Breeding of new strains of Gracilariopsis lemaneiformis with high agar content by ARTP mutagenesis and high osmotic pressure screening

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Research Article

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Abstract

ARTP (atmospheric and room temperature plasma mutagenesis) mutagenesis was tried on *G·lemaneiformis*, and mutagenesis conditions were confirmed. An osmotic pressure screening program was established. Mutants were identified and characterized of relevant physiological traits. The aim of the study is to try to use ARTP mutagenesis and osmotic pressure screening for the breeding of high agar *G·lemaneiformis*. Treatment time of 46S was found to be optimal mutagenesis time and used in subsequent experiment. The mutagenized spores were initially screened with 58‰ artificial seawater, and then the surviving spores were screened twice with 60‰ artificial seawater in their vertical growth phase and branch growth phase respectively. Four fast-growing and hypertonic resistance gametophytes were selected. The actual photosynthetic efficiency (Y(PSII)), photochemical quenching (QL), and non-photochemical quenching (NPQ and QN) of four mutants were measured. Both Y(PSII) and QL of the mutant were significantly higher than Ctr. When subjected to salinity stress, the values of Y(PSII) and QL of X3 and X5 were higher than those of Ctr in the early stage of salt stress, which indicated that mutants X3 and X5 can more effectively protect the photosynthetic active reaction center PSII and improve the photochemical quenching in the early stage of salt stress. NPQ and QN of X3 and X5 were higher than Ctr in most of the times, which indicated that the photoprotection system of mutants X3 and X5 was more effective than Ctr. The growth rate of the four mutants was higher than that of the control. X4 was the highest. The agar content was measured, X5 displayed the highest agar content among the tested strains. X5 was more in line with expectations, because of its high agar content and good hypertonic resistance. In this study, the mutant of *G·lemaneiformis* with high agar content was obtained by the procedure, which provided a certain reference for the selection of high agar content in *G·lemaneiformis* breeding.

Introduction

Gracilariopsis lemaneiformis is a kind of marine red algae, which has very important economic value (Sui et al. 2020; Zhang et al. 2005; Gao 2010). It is source of agar, an indispensable additive in medical and food industry (Pan et al. 2010). The main purpose of *G·lemaneiformis* breeding in the past was to obtain varieties with high temperature tolerance, fast growth, and high biomass accumulation, such as the variety 981 and Lulong No. 1 (Zhang et al. 2009; Zhou et al. 2016). However, there is no precedent for the selection and breeding of high agar varieties. This may be partly because of lacking of certain technical means for breeding of the *G·lemaneiformis* with high agar.

There were many cases from yeast and microalgae of breeding of high polysaccharides contenting strains via hyperosmotic screening, for example, the breeding of yeast with high-yield trehalose. After mutagenesis and multiple hypertonic screening, yeast mutants with thickened cell walls and increased trehalose content were selected. In microalgae, there are also examples of breeding of high-yield polysaccharide strains based on the same principle, such as the breeding of high-yielding polysaccharide *Spirulina* species (Ma 2003; Li 2014). It was also reported that the agar content of *G·lemaneiformis* was increased in both hypertonic and hypotonic cultures, which suggested that the agar content has a certain

correlation with the physical quantity of osmotic pressure (Bird 1988; He et al. 2002; Hurtado-Ponce et al. 1997; Hu et al.2019) Therefore, it is reasonable to exploit osmotic pressure screening for breeding of *G·lemaneiformis* strains with high agar contents.

With appropriate equipment, physical methods achieve mutagenesis conveniently, efficiently, and safely. Based on its penetrating power, there are no restrictions on the material, including which atmospheric and room temperature plasma (ARTP) is one of the widely used in recent years. The ARTP developed in recent years has gradually become the preference choice of physical mutagenesis mean due to its safety, efficiency and non-polluting advantages (Li 2007; Li 2011). ARTP acted on the DNA of the organism and caused DNA damage, which leading to SOS repair and mutagenesis. In recent years, ARTP mutagenesis has been widely used in microorganisms and microalgae, such as breeding of *Streptomyces viridochromogene* producing high-yield avilamycin, L-glutamic acid poducing strain of *Corynebacter- ium glutamicum* GY1 and improving of lipid productivity of the oleaginous microalgae *Chlorella pyrenoidosa* et al. (Jiang. 2018; Liang et al. 2020; Cao et al. 2017).

In the present study, ARTP mutagenesis was tried on *G·lemaneiformis*. Mutagenesis conditions of ARTP were confirmed. An osmotic pressure screening program was established. Mutants were identified and characterized of relevant physiological traits. The aim of the study is to try to use ARTP mutagenesis and osmotic pressure screening for the breeding of high agar *G·lemaneiformis*. The research laid a foundation for the technical approach for ARTP mutagenesis and further cultivar development of *G·lemaneiformis*.

Methods And Materials

Experimental materials

G·lemaneiformis used in the experiment was WLP-1 (Wei et al. 2016), which was tetrasporophyte and cultivated in Key Laboratory of Marine Genetics and Breeding Ministry of Education (Ocean University of China). Culture conditions was under the temperature of 20℃, with light intensity of 15μmol/(m^2·s), and the light-dark cycle of 12L:12D.

Cultivation of experimental materials and collection of tetraspores of *G·lemaneiformis*.

The young branches were cut from WLP-1 and cultured with seawater supplemented with Pro medium at a temperature of 20°C and a light intensity of 15μmol/(m^2·s). The light-dark cycle is 12L:12D, and the medium is changed every three days. After the shoots mature, they were placed under the conditions of 30μmol/(m^2·s) light intensity to be induced to release of spores. The released spores were centrifuged at 500G for 15 minutes, and then 200G for 10 minutes for concentration. The precipitated spores were re-suspended in certain volume of sea water for quantitation using a hemocytometer.

ARTP treatment

The mutagenic power of ARTP (Wuxi Yuanqing Tianmu Biological Technology Co., Ltd, China) was 120W. The high-purity helium gas volume was set to 10 SLM, the air pressure was controlled between 0.1MPa and 0.2Mpa and processing distance was 2mm. The temperature of the instrument is set to 20°C.

Determination of survival rate and death rate

The time-lethal rate curve was drawn using collected spores with a concentration of approximately 10^5~10^6/ml. Six time gradients of 0S, 10S, 20S, 30S, 40S, and 50S were adopted, and three parallels were set for each mutagenesis time. After mutagenesis, the tetraspores were put into a six-well plate, and then 10ml of sterilized seawater was added. Spores were cultured statically under 20°C, a light intensity of 15μmol/(m^2·s), and a light-dark cycle of 12L:12D. The sterilized seawater was changed every two days. After one week of cultivating, death rate was calculated under a microscope by the following formula: F (%) = (1-S/T) × 100%, F (%) represents the mortality rate, S represents the number of surviving spores, and T represents the total number of spores.

Determination of screening salinity

Artificial seawater with high salinity was used for hypertonic screening after mutagenesis. The artificial seawater formula was from Shuping Zhu artificial seawater formula (Zhu 1964), and the salinity was 50‰, 52‰, 54‰, 56‰, 58‰, 60‰, respectively. Three parallel were operated for each salinity gradient. About 6000 spores was added to six-well plates in artificial seawater with different salinities. The sterilized seawater was changed every two days. After one week of cultivating, they were placed under a microscope and counted for survival and mortality using the following formula: S (%) = (1-F/T) × 100%, S (%) represents the survival rate, F represents the number of dead spores, and T represents the total number of spores.

Confirmation of the high osmotic pressure tolerant of screen-out strains

The tested strains were cut, each with 30 algae tips, each 1cm and divided into 3 parallel, each with 10 tips in parallel. Individual growed-up from releasing of WLP-1 was used as control and treated in the same way. The algae tips were put in artificial seawater with salinity of 60‰ and cultivated statically under 20°C, a light intensity of 15μmol/(m^2·s), and a light-dark cycle of 12L:12D for 24 days. Culture medium was changed every two days. The actual photosynthetic efficiency (Y(PSII)), photochemical quenching QL and non-photochemically quenched QN and NPQ of the above algae tips were measured every two days.

Determination of chlorophyll fluorescence parameters

Algal tips with the length of 1 cm were cut and treated under a salinity of 30‰ of artificial seawater for 1 day and then transferred to 60‰ of artificial seawater. Determination of chlorophyll fluorescence parameters of mutants and controls was performed every two days, generally at 8 am, start time point of light phase. Three parallel were set for each strain and each parallel included ten algal tips. Parameters

for determining photosynthetic efficiency (Y(PSII)), photochemical quenching (QL), non-photochemically quenched QN and NPQ were set as 10% culture light intensity, 35% saturated light intensity, 1 exposure, and 5% sensitivity.

Determination of the growth rate

Thirty algal tips were cut from each sample, each with a length of 1 cm. The 30 algae tips were divided into three parallels, each with 10 tips, and a control group was set to do the same treatment. Culture conditions of the material was under the temperature of 20℃, with the light intensity of 15μmol/(m2·s), and the light-dark cycle of 12L:12D. The sea water was changed every three days. The length of each algae tip was measured once a week and the algae tip was cut back to 1cm again. The material were measured for four weeks, and then the growth rate of the mutant and the control group was calculated using the formula V_L (cm/week) = $(L-L_0)/T$. In the formula, L is the length of the algae at the end of the experiment (cm); L_0 is the length of the algae at the beginning of the experiment (cm); T is the culture time (weeks).

Determine of the agar content (Xue et al. 2006)

The agar extraction method followed those in the literature with slightly modified. 0.5 g of fresh algae were taken and placed in an oven at 60°C to a constant weight (about 30 min). 2.5mol/L of NaOH was added at the rate of 400μL per 0.01 g of algae (dry weight), and then the material was treated in a water bath at 85°C for 2h. The processed material was filtered through 4 layers of gauze to remove the lye and washed three times with distilled water. The algae was put into a beaker containing double-distilled water and 0.1mol/L HCl was added. After adjusting the pH of the algae to 6.5, the algae was washed three times with double-distilled water. The processed algae body was added with distilled water (1g to 60 mL double distilled water) and placed in a pressure cooker at 120°C for 2 hours. The material was centrifuged quickly at 15000rpm for 1.5 minutes. The collected supernatant was placed in a rectangular parallelepiped container folded with foil paper which was weighed in advance. The foil paper was put into a clean petri dish and frozen overnight at -20°C. The solidified agar was melted at room temperature and rinsed several times with distilled water. The above-mentioned materials were then placed in an oven at 60°C to be dried to a constant weight. The extract together with the foil paper were weighed and recorded, and then the dry weight of the agar were obtained by subtracting the weight of the foil paper.

Statistical processing

Excel 2016 was used to organize the original data. SPSS statistical software was used for data processing and difference significance analysis, and the significance level was set as $P<0.05$.

Results

The determination of the optimal mutagenesis time and screening salinity

The time-lethal curve (a) of the ARTP tetraspores of *G·lemaneiformis* was shown in figure 1. It was shown that with the increase of the mutagenesis time, the mortality of the spores gradually increased. A formula Y=1.8227X-3.3156 and R^2=0.9778 was obtained. The half-lethal action time of the ARTP mutagenesis was 29S, and when the death rate of tetraspores was 80%, the action time of the ARTP mutagen was 46S. To obtain a higher mutation rate, treatment time of 46S was used in subsequent mutagenesis in the experiment.

The survival rate (b) of tetraspores decreased successively with the increasing of salinity. When the salinity was 60‰, the survival rate of tetraspores was about 4.10%. When the salinity was 58‰, the survival rate of tetraspores was 22.67%. In subsequent experiments, salinity of 58‰ as well as 60‰ were chosen as the osmotic pressure screening condition for the mutants.

Mutagenesis and series salinity screening

Mutagenesis time of 46 seconds was used to treat spores with a scale about 2.7×10^5, and then artificial seawater with salinity of 58‰ was exploited for screening for one week. 8,680 survived spores were obtained and transferred to normal sea water for culture. The spores entered the vertical stage after two weeks in normal culture.

In order to further reduce the number of tetraspores, 60‰ salinity artificial seawater were used for the second hypertonic screening, and the screening time was 3 weeks. 17 dominant individuals were selected from the surviving individuals for subsequent cultivation.

The third hypertonic screening of the spores started after they had branched out with an artificial seawater treatment of 60‰ salinity for three weeks. A total of 9 hypertonic tolerant mutants were screened. After two months of cultivation, four mutants were selected which displayed better growth trends.

Confirmation of the high osmotic pressure tolerant of screen-out strains

4 screen-out strains were further validated for their tolerance aganist the high salinity treatment in 60‰ salinity within a treatment time of 21 day (table 1). In the control group, only about 3 algae tips remained in good condition showing bright red color and a complete body. About 15 algae tips in HAGL-X5 remained bright red. HAGL-X3 maintained a bright red state for 9 algae tips, followed by HAGL-X4 with 5, and finally HAGL-X2 with 3. Compared with the control group, t HAGL-X5 and HAGL-X3 displayed superior osmotic pressure resistance properties.

Tab.1 Number of algae tips survived after 60‰ salinity treatment for 21 days

strains	Control	HAGL-X2	HAGL-X3	HAGL-X4	HAGL-X5
Surviving tips	3	3	9	5	15

The actual photosynthetic efficiency (Y(PSII)), photochemical quenching QL, non-photochemically quenched NPQ and QN

The actual photosynthetic efficiency (Y(PSII)) and photochemical quenching QL of Ctr as well as the mutants treated in artificial seawater with a salinity of 60‰ for 24 days were shown in figure 2 (a and c). On day zero, the Y(PSII) and QL values of all mutants were significantly higher than those of the control ($P<0.05$), and those for X3 and X4 were significantly higher than those of X2 and X5 ($P<0.05$). When entering the high salinity treatment (after the second day), the Y(PSII) and QL values of all strains decreased sharply to the lowest values, and gradually recovered between the second and tenth days. Among them, the Y(PSII) of X5 on the 2nd, 6th, 8th and 10th days was significantly higher than that of Ctr ($P<0.05$), and the Y(PSII) of X3 on the 2nd and 8th days was significantly higher than that of Ctr ($P<0.05$). The QL values of X5 on the 2nd, 4th, 8th, and 10th days were significantly higher than Ctr ($P<0.05$), and the QL values of X2, X3, and X4 on the second day were all significantly higher than Ctr ($P<0.05$). After the tenth day, between the 12th and the 16th day, the Y(PSII) of all the strains fluctuated around 0.20, and they were all very close, with no significant difference ($P>0.05$). From day 18 to day 24, Y(PSII) of all strains started to decline slowly. At this stage, only Ctr was significantly higher than that of X4 on the 24th day ($P<0.05$), there was no significant difference in Y(PSII) between Ctr and other mutants at this stage ($P>0.05$). From the 12th to the 22nd day, the QL of all the strains fluctuated around 0.65, and they were also very close, with no significant difference ($P>0.05$). On day 24, the QL values of all strains began to decline. There was no significant difference in QL between Ctr and X2, X3 and X5 on the 24th day ($P>0.05$).

The NPQ (b) of HAGL-X5 and HAGL-X3 were higher than those of the control group before 16 days. The NPQ of HAGL-X3 at the 2nd, 8th day was significantly higher than that of the control group. The NPQ of HAGL-X5 at the 16th day was significantly higher than that of the control group ($P<0.05$). After 16 days, the NPQ of the control and HAGL-X5 were close to each other ($P>0.05$). The NPQ of HAGL-X2 was not significantly different from that of the control group, while the NPQ of HAGL-X4 was even lower than that of the control group as a whole ($P<0.05$). From a trend point of view, the NPQ values of the mutant groups HAGL-X5 and HAGL-X3 were first reduced to the lowest value after being stressed, and then the NPQ value quickly returned to the maximum value (on the sixth day). The control group also decreased. Then it rose again, returning to a higher level on the sixth day. Both HAGL-X5 and the control decreased slightly, and rose to a higher point again at twelve days, and then slowly decreased. The overall trend was similar, but the value of HAGL-X5 was higher than the control overall. After HAGL-X3 rose to its highest point on the sixth day, it declined at a faster rate, but overall it was also higher than the control group.

D in Figure 2 shows the measurement results of non-photochemically quenched QN in artificial seawater with a salinity of 60‰ for 24 days. On day zero, the QN values of X3 and X4 were significantly lower than those of Ctr, X2 and X5 ($P<0.05$). After entering the high salinity treatment (after the second day), the QN values of Ctr, X2, and X5 also decreased sharply, while those of X3 and X4 increased. The QN values of the mutants and Ctr increased gradually and reached the maximum between the second and sixth days. The QL value of X5 on the 6th day was significantly higher than that of Ctr ($P<0.05$), and the QL value of

X3 on the 2nd and 6th day was significantly higher than that of Ctr ($P<0.05$). After the rising period, between days 12 and 24, the QN values of all lines began to decline slowly. After 12 days, the QN values of X2 and X5 were higher than Ctr. The QN value of X5 on the 16th and 18th day was significantly higher than that of Ctr ($P<0.05$), and the QN value of X2 on the 16th and 22nd day was significantly higher than that of Ctr ($P<0.05$). The values of the mutants were higher than those of the control, though the difference was insignificant.

The growth rate

Fig.3 showed the results of the growth rate measurement of each strain. The growth rate of all mutants was higher than that of the control group, which suggested that the selected mutants had obvious growth advantages. The maximum growth rate of mutant HAGL-X4 was 0.45 cm per week, followed by HAGL-X3 at 0.37 cm per week, followed by HAGL-X5 at 0.32 cm per week, and HAGL-X2 at 0.30 cm per week. The growth rate of HAGL-X3 and HAGL-X4 was significantly higher than that of the control group ($P<0.05$), while those of HAGL-X2 and HAGL-X5 had no significant difference with control.

Agar content

Fig.4 showed the measurement results of the agar content of each strain. The highest agar content was evidenced in HAGL-X5 with 7.74%, followed by HAGL-X4 which was 6.01%. The agar content of HAGL-X5 and HAGL-X4 was about 1.5 and 1.2 times that of control. The agar content of HAGL-X5 was also significantly higher than that of other mutants ($P<0.05$), while those of HAGL-X2 and HAGL-X3 was even lower than that of the control. Therefore, HAGL-X5 is a mutant that meets the expectations of the screen.

Discussion

This experiment successfully screened out HAGL-X5 strain with high agar content via hypertonic screening. It was reported that plants (Kumar et al. 2017; Munns et al. 2019), fungi, yeast (Mager et al. 2002; Hohmann et al. 2007), cyanobacteria and other microalgae (Bisson et al. 1995), will response with a series of physiological and biochemical changes when they are subjected to high osmotic stress. When cells are under hypertonic stress, they will lose water quickly, which will cause cell shrinkage and cell membrane invagination (Park et al. 2016). The forces balance of cell wall will be changed due to cell contraction, which lead to structure shift of the cell wall to enhance its mechanical strength to deal with hypertonic stress (Hohmann et al. 2016). Secondly, in order to avoid further loss of water in the cells, which will affect the normal physiological and biochemical reactions, the cells will form a large number of osmotic regulators to adjust the osmotic pressure. These osmotic regulators mainly include mannitol, proline, betaine, trehalose, etc. Some of these osmotic regulators not only regulate cell osmotic pressure, but also play an important role in maintaining the normal conformation of proteins in cells (Singh et al. 2015). Through the above analysis, it can be seen that when cells are subjected to high osmotic pressure treatment, on the one hand, cells maintain the basic shape of cells by producing osmotic pressure regulators, and provide a good ionic environment for normal metabolism. On the other hand, the cell walls of cells thicken in response to high osmotic pressure. (Karandashova et al. 2005; Wang et al. 2019; H et

al. 2021; Li et al. 2019). Therefore, when we use high-salinity artificial seawater for screening, it is high possible to obtain strains with *G·lemaneiformis* physiological changes such as thickening of the cell wall and increase of cell content. *G·lemaneiformis*

At the same time, we carried out high osmotic pressure screening at different developmental stages of *G·lemaneiformis* to ensure the stability of the mutational traits.

Compared with the 6.91% agar content of cultivar 981 (Chen et al. 2020), the agar content of HAGL-X5 was 7.74% which is applaudable. This experiment also provided a valuable reference for the subsequent breeding of *G·lemaneiformis* with, high polysaccharide contains.

At present, ARTP mutagenesis has been successfully applied to the breeding of more than 40 kinds of microorganisms, including bacteria, actinomycetes, fungi, yeasts, microalgae, etc. (Ottenheim et al. 2018; Li et al. 2020). In algae breeding, it was mainly applied in microalga, such as *Crypthecodinium cohnii* and *Haematococcus pluvialis* where high polysaccharide and astaxanthin contains were mainly targeted (Liu et al. 2015; Wu et al. 2016). In terms of power and optimal mutagenesis time, the former was 150W, 70S, and the latter 100W, 40S, while those of *G·lemaneiformis* spores was 120W, 46S, which was not very different from those of microalgae. In terms of mutation rate, a total of 50,000 microalgae cells were mutagenized in the polysaccharide mutagenesis experiment, and 12 mutants that met the requirements were obtained, and the mutation rate was 0.024%. The mutation rate in astaxanthin breeding was 66% (Liu et al. 2015; Wu et al. 2016). In this breeding, the total number of mutagenized spores was about 2.7×10^5, and then the artificial seawater of 58‰ was used for one week screening. The number of surviving individuals was 8680. Referring to hypertonic resistance, and the mutation rate was 3.21%. After the second hypertonic screening, there were about 6505 remaining individuals, from which 17 individuals with better growth were selected by observation method. Referring to growth trend, the selection rate was 0.26%. It can be found that the mutation/selection rate of ARTP either on macroalgae or on microalgae varies. However, the target mutants can be effectively obtained by ARTP mutagenesis.

In terms of maximum photosynthetic efficiency, it has been reported that algae photosynthesis is inhibited when they are in high salinity seawater (Masojídek et al. 2000). High osmotic pressure can change the thylakoid structure and block the electron transport chain of the photosynthetically active reaction center PSII, thereby reducing the maximum photosynthetic efficiency. At the same time, due to the ROS generated by hyperosmotic and high-salt stress, lipid peroxidation, DNA damage, and enzyme activity are impaired, and the phytochrome is decomposed (Demidchik, 2015; Fatma et al 2014; Yoshioka-Nishimura, 2016). In this experiment, mutants and controls were treated with high salinity for three weeks. The actual photosynthetic efficiency (Y(PSII)), photochemically quenched QL and non-photochemically quenched NPQ, QN of mutants and controls were determined. It can be found that most of the difference of Y(PSII) and QL among strains were observed in the first ten days. The Y(PSII) and QL values of the mutants were significantly higher than those of the control without salt stress. Further analysis found that from the start point, Y (PSII) and QL of X3 and X4 were the highest and significantly higher than other strains, which indicated that the actual photosynthetic efficiency and photochemical quenching of

X3 and X4 were higher than those of other strains. Therefore, the light energy conversion rate of these two strains is the highest, which can explain the reason why X3 and X4 were higher than other strains in the growth rate. From the second day to the tenth day, the Y(PSII) and QL values of X3 and X5 were higher than those of Ctr, indicating that X3, especially X5, can more effectively protect the photosynthetic rental active reaction center PSII and enhanced photochemical quenching under stress. The non-photochemical quenching of NPQ and QN indicates the strength of the photoprotective mechanism. On the start point, both NPQ and QN of X3 and X4 were significantly lower than other strains, which was just opposite to the status of QL. The NPQ value of X5 was higher than Ctr from the second day to the eighteenth day, though with insignificant difference($P > 0.05$). The NPQ value of X3 was higher than Ctr from the second day to the fourteenth day, and the difference was not significant between the sixteenth day and the twenty-fourth day. The QN value of X5 was higher than Ctr from the second day to the twenty-fourth day, except that it was lower than Ctr on the 8th and 10th day. The QN value of X3 was higher than Ctr from the second day to the twenty-fourth day except that it was lower than Ctr on the eighteenth day. The above results indicated that the photoprotection mechanism of the mutants was stronger than that of Ctr in most days except for some days which were lower than Ctr. The better photosynthetic parameters of the mutants than the control may be related to the higher growth rate and better state of the mutants.

In short, this experiment was the first to successfully select individuals with high agar content using the method of hypertonic screening. In this experiment, individuals with high agar content were screened out by hypertonic screening, and a feasible breeding system was reported. The study contributed to breeding work of *G·lemaneiformis*, especially for the purpose of a strain selection with high agar contents.

Declarations

Data Availability

Methods, materials, and data used in this study are fully delineated in the text.

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Declaration of interests

The authors declare no conflict of interest

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Author Contributions

All authors contributed to study conception and design. Baoheng Xiao and Yiyi Hu were responsible for the experimental design. The whole experimental process was carried out by Baoheng Xiao and Yiyi Hu and Xiaoqing Feng. The first draft was written by Baoheng Xiao. Supervision, review and editing, and funding were provided by Professor Zhenghong Sui. Final manuscript read and endorsed by all authors.

References

1. Bird KT (1988) Agar production and quality from *Gracilariasp*. strain G-16: Effects of environmental factors. Botanic Marina 31:33-39 https://doi.org/10.1515/botm.1988.31.1.33
2. Bisson MA, Kirst GO (1995) Osmotic acclimation and turgor pressure regulation in algae. Naturwissenschaften 82(10):461-471 https://doi.org/10.1007/BF01131597
3. Chen Y, Cai XL, Sun X, et al (2020) "Agar accumulation and physiology and biochemistry changes of *Gracilariopsis Lemaneiformis* under different salinity stress." Acta Hydrobiology Sinica 44(06):1322-1329 https://doi.org/10.7541/2020.153
4. Cao S, Zhou X, Jin W, et al (2017) Improving of lipid productivity of the oleaginous microalgae *Chlorella pyrenoidosa* via atmospheric and room temperature plasma (ARTP). Bioresource technology 244:1400-1406 https://doi.org/10.1016/j.biortech.2017.05.039
5. Demidchik V (2015) Mechanisms of oxidative stress in plants: From classical chemistry to cell biology. Environmental and Experimental Botany 109:212-228 https://doi.org/10.1016/j.envexpbot.2014.06.021
6. Ekman P, Yu S, Pedersen M (1991) Effects of altered salinity, darkness and algal nutrient status on floridoside and starch content, α-galactosidase activity and agar yield of cultivated *Gracilaria sordida*. British Phycological Journal 26(2):123-131 https://doi.org/10.1080/00071619100650091
7. Freile-Pelegrín Y, Robledo D, Pedersén M, et al (2002) Effect of dark and salinity treatment in the yield and quality of agar from *Gracilaria cornea* (Rhodophyceae). Ciencias Marinas 28(3):289-296 https://doi.org/10.7773/cm.v28i3.223
8. Gao YX (2010) Research progress on the polysaccharides and phycobiliproteins of *Gracilariopsis Lemaniformis*. Journal of Chinese Medicinal Materials 33(003):474-477 https://doi.org/10.13863/j.issn1001-4454.2010.03.051
9. Hurtado-Ponce AQ, Pondevida HB (1997) The Interactive Effect of Some Environmental Factors on the Growth, Agar Yield and Quality of *Gracilariopsis bailinae* (Zhang et Xia) Cultured in Tanks. Botanica Marina 40(1-6):217-224 https://doi.org/10.1515/botm.1997.40.1-6.217
10. Hohmann S, Krantz M, Nordlander B (2007) Yeast osmoregulation. Methods in Enzymology 428:29-45 https://doi.org/10.1016/S0076-6879(07)28002-4

11. He LH, Shen SD, Wang DL (2002) Effect of salinity on growth, agar productivity and composition of *Gracilaria tenuistipitata* var *liui* Zhang *et* Xia. Marine Science Bulletin (03):39-43 https://doi.org/10.3969/j.issn.1001-6392.2002.03.006
12. Hu YY, Du QW, Mi P, et al (2019) Gene cloning and expression regulation in the pathway of agar and floridean starch synthesis of *gracilariopsis lemaneiformis* (rhodophyta). Journal of Applied Phycology 31(3):1889–1896 https://doi.org/10.1007/s10811-018-1690-0
13. Jiang SJ (2018) Protoplast mutagenesis breeding of *streptomyces viridochromogene* producing high-yield avilamycin by artp. Chinese Journal of Antibiotics 43(07):831-836 https://doi.org/10.13461/j.cnki.cja.006302
14. Karandashova IV, Elanskaya IV (2005) Genetic control and mechanisms of salt and hyperosmotic stress resistance in cyanobacteria. Russian Journal of Genetics 41(12):1311-1321 https://doi.org/10.1007/s11177-006-0001-z
15. Kumar J, Singh S, Singh M, Srivastava PK, et al (2017) Transcriptional regulation of salinity stress in plants: A short review. Plant Gene 11:160-169 https://doi.org/10.1016/j.plgene.2017.04.001
16. Li XE (2014) Screening of stress-resistant brewing yeast based on cell wall polysaccharides composition (Master's thesis, Jiangnan University) https://kns.cnki.net/KCMS/detail/detail.aspx?dbname=CMFD201501&filename=1014380650.nh
17. Li HP, Wang LY, Li G, et al (2011) Manipulation of lipase activity by the helium radio-frequency, atmospheric-pressure glow discharge plasma jet. Plasma Processes & Polymers 8:224-229 https://doi.org/10.1002/ppap.201000035
18. Li HP, Sun WT, Wang HB, et al (2007) Electrical features of radio-frequency, atmospheric-pressure, bare-metallic-electrode glow discharges. Plasma Chemistry & Plasma Processing 27:529-545 https://doi.org/10.1007/s11090-007-9079-x
19. Li L, Zhang X, He N, et al (2019) Transcriptome profiling of the salt-stress response in the halophytic green alga *dunaliella salina.* Plant Molecular Biology Reporter 37(5):421-435 https://doi.org/10.1007/s11105-019-01168-z
20. Lee WK, Lim YY, Leow TC, et al (2016) Factors affecting yield and gelling properties of agar. Journal of Applied Phycology 29(3):1-14 https://doi.org/10.1007/s10811-016-1009-y
21. Li JT, Liu XH, He YD, Wang GY (2020) Mutagenesis Breeding in DHA Production by Oleaginous Microorganisms. Biotechnology Bulletin 36(1):110-115 https://doi.org/10.13560/j.cnki.biotech.bull.1985.2019-0637
22. Liang L, Huang QG, Weng XQ, et al (2020) Breeding L-Glutamic Acid Producing Engineering Strain by Mutagenesis and Its Fermentation Efficiency. Biotechnology Bulletin 36(6):143-149 https://doi.org/doi:10.13560/j.cnki.biotech.bull.1985.2019-084
23. Liu B, Sun Z, Ma X, et al (2015) Mutation Breeding of Extracellular Polysaccharide-Producing Microalga *Crypthecodinium cohnii* by a Novel Mutagenesis with Atmospheric and Room Temperature Plasma. International Journal of Molecular Sciences 16(4):8201-8212 https://doi.org/10.3390/ijms16048201

24. Lv H, Kim M, Park S, Baek K, et al (2021) Comparative transcriptome analysis of short-term responses to salt and glycerol hyperosmotic stress in the green alga *dunaliella salina*. Algal Research 53:102147 https://doi.org/10.1016/j.algal.2020.102147
25. Ma MP (2003) Studies on germplasm and optimal of production conditions of high-yield polysaccharide in Spirulina and investigation of its molecular mechanism (Master's thesis, Zhejiang University) https://kns.cnki.net/KCMS/detail/detail.aspx?dbname=CMFD9904&filename=2003071040.nh
26. Munns R, Passioura JB, Colmer TD, et al (2019) Osmotic adjustment and energy limitations to plant growth in saline soil. New Phytologist 225(3):1091-1096 https://doi.org/10.1111/nph.15862
27. Mager WH, Marco S (2002) Novel insights into the osmotic stress response of yeast. Fems Yeast Research (3):251-257https://doi.org/10.1016/S1567-1356(02)00116-2
28. Monniaux M, Hay, A (2016) Cells, walls, and endless forms. Current opinion in plant biology 34:114-121 https://doi.org/10.1016/j.pbi.2016.10.010
29. Masojídek J, Torzillo G, Kopecky J, et al (2000) Changes in chlorophyll fluorescence quenching and pigment composition in the green alga *chlorococcum* sp. grown under nitrogen deficiency and salinity stress. Journal of Applied Phycology 12(3-5):417-426 https://doi.org/10.1023/A:1008165900780
30. Ottenheim C, Nawrath M, Wu JC (2018) Microbial mutagenesis by atmospheric and room-temperature plasma (ARTP): the latest development. Bioresources and Bioprocessing 5(1):1-14 https://doi.org/10.1186/s40643-018-0200-1
31. Pan JQ, Li SD (2010) Development and Utilization of Gracilaria Resources. Chinese Journal of Tropical Agriculture (10):47-50 https://doi.org/10.3969/j.issn.1009-2196.2010.10.013
32. Park HJ, Kim WY, Yun DJ (2016) A new insight of salt stress signaling in plant. Molecules & Cells 39(6):447-459 https://doi:10.14348/molcells.2016.0083
33. Sui ZH, Hu YY, Zhou W, et al (2020) Review on the cultivation and genetic breeding of *Gracilariopsis Lemaniformis* (Rhodophyta). periodical of Ocean University of China 50(9):98-104 https://doi.org/10.16441/j.cnki.hdxb.20200026
34. Singh M, Kumar J, Singh S, et al (2015) Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. Reviews in Environmental Science and Bio/Technology 14(3):407-426 https://doi.org/10.1007/s11157-015-9372-8
35. Siow RS, Teo SS, Ho WY, et al (2012) Molecular cloning and biochemical characterization of galactose-1-phosphate uridylyltransferase from *gracilaria changii* (rhodophyta) 1. Journal of phycology 48(1):155-162 https://doi.org/10.1111/j.1529-8817.2011.01105.x
36. Teo SS, Ho, C, Teoh S, et al (2009) Transcriptomic analysis of *gracilaria changii* (rhodophyta) in response to hyper- and hypoosmotic stresses. Journal of Phycology 45(5):1093-1099 https://doi.org/10.1111/j.1529-8817.2009.00724.x
37. Wei HH, Sui ZH, Wang JG, et al (2016) X-ray-irradiation mutation on carpospores of *Gracilariopsis Lemaniformis* and screening of mutants with superior traits. periodical of Ocean University of China

(09):50-58 https://doi.org/10.16441/j.cnki.hdxb.20150428

38. Wang W, Xu Y, Chen T, et al (2019) Regulatory mechanisms underlying the maintenance of homeostasis in *Pyropia haitanensis* under hypersaline stress conditions. Science of the Total Environment 662:168-179 https://doi.org/10.1016/j.scitotenv.2019.01.214
39. Wu XY, Liu ZS, Jiang Y (2016) Mutation breeding of *Haematococcus pluvialis* by atmospheric and room temperature plasma and isolation of high-producing mutants. Journal of Food Safety and Quality (09):3781-3787 https://doi.org//10.19812/j.cnki.jfsq11-5956/ts.2016.09.060
40. Xue ZX, Yang GP, Wang GC (2006) Research on the extraction method of *Gracilaria lemaneiformis*. Marine Sciences (08):71-77 https://doi.org/10.3969/j.issn.1000-3096.2006.08.016
41. Xu Y, Lu K, Guan B (2006) Effects of concentrations and ratios of nitrogen and phosphorus on the growth and agar content of *gracilaria lemaneiformis* (rhodophyta). Transactions of the Chinese Society of Agricultural Engineering 22(8):209-213 https://doi.org/10.1016/j.ejps.2006.05.004
42. Yoshioka-Nishimura M (2016) Close Relationships Between the PSII Repair Cycle and Thylakoid Membrane Dynamics. Plant Cell Physiol 57(6):1115-1122 https://doi.org/10.1093/pcp/pcw050
43. Zhang YY, Chen MZ, Yu J, et al (2005) Studies on the antimutagenic and antitumour effects of phycobiliproteins from *Gracilaria lemaneiformis*. Chinaese Journal of Marine Drugs (03):36-38 https://doi.org/10.3969/j.issn.1002-3461.2005.03.010
44. Zhang XC, Fei XL, Wang GC, et al (2009) Basic research and large scale cultivation of *Gracilariopsis lemaneiformis*. Periodical of Ocean University of China (05):947-954 https://doi.org/10.16441/j.cnki.hdxb.2009.05.024
45. Zhou W, Sui ZH (2016) *Gracilariopsis lemaneiformis* "Lulong No. 1". Chinese Acquaculture (03):56-57 https://doi.org/10.3969/j.issn.1002-6681.2016.03.026
46. Zhu SP, Liu Z, Xiang BQ, et al (1964) Effects of soil extract, its microelements and different concentrations of vitamin B12 and cobalt on the Nitzschia Closterium W.Smith. Journal of Fisheries of China (1):19-38 http://qikan.cqvip.com/Qikan/Article/Detail?id=15120654
47. Zhang XC, Qin S, Ma JH (2005) Seaweed Genetics. Agriculture Press

Figures

Figure 1

The time-lethal curve by ARTP treatment and salinity-survival curve of the spores of *G·lemaneiformis*

Figure 2

Determination of chlorophyll fluorescence parameters Y(PSII) (A), NPQ (B), QL (C), QN (D) for controls and mutants

Figure 3

The measurement results of the growth rate of each strain

Figure 4

Measurement results of agar content in each strain