

GROWTH OF SPORE *GRACILARIA* SP. BECOME A PLANLET USING PROVASOLI'S ENRICH SEAWATER MEDIA

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Abstract

This research aims to determine the production technique for *Gracilaria* sp seaweed plantlets produced from spores, conducted in August - November 2024 at the Fish Reproduction and Genetics Laboratory, Pangkep State Polytechnic of Agriculture. Parameters that influenced spore growth were observed including light intensity, temperature and salinity of the culture medium. The research began by taking the thallus of *Gracilaria* sp. containing cystocarp and it was cut 1.5-2 cm long and soaked in sterile sea water with a salinity of 30 ppt. The thallus were maintained in a petri dish containing a culture medium PES (Provasoli's Enrich Seawater) fertilizer at a dose of 5 mL PES in 1000 mL seawater. Observation of the release of spores from the cystocarp was carried out 24 hours after planting. Observation of the growth of spores into plantlets was carried out at room temperature 26-27°C, using a microscope with 10-20X magnification. The results were that spore release occurred at DOC-2 and spore attachment occurred at DOC-9. The spore size increases up to DOC-14. Spore buds began to appear at DOC-18 and spore buds began to grow at DOC-22. The increase in the length of the spore shoots began at DOC-32 and the shoots began to produce branches at DOC-40. In DOC-52 the spore shoots have become plantlets. This research indicates that PES fertilizer can be a good medium for the growth of *Gracilaria* sp spores to become plantlets at a light intensity of 12 hours of light and 12 hours of darkness.

Keywords: Cystocarp, *Gracilaria* sp, PES, Plantlets, Spore.

INTRODUCTION

Seaweed is one of the important aquaculture commodities that has a role in reducing the impact of environmental pollution due to inorganic pollutants and climate change (Erlania & Radiarta, 2014). Seaweed is one of the marine resources that has various benefits for food, pharmaceutical, beauty and other industrial activities (Asni, 2015). Seaweed also contains carbohydrates, proteins, fats, and several other bioactive compounds (Safia, 2020).

Seaweed is an algae that lives in the sea and belongs to the thallophyta division. Seaweed can be classified into 4 classes based on pigment content, namely green seaweed (Chlorophyta), red seaweed (Rhodophyta), brown seaweed (Phaeophyta) and blonde seaweed (Chrysophyta) (Nadhif, 2021).

Seaweed *Gracilaria* sp. It generally contains agar or also called gelatin as a result of its primary metabolism. Agar is obtained by extracting seaweed in an acidic atmosphere after being treated alkaline and produced and marketed in various forms, namely: flour gelatin, paper gelatin and bar gelatin and processed into pharmaceutical industry additives (Sipahutar *et al.*, 2021).

However, the problems faced by seaweed cultivators of *Gracilaria* sp. Currently, it is the availability of quality and sustainable seeds. One way to produce quality seaweed seeds that are available at all times is by tissue culture techniques and generatively (spores) (Asrul *et al.*, 2020). To support the growth and development of seaweed spores, appropriate and sufficient nutrients are needed, especially the elements Nitrogen and Phosphorus (Lideman *et al.*, 2013).

The use of spores for seed sources is one of the ways to increase production and improve cultivation techniques. Carpospore-type spores are easier to use as a source of seedlings because the spore sacs can be seen with the naked eye. The use of spores as a source of seeds has been successfully carried out in several countries, especially in Japan and Korea, namely in producing *Porphyra* seeds (Lideman *et al.*, 2016)

LITERATURE REVIEW

The results of research on the production of marine gracilaria seeds (*Gracilaria* sp.) through spore culture on ropes carried out by the Takalar Brackish Water Aquaculture Fisheries Center (BPBAP Takalar) since July 2013 have started efforts to produce seeds derived from spores attached to ropes , and ropes that already have seeds can be directly used to stretch in the sea. The advantages of using spore seeds compared to tied seedlings include that after harvesting, the seedlings can be raised again for the next cycle, thereby reducing the cost of purchasing seeds and reducing the preparation time for seedlings. The results of the study showed that *Gracilaria* sp. which comes from spores raised at the cultivation site can adapt and develop to reach a size of 20 – 25 cm at the age of 70 days. This will be very beneficial for farmers in terms of the fulfillment of seeds that can be adjusted according to needs, both in terms of planting season, quantity and quality. Seedlings derived from these spores after reaching the size ready for harvest can be harvested by cutting the talus (pseudo-stem) and the remaining pieces that are still attached to the rope can be used again as seedlings for the next cycle (Lideman *et al.*, 2013)

Based on the results of the study Hirijal (2024) about the effect of irradiation on the release and attachment of seaweed spores *Gracilaria* sp. In the release and attachment of *Gracilaria* sp. seaweed spores, it can be identified that the difference in the length of irradiation, can indicate the number of spores released from the spore sac. The results showed that the spores released were observed for 6 days fluctuated or fluctuated. The average value of spores released was highest in treatment (B) with an irradiation duration of 8 hours of light and 16 hours of darkness on day 5 of 560.00 ± 295.97 spores/Cystocarp. The lowest mean value was obtained in the control treatment (12 hours of light and 12 hours of dark), which was 293.33 ± 50.33 spores/cystocarp.

RESEARCH METHOD

Place and Time

This activity was carried out at the Fish Reproduction and Genetics Laboratory, Pangkep State Polytechnic of Agriculture. The activity was conducted from August to December, 2024.

Tools and materials

The tools used in this activity include petridish, erlenmeyer, beaker glass, laboratory vacuum pump, razor, cutting board, spoit, lamp, autoclave, oven, microscope and hand refractometer. The ingredients used include seaweed *Gracilaria* sp., seawater, fresh water, PES fertilizer, alcohol, betadine, dish soap and tissue.

Design Activity

This activity was devoted to the process of planting *Gracilaria* sp. Broodstock, in order to study development techniques or production methods of *Gracilaria* sp. seaweed plantlets from spores. The containers that will be used in this activity are 70 petri dishes. The equipment to be used such as

petri cups, erlenmeyer, and beaker glass are washed first and then dried. *Furthermore, the equipment to be used is wrapped in aluminum foil and then tied using a rubber band and sterilized* using an autoclave at a temperature of 121°C for 20 minutes with a pressure of 1 atm. The seawater medium to be used is deposited in the reservoir for 1-2 days. Furthermore, salinity is adjusted at 30 ppt for preparation of acclimatization of broodstock and for spore release media. The seawater is filtered first using 0.45 µm whatman paper then put into the autoclave then the temperature is set at 121°C for 20 minutes at a pressure of 1 atm. Next, the seawater is cooled and ready for use.

Gracilaria sp. cleaned with seawater, the goal is to clean the spores from dirt or moss that stick to them, then be maintained as an acclimatization stage in a diving aquarium for 2-7 days. This acclimatization aims to adjust the physiology of Gracilaria sp. to their new environment. After acclimatization, the broodstock is transported to a tissue culture laboratory for the broodstock cutting process. The cutting was carried out using a scalpel or razor by cutting the sea algae thallus containing 5 spore bags that were clamped using tweezers on a cutting board, then pieces of thallus with a length of 1.5-2 cm and re-immersed in a glass beaker that had been filled with sterile seawater.

The fertilizer used for maintenance is Provasoli's Enrich Seawater. The composition of PES fertilizer is, Sodium nitrate, tris base as much as 2.5 gr/liter, cyanocobalamin as much as 0.5 ml/liter, biotin as much as 1 ml/liter, thiamine as much as 1 ml/liter and distilled water as much as 1000 ml. The preparation of grund media uses 1000 ml of sterile seawater that has been filtered and then sterilized and 5 ml of stock PES fertilizer is added aseptim.

The result of cutting the talus with spore sacs (cystocarp) is then put into 100 ml erlemeyer mixed with 1 drop/100mL of sunlight and then shaken for 8-10 minutes, then rinsed up to 3 times or until there is no smell of soap. Next, put the pieces of thallus in 100 ml of sterile seawater and add 10 ml of betadine then soak while shaking for 2-3 minutes, then rinse up to 3 times or until there is no smell of betadine. After that, the talus is rinsed with sterile seawater, then the talus is dried by arranging it on a tissue until the water attached to the talus is gone. After that, the talus is put into a petri dish that has been given media, and waited for 24 hours for the spore bag on the talus to release the spores.



Figure 3.1 Talus with spore sacs

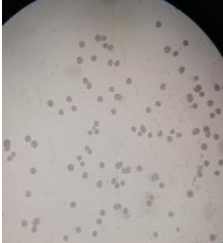
Observation to see if the spores have come out of the spore bag is carried out 24 hours after planting. If the spores have been released, the talus is transferred to a new medium, while the spores are allowed to remain in the medium until they stick to the maintenance container. The talus that was transferred to the new medium was observed again after 24 hours, and if it was found that there were still spores released, the talus was transferred to the new medium. And so on until all spores have been released from the spore bag, or according to the needs of the desired number of spores. Talus that is no longer used can be discarded. The spores that have escaped from the spore sac are

observed using a microscope with magnification of 10× and a magnification of 20×. Spores are observed daily during activities at room temperature 26-27°C.

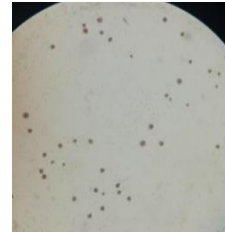
Spore maintenance is carried out by replacing the maintenance medium every 2 days, replacing the medium is carried out with a composition of 5 ml of PES fertilizer and 1000 ml of sterile seawater with a water salinity of 30 ppt. If seawater has a salinity of 34 ppt, then dilution is carried out using sterile fresh water. Seawater dilution is carried out with a composition of 1000 ml of seawater, 133 ml of fresh water and 5.6 ml of PES fertilizer. The replacement of the media is carried out by disposing of the old maintenance media, then spraying using sterile seawater that has been filled in a spray bottle to remove dirt and diatoms attached to the container. After all the dirt is removed, it is rinsed using sterile seawater until all the dirt is gone. After that, a new maintenance medium is added and placed back on the maintenance rack. Observation of bud growth from spores is done daily until the buds reach a condition or size called a plantlet.

RESULTS AND DISCUSSION

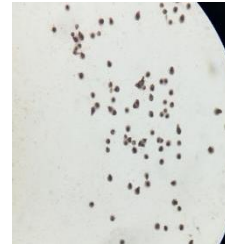
The results obtained in this activity include the development of seaweed spores *Gracilaria* sp. be the plantlet and maintenance life of each development of seaweed spores *Gracilaria* sp. to be a plantlet can be seen in the following table.

No	Stadium	Age Maintenance	Documentation
1.	Planting parent	DOC 1	
2.	Release spores	Doc 2	
3.	Attachment Spore	Doc 9	

4. Spore increase size Doc 14



5. Spore buds Doc 18



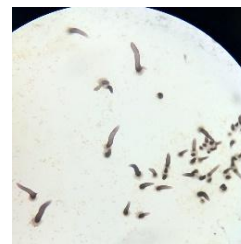
6. Spore buds start grow Doc 22



7. Spore buds grow Doc 26



8. Spore buds grow and increase long Doc 32



9. Spore buds grow and increase long Doc 36



10. Spore buds increase long and start emit branch Doc 40



11. Spore bud branch grow Doc 44



12. Spore bud branch increase long DOC 48



13. Spore buds develop , shoot branches develop , and spore buds Already become planlets . DOC 52



Production planlet grass sea gracilaria sp. from spores can be seen in the results the resulting study that release spores from pocket spores happen namely 24 hours after done planting parent in petri dishes. The treatment given that is giving lighting 12 hours of light and 12 hours of darkness. According to Choi *et al.* (2020), the rhythm Circadian in algae is an internal and natural process that regulates cycle algae activity and sleep, taking place about 24 hours. This process can respond especially to change light, temperature, and environment, as well as influence various function body like photosynthesis, growth, and reproduction.

Spores that have been let go will stick to the container maintenance, attachment spores happened 9 days ago after spores succeed let go from pocket spores. Attachment spores can observed below microscope with 10x and 20x magnification, attachment spores marked with spores that are not move place if the maintenance media is shaken, so can made into reference that spores has stick with good in the container maintenance. At the time attachment spores so done maintenance media replacement For First time, and will done maintenance media replacement in a way periodic with prone to time Water changes 2 times a day. Replacement of maintenance media done until spores reach the size called planlet or can also be up to planlet will moved to receptacle Erlenmeyer. More irradiation long can increase backup energy spores through more photosynthesis intensive, so that facilitate the pasting process more spores efficient (Guo *et al.*, 2016). Such as what was stated by Lobban and Harrison (1994) in (Hesti Mulyaningrum *et al.*, 2015) who stated Lots

factor affecting attachment from spores until grow become thallus such as pH, salinity, temperature, intensity light, active or whether or not spores, viscosity from waters, microfilm layers on the substrate, roughness substrate, ability polarisari spores to substrate, and capability adhesion spores to substrate.

Development spores become planlet need 52 days maintenance, development spores since attachment spores until spores have shoots and reach size planlet. Since the occurrence attachment to container maintenance, spores will increase size with look at the diameter of the spores. Spores that have a diameter greater big will more fast the occurrence growth of spore buds, spores that have grow will increase size and branching, but not all spores have branches and number the same branch. Spores that have increase long and branched will done observation routinely and replacement of maintenance media in a way periodically, until the spores sprout reach the size called planlet and ready to be moved to receptacle Erlenmeyer. In doing studies production planlet so need be noticed salinity of the water used as maintenance media that is is at 30 ppt and maintaining temperature room stable namely at a temperature of 26-27 °C. The use of PES media shows that development spores become *Gracilaria* sp. young who have thallus occurs at the age of 30 - 40 days, this This give more results fast If compared to with study previously conducted on *Gracilaria gigas* (Dworjanyn, 2020).

CONCLUSION

Based on the study conducted, it can be concluded that the generative (spore) cultivation of gracilaria sp. grass can be done by carrying out the appropriate procedure and aseptically. Seeding using spores can also be a source of quality and sustainable seeds in the provision of gracilaria sp. seaweed seeds.

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