased and became stable at 6thd in No.1 flasks (Fig. 4A), 5thd in No. 2 flasks (Fig. 4B) and 3rd d in No. 3 flasks (Fig. 4C), respectively. With increasing elapsed incubation time, the concentrations of *O. marina* in No. 1 flasks continuously increased and reached to the maximum 0.20×10^5 cells/ mL at 9thd, but then continuously decreased and down to 0.15×10^5 cells/mL at 15th d (Fig. 4A). With increasing elapsed incubation time, the concentrations of *O. marina* in No. 2 flasks continuously increased and reached to the maximum 0.26×10^5cells/ mL at 12th d, but then continuously decreased and down to 0.21×10^5 cells/ mL at 15th d (Fig. 4B). With increasing elapsed incubation time, the concentrations of *O. marina* in No. 3 flasks continuously increased and reached to the maximum 0.25×10^5 cells/ mL at 11thd, but then continuously decreased and down to 0.20×10^5 cells/ mL at 15thd (Fig. 4C).

With the increase of the initial density of *O. marina* cell (0.17×10^4 cells/mL, 0.50×10^4 cells/mL and 0.64×10^4 cells/ mL in *C. marina* culture media, respectively), the time, that the populations of *O. marina* reached the stationary phases, required shorter after inoculated by *O. marina* respectively, and the death time of all cells of *C. marina* became shorter after inoculated with *O. marina* respectively. During the 15 days culture period, all *C. marina* populations were evolved to *O. marina* populations within the concentrations designed in the co-culture in this experiment.

Jeong et al. (2003) revealed that, with increasing elapsed incubation time, the concentrations of *Heterosigma akashiwo* in the experimental esocosm (MC1) varied from 1.38×10^4 to 1.76×10^4 cells/mL, and did not markedly change over the first 18 h, but continuously decreased between 18 and 58 h and down to 10 cells/mL by 73 h. With increasing elapsed incubation time, the concentration of *H. akashiwo* in control mesocosm (MC2) increased from 1.90×10^4 to 5.04×10^4 cells/mL with a depression between 50 and 66 h. The concentration of *O. marina* in MC1 increased from 2.10×10^3 to 9.03×10^3 cells/mL, between 0 and 50 h, but decreased to 4.15×10^3 cells/mL by 73 h, probably due to food limitation. As revealed in our study, with the increase in the initial densities of *O. marina* cell (0.17×10^4cells/mL, 0.50×10^4cells/mL and 0.64×10^4 cells/mL in *C. marina* culture media), the time that the populations of *O. marina*

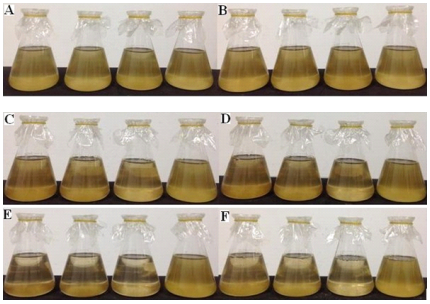

Fig. 3: Colour changes of culture media of *Chattonella marina* after rotated. Notes: A: culture medium of *C. marina*, B: culture medium inoculated by *O. marina* for 1 day, C: culture medium inoculated by *O. marina* for 6 days, D: culture medium inoculated by *O. marina* for 7 days, E: culture medium inoculated by *O. marina* for 12 days, F: culture medium inoculated by *O. marina* for 14 days.

reached the stationary phases required shorter, were 6d, 5d and 3d after inoculated with *C. marina* respectively, and the death time of all cells of *C. marina* became shorter, were 7d, 6d and 4d after inoculated with *O. marina* respectively. These results implied that the growth of *O. marina* and *C. marina* were affected by the initial concentration ratio of the two marine microalgae. It also revealed that during the 15 days culture period, all *C. marina* populations were evolved to *O. marina* populations.

The results of the present study indicated that disturbance feeding was one of the important reasons for succession of *C. marina* to *O. marina*. It has not been reported whether *O. marina* can produce toxic substances to *C. marina*. According to Kuroda et al. (2005), Marshall et al. (2005a) and Marshall et al. (2005b), *C. marina* can produce reactive oxygen species (ROS), hemolysins and so on, but allelopathic effects of *C. marina* on *O. marina* have not been reported.

CONCLUSIONS

1. During the 15 days culture period, the colour of culture media had changed and their transparencies had increased, with increasing elapsed incubation time after inoculated by *O. marina* under different initial cell densities.
2. Within the concentrations designed in this experiment, the feeding of *O. marina* on *C. marina* was affected by the initial concentration of *O. marina*. With the increase of the initial density of *O. marina*, the time that the populations of *O. marina* reached the stationary phase required shorter after inoculated with *C. marina* respectively, and the time fed up of *C. marina* by *O. marina* became shorter after inoculated with *O. marina* respectively. During the 15 days culture period, all *C. marina* populations were evolved to *O. marina* populations.

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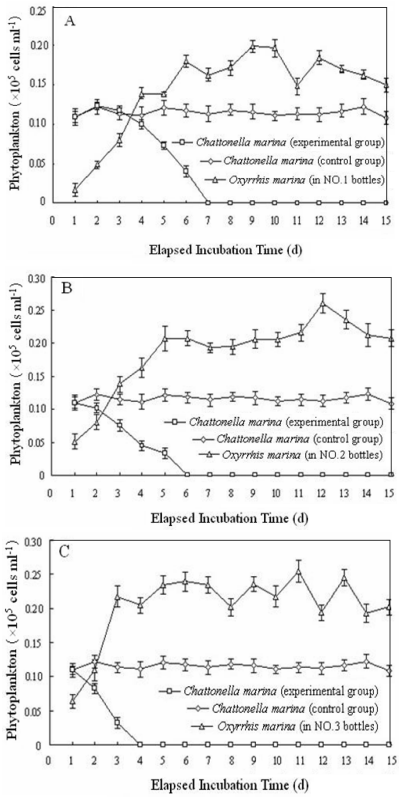

Fig. 4: Cell concentrations of *Oxyrrhis marina* and *Chattonella marina* as a function of elapsed incubation time.

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