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Short communication

Selection and characterization of an improved strain produced by inter-species hybridization between *Pyropia* sp. from India and *Pyropia haitanensis* from China

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ABSTRACT

A crossing-experiment between *Pyropia* sp. from India and *P. haitanensis* from China was carried out for breeding improved strains. The male parent was the wild-type strain (*Ps-WT01)* of *Pyropia* sp., which was characterized by thick blade, slow growing, good toughness and more production of conchospores, and the female parent was the mutation strain (*Ph-HMC5*) of *P. haitanensis*, which was characterized by thin blade, fast growing, poor toughness and low production of conchospores. At first, four improved strains with obvious recombinant advantages were obtained from the F_1 color-sectored blades, and finally the most improved strain (*HR-5*) was selected from them. The maximum of absolute growth rate of the male and female parents were 0.39 and 5.24 cm/d, respectively, while that of *HR-5* strain was 10.11 cm/d and its absolute growth rate was greater than the maximum growth rate of the female parent during 41–55 days of culture. After 60 days, the mean blade length of *HR-5* was 168.67 cm, which was 12.80-fold of that (13.18 cm) of its male parent and 1.97-fold of that (85.67 cm) of its female parent, respectively. The chlorophyll *a* content in the 35-day-old blades of *HR-5* strain was 10.57 mg/g, which increased by 68% and 35% compared with that of its male and female parents, respectively. The phycobiliprotein content of *HR-5* blades was 114.88 mg/g, which was 2.51- and 2.01-fold of that of its male and female parents, respectively. The mean thickness of the 35-day-old blades of *HR-5* was 25.06 μm, which was 60% and 124% of that of its male and female parents, respectively. The total number of conchospores per shell released from *HR-5* was 404.14×10^4, which was 2.73- and 6.38-fold of that of its male and female parents, respectively. The above results confirmed that *HR-5* strain was characterized by fast growth, high quality as well as large amount of conchospores, and may offer an alternative for the nori industry.

1. Introduction

Pyropia haitanensis is an important economic algae. Its total output accounts for approximately 75% of the total *Pyropia* production in China (Ma and Cai, 1996). In the 1960s, artificial breeding and cultivation of *P. haitanensis* was succeeded, leading to the establishment and development of culture industry of *P. haitanensis* (Fujian Fisheries Bureau, 1979). However, the wild-type strain of *P. haitanensis* has been cultivated without any selection or improvements for over 50 years, thus, breeding new cultivars has been placed in the important agenda (You, 1999). Until today, numerous stable artificial mutants with inheritable and valuable characteristics, such as heat tolerance, low-salt resistance and low-nitrogen and phosphorus resistance have been isolated (Yan and Ma, 2007; Yan and Chen, 2008; Liu et al., 2009; Yan et al., 2010; Tan et al., 2014). Meanwhile, several improved strains with high quality and yield have been obtained and received the certification of new cultivar from Chinese government (Wang et al., 2011). In addition, through the intra-species hybridization of artificial pigment mutants of *P. haitanensis*, some strains with better characteristics than their parents have been obtained (Xu et al., 2008; Wang et al., 2010), but their cultivation in large-scale has not been reported.

Crossbreeding of *Pyropia* mainly utilize the hybrid recombination advantage, that is, the hybrid progeny produced by interbreeding between individuals with different characters is superior to parents in growth and biochemical characteristics (He et al., 2007). One of the important reasons for generated hybrid recombination advantage in

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intraspecific cross is the significantly different characteristics of parental strains (Xu et al., 2008). The *Pyropia* sp. was derived from the India peninsula, we have previously (Zhang and Yan, 2014) reported that the morphological structure, chromosome counts, sex and 5.8S rDNA-ITS region of this species were very similar to those of *P. haitanensis* from China. This indicated that the genetic relationship between them is very close although they were isolated so long distance from China to India, But, there are still no enough evidences to identify they are the same species. Therefore, we think these two species are different species in this research. In addition, we have previously (Liu et al., 2013) reported that the gametophytic blades of heterozygous conchocelis produced by crossing *Pyropia* sp. from India and *P. haitanensis* from China were fertile. The wild-type *Pyropia* sp. (*Ps-WT01*) was characterized by thick blades, slow growing, good toughness in elasticity, maroon thallus with greenish basal part and reddish apical part, and many marginal denticles. The mutation strain of *P. haitanensis* (*Ph-HMC5*) was characterized by thin blade, fast growing, poor toughness in elasticity and thallus with uniform pale red brown color, and no marginal denticles. In this paper, we further screened the improved hybrids strains with recombinant advantages through the hybridization between *Ps-WT01* and *Ph-HMC5* strains.

2. Materials and methods

2.1. Materials

The male parental stain was wild-type *Pyropia* sp. (*Ps-WT01*) originated from the coast of Indian peninsula. In detail, its free-living conchocelis were developed from carpospores released by a gametophytic blade (Zhang and Yan, 2014) and cultured in our laboratory to obtain conchospores and blades. These blades were prepared as single somatic cell suspensions using enzymatic hydrolysis technology and then cultured and developed into regenerative blades (Wang et al., 1986). After cultured for a period of time, single regenerative blades underwent parthenogenesis (Yan et al., 2007) and produced homozygous conchocelis (strains). The female parent was a mutation strain (*Ph-HMC5*) of *P. haitanensis*. Its homozygous conchocelis were also obtained through blade parthenogenesis. The conchocelis filaments were stored at 18 °C in the laboratory.

2.2. Inter-species crossing experiment, heterozygous conchocelis isolation and F_1 gametophytic blades culture

Crossing experiments, heterozygous conchocelis isolation and F_1 gametophytic blades culture were conducted as previously described by Liu et al. (2013) with slight modification. In brief, 3–5 vinylon monofilaments (3 cm long) were placed in a flask for attachment of conchospores released from conchocelis. Then, the conchospores were cultured with aeration to develop into gametophytic blades. When the blades grew to 1 cm, they were detached from the monofilaments and sequentially cultured with aeration at 23 °C in an incubator (250 mL) with 50 μmol photons $m^{-2} s^{-1}$ and 10:14 h light/dark cycle (10 L:14D). The culture medium was rich in MES nutrient (Wang et al., 1986) and refreshed every 5 days.

2.3. Selection of the improved strains

After cultured for 5–7 weeks, the color-sectored F_1 gametophytic blades were developed from the conchospores of heterozygous conchocelis. Four sectors with fast growth rate and good color were collected and treated with sea snail enzyme to obtain single somatic cells, respectively (Yan and Wang, 1989). These cells were respectively cultured to regenerate into blades and obtain the homozygous free-living conchocelis (strain) by blade parthenogenesis. By comparing the morphology, color, growth rate, pigment content of their F_1 gametophytic blades of each strain with those of their parental strains, one strain with the most obvious hybrid recombinant advantages was selected, namely the improved strain.

2.4. Characteristics of the improved strain

2.4.1. Blade growth

After cultured for 30 days, twenty blades of each strain were randomly selected and cultured in three aerated flasks (1 L), respectively. The blade length, width and wet weight were measured every 5 days in a 30-day period. The specific and absolute growth rates of the blades were calculated as previously reported by Stein (1973).

2.4.2. In vivo absorption spectra and contents of chlorophyll a as well as the contents of phycobiliproteins

After being cultured for 35 days, the *in vivo* absorption spectra and contents of chlorophyll *a* (Chl. *a*) of the F_1 blades were measured (Yan and Aruga, 1997). The contents of phycoerythrin (RPE), phycocyanin (RPC) and allophycocyanin (APC) were measured according to the methods described by Gao (1993). Specifically, the fresh blades were dried at 80 °C for 6 h, and ground after three freezing and thawing cycles (between −20 °C and room temperature). The extract was centrifuged at 15,000 × *g* for 20 min, and the supernatant was used to determine the OD562, OD615, OD650, and OD652. The absorbance and OD were measured by using an ultraviolet spectrophotometer (UV-2600, Shimadzu). The contents of Chl. a, PE and PC were calculated using the following formulae:

$$\text{Chl. } a = (11.6 \times \text{OD665} - 1.31 \times \text{OD645} - 0.14 \times \text{OD630}) \times \text{V/W} \quad (1)$$

$$\text{PE} = (0.123 \times \text{OD562} - 0.07 \times \text{OD615} + 0.015 \times \text{OD652}) \times \text{V/W} \quad (2)$$

$$\text{RPC} = (0.162 \times \text{OD615} - 0.099 \times \text{OD650} - 0.001 \times \text{OD562}) \times \text{V/W} \quad (3)$$

$$\text{APC} = (0.171 \times \text{OD650} - 0.0006 \times \text{OD562} - 0.004 \times \text{OD615}) \times \text{V/W} \quad (4)$$

$$\text{PC} = \text{RPC} + \text{APC} \quad (5)$$

where V (in mL) is the volume of grinding fluid and W (in mg) is dry weight of sample.

2.4.3. Blade thickness

After being cultured for 35 days, the blades were cut into three parts with bare-handed, double-edges blades. Blade thickness of every part was measured with an optical microscope (OLYMPUS-BH) and expressed as the mean thickness of thirty slices.

2.4.4. Amount of released conchospores

One clam shell was inoculated with free conchocelis weighing 0.5 mg. When conchosporangias were formed, one conchocelis shell of each strain was placed in a 250 mL flask containing 50 mL culture media to collect conchospores and conchospores per strain were collected and inoculated in three Petri dishes (9 cm in diameter) to measure the amount of released conchospores. As the releasing peak of conchospores of *P. haitanensis* usually appeared at 7–12 am (Fujian Fisheries Bureau, 1979), we collected the conchospores released from each strain at 1 pm every day and inoculated them into three Petri dishes containing culture media. At 24 h post-incubation, the amount of conchospores of 20 fields (10×) selected randomly in cross along the dish center was counted under light microscope and calculated as mean amount per field. According to the areas of the single field and Petri dish, the amount of conchospores in one Petri dish was calculated and used to calculate the daily amount of conchospores from one conchocelis shell. The number of conchospores was counted for twenty days and the number of conchospores released in twenty days was calculated as the total amount of one conchocelis shell. The experiments at each strain were repeated three times.

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