Effect of Ecological Factors on Liberation and Development of Carpospore *Gracilaria*

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Abstrak

Karpospora pada Gracilaria sebagai alat reproduksi berpotensi sebagai penyedia bibit. Diantara beberapa faktor lingkungan lainnya, intensitas sinar dan salinitas merupakan faktor yang berpengaruh terhadap pelepasan spora. Penelitian ini bertujuan mengetahui pengaruh intensitas sinar dan salinitas terhadap pelepasan karpospora dari G. gigas. Rancangan Acak Lengkap digunakan dalam penelitian pelepasan karpospora dengan perlakuan intensitas sinar (1000, 1500 dan 2000 lux)dan salinitas (25, 30, dan 35 /oo). Jumlah terbesar kapospora terlepas dicapai dalam kombinasi intensitas sinar 1500 lux dan salinitas 30 /oo. Hal ini berarti bahwa intensitas sinar dan perbedaan salinitas memberikan pengaruh terhadap pelepasan karpospora G. gigas. Karpospora dari Gracilaria mempunyai fungsi penting dalam reproduksi. Terdapat beberapa faktor lingkungan yang menginduksi proses persporaan baik dalam kondisi di laboratorium maupun di alam. Namun begitu perkembangan dari karpospora G. vermiculophylla, species dari daerah 4 musim, telah menunjukkan suatu karakteristik tersendiri selama periode budidaya.

Kata kunci : *karpospora, G. vermiculophylla, G. gigas, pelepasan*

Abstract

Carpospore as reproduction device of Gracilaria is potential seeds provider. Among several other environmental factors, light intensity and salinity are suspected influencing the release of the spore. This study is aimed to define this light intensity and salinity effects on liberation of G. gigas. Completely Randomized Design is employed on the carpospore liberation with series light intensity (1000, 1500, 2000 lux) and salinity (25, 30, 35 /oo). The highest number of carpospore was released in 1500 lux and 30 / oo. This means, the light intensity and diferent salinity effected the liberation carpospore of G. gigas. Carpospores of Gracilaria have an important function for reproduction. There are some ecological factors which could induce sporulation in the field or under laboratory condition. But development of the carpospores of temperate G. vermiculophylla have showed a characteristics during the culture period.

Key words : *carpospore, G. vermiculophylla, G. gigas, liberation*

Introduction

The growing in development of the commercial algae resource is primarily due to recognition of its economic importance as a source of food, as raw material for the manufacture of commercial products such as agar, alginates and carragenan and as an additional source of livelihood for communities that inhabit the coastal areas (Jayasuriya, 1989). *Gracilaria* is one of the commercial algae, that produce agar, haversted and also cultivated world wide (Rebello *et al.*, 1996). Recently, the demand for agar has fastly increased, while the supply of *Gracilaria* from natural population has not. Consequently, cultivation of this red alga both in pond and sea as a problem solving of balance between demand for agar and suppling of this alga (Kalkman *et al.*, 1991).

On spore liberation and culture using spores of this commercial alga has been described by few authors (Tsekos and Karataglis, 1974; Jayasuriya, 1989; Trono C,Jr., 1989; Ampili *et al.*, 1991; Oliveira and Alveal, 1990). After the spores liberated, both from tetraspores and carpospores, they grew and

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attach on the substratum. There are many ecological factors, which affected during their sporeling growth, but almost no information is available on the carpospore germination, early stages of development and growth rate of sporelings of *Gracilaria* (Rabanal *et al.*, 1997).

In cultivating algae, it is important to maintain growth conditions in the optimal range to reduce the duration of cultivation to ensure in the greatest yield (Wong and Chang, 2000). That means, target of the cultivating of the commercial algae is a high biomass. To obtain high biomass of this algae as *Gracilaria*, we have to consider their ecological factors such as temperatur, pH, salinity, light intensity, water motion, etc. Because of *Gracilaria* is one euhialyne algae, thus, this algae can tolerate wide range of salinity (Wong and Chang, 2000). There are many authors which reported the influence of salinity on the growth of red algae (Ganesan and Rao, 1997; Wong and Chang, 2000, Dawes *et al.*, 1999). On the other hand, there are also many publications about germination, early state and liberation of spore from marine algae (Oza and Krishnamurthy, 1967; Rao, 1974; Oza, 1975; Kaliaperumal and Rao, 1987, Rao and Subbarangaiah, 1986; Rao and Kaliaperumal, 1976). Salinity and light intensity are critical factors for growth of *Gracilaria* both in laboratory or in the field. This study is aimed to define this light intensity and salinity effect on liberation and the carpospores development of *Gracilaria*. In addition, this paper reported study of *Gracilaria* from tropical and sub tropical waters.

Materials and Methods

Carpospores liberation

Living material of *Gracilaria gigas* Harvey was collected on March 1999 from western Lombok waters, Indonesia and transported to the Marine station of FFMS-UNDIP (Faculty of Fishery and Marine Science, Diponegoro University) at Jepara, where the experiments of carpospore liberation were carried out. The cystocarpic plants containing a single mature cystocarp were selected and washed throughtly using seawater. They were placed in petridish with 9 cm diamter each containing 10 ml of steril seawater.

Different salinity was produced by diluting of the seawater or addition NaCl (Stein, 1979) and was checked by a handrefractometer. The plants were maintained in black box (40 x 40 x 60 cm^3). A series of light intensity of 1000, 1500 and 2000 lux from flourescent lamp (10 and 20 watt) and salinity of 25, 30 and 35 $^{o}/_{oo}$ were employed as factor in factorial experimental design with 6 replications. Carpospores released after 24 hours incubation were counted under microskop. Statistical analyse was employed to know effect of the treatment and also duncan multiple range test (DMRT) if necessary (Gomez and Gomez, 1995).

Development of Carpospores

This study was carried out at Marine Botany Laboratory, Universität Bremen with carpospores from cystocarpic plants of *G. vermiculophyllla* (Ohmi) Papenfuss from Shimoda City, Shizuoka Pref. Japan. This species was collected by Ryuta Terada from the Laboratory of Deep Seawater Science, Fac. of Agriculture, Kochi University, Japan on March 29, 1998. Cystocarpic plants containing a single mature cystocarp were selected and washed throughly in seawater. The cystocarp was placed on object glass in a petri dish of 9 cm diameter containing 10 ml of filtered North Sea water with a salinity of 31 ppt. The seawater was enriched with some vitamines according to the formula from Provasoli enriched seawater (PES). The medium was changed every two days when the spores were investigated.

The salinity was checked with an Condukto-meter LF 191 (Juergens, Germany). The Petri dish were maintained in an incubator under the same conditions as the stock culture. The Petri dish contained 90 carpospores on one glass slide. The culture was illuminiated with 75-100 $\mu molm^{-2}sec^{-1}$ photosynthetic active radiation (determined using Quantummeter LI-185B) provided by cool-white fluorescent tubes under a 14:10h L:D cycle at a temperatures of 17°C. The cultures were not aerated. Germanium dioxide was added to eliminate diatoms. Growth rate of all spores were not determined, but the cell division was noticed and photographed.

Table 1. Total carpospores liberated by *G.gigas* Harvey in differential light intensity (LI) and salinity (S) with 6 replications (a, b, c, d, p, q, x, y show no significance different)

LI (lux)	S25°/oo	S30°/oo	S35°/oo	Average
1000	$179,67^{cd}$	$218,00^{bcd}$	$206,83^{bcd}$	$199,83^{p}$
1500	$292,50^{a}$	$343,00^{a}$	$232,50^{b}$	$289,33^{q}$
2000	$167,00^{d}$	$237,67^{b}$	$225,83^{bc}$	$210,17^{p}$
Average	$211,39^{x}$	$266,22^{y}$	$221,72^{x}$	

Results and Discussion

Carpospores liberation

Total carpospores liberated of *G. gigas* are presented in Table 1. This table shows that the carpospore liberation of the alga was effected by light intensity and salinity with optimum results on 1500 lux and 30 °/$_{oo}$.

Carpospore liberation was effected by several ecological factors i.e. light intensity, salinity, temperature and pH and the carpospore of *G.verrucossa* was optimum liberated by light intensity 1000 lux (Kim, 1970). Tetraspores, however, of *Gelidium pusillum, Pterocladia heteroplatos* and *Gelidiopsis variabilis* were released by light intensity at 500 lux. Results of spores liberated on this differential light intensity may be affected by different their species and habitat (Rao and Kaliaperumal, 1983). This study showed that carpospore of *G.gigas* optimum liberated on 1500 lux. Because maturation of cystocarp can be done optimum and also there is no inhibite of their metabolism spore leberation.

Salinity is a ecological factor important for the marine organism such as marine algae. They need salinity for rearing the balance osmotic pressure with their ecosystem (Kim, 1970; Reed, 1990; Lobban and Harrisom, 1997). Salinity 10-40 °/$_{oo}$ is good for tetraspore leberation of some marine red algae in Visakhapatnam, India and mainly for *Gelidium pusillum, Pterocladia heteroplatos* and *Gelidiopsis varibilis* can optimum liberate their tetraspore on 30 °/$_{oo}$ (Rao and Kaliaperumal, 1983) and tetraspores of *Dictyota dichotoma* is also released on same salinity (Rao and Reddy, 1997). This similar to the present study, where the carpospores of *G. gigas* were liberated with a peak of 343 pro cystocarp on salinity 30 °/$_{oo}$. In addition, carpospores of Asparagopsis delilei are optimum liberated between 20 and 40 °/$_{oo}$ (Ganesan and Rao, 1997).

G. gigas lifes in Lombok waters with salinity of 31 °/$_{oo}$. If they can release carpospores with a peaks on 30 °/$_{oo}$, thats means on this salinity there is no different osmotic pressure between internal and outside cell. Because of that, they could not optimum release their carpospore on lower and higher salinity. If the salinity in outside cell is higher than internal cell, the water will flow out of the cell with diffusion. So the concentration of salt compound will increase. That means it will reduction of turgor pressure and the cell will become flaccid. If the waters flow out of the cell so much, it will damage membrane, organela, enzim and osmotic balance. It will be happen also, if the concentration of salinity in outside cell is lower than intenral cell (Lobban and Harrison, 1997). Occurence of mucus which produced by the mature cystocarp effected in increasing of the osmotic pressure, therefore the water flow in interneal cystocarp. The osmotic pressure in cystocarp will be increase and bigger than outside. That effect the carpospores released throught out their ostiol (van de Hoek *et al.*, 1995). If the salinity is not optimum for the algae, that will effect on the production of mucus, as a consequence, the carpospore released will be inhibited. The present study supported of this idea with the statistical calculation such as on Table 2.

Table 2. Carpospores liberation of *G. gigas* Harvey from Lombok waters at differential light intensity and salinity (P<0,05)

SK	DB	JK	KT	F	F_{tab}5%
Light Intensity	2	86306,33	43153,17	20,94**	5,15
Salinity	2	30562,33	15281,17	7,41**	5,15
Interaction	4	29423,33	7355,83	3,57*	3,78
Galat	45	92741,33	2060,91		
Total	53	239033,33			

Development of Carpospores

Development of carpospores in *G. vermiculophylla* can be measured by divisions of the spore. After the carpospores were released, they attached to the surface of object glass and began their division of spore 24 hours later. Eight-celled forms were reached after 48 hours in culture. The 16-celled structure or more was found after 72 hours in culturing. After 7 days of cultivation, the spores have formed a morula-like structure, and by 10-13 days in culture, epidermis hairs were obtained. A thallus smaller than 0.1 mm could be detected after 15 days of cultivation. After 48 days of cultivation, a thallus with an average length of 6.5 mm was found. Fig.1. represents a development and division of carpospores from *G. vermiculophylla*.

After the carpospores were released, they attached on the surface of an object slide. Twenty four hours later they were divided into two cells. The formation of the four-cell state was found parallel or transversal division. The perpendicular divison in two discs giving rise to the formation of three and four-cell states were not found. The division four-celled of carpospores of *G. vermiculophylla* proceeds in another form (Fig.1). The formation of four-cell state of *G. corticata* was perpendicular division in two discs to the formation three or four-celled (Oza, 1975). This study was also different from Rabanal *et al.* (1997). They reported that four-cell state of *Gracilariopsis bailinae* divided transversely and longitudinally. Rao (1974) described also that germination of tetraspores of *Gelidiella acerosa*, which occupied the Gulf of Mannar-India, had no pattern. The tetraspores divided into 3 to 5 celled filaments and cells of the upper part divided further to give rise to a sporulation.

The 16-celled stages of *G. vermiculophylla* were obtained after 72 hours in culturing. The spores developed into a morula-like structure after 168 hours cultivation. The hyaline hairs were formed after 10-13 days. In the present study formation of carpospores of Gracilariopsis bailinae was rather different from that reported by Rabanal *et al.* (1997). They have found morula-like stages with hyaline hairs of the red alga after 5 days in culturing. It means, the development of carpospores in *G. vermiculophylla* was later than in *Gracilariopsis bailinae*. The function of the hyaline-hairs are not yet known (Rabanal *et al.*, 1997). The branchlets of *G. vermiculophylla* have just been organised after 48 days cultivation with an average length of 6.5 mm. This was also later than with *Gracilariopsis bailinae* (Rabanal *et al.*, 1997) and *Gelidium canariensis* (Garcia-Jimenez *et al.*, 1999) but faster than the tetraspore germination of *G. acerosa* which occurred after one to two months in culture (Rao, 1974).

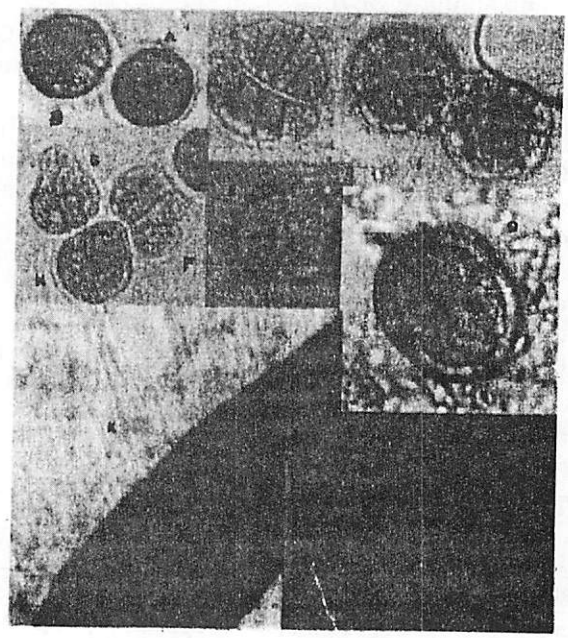

Figure 1. Carpospores development of G. vermiculophylla (A=one cell, B=two cells, C=longitudinal division, D=transversal division, E=four cells, F=three cells, G-H=16-celled stage, I=morula-like stage, J=branchlet, K=hyaline hair)

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