Mar. Res. Indonesia Vol.34, No.2, 2009: 87-89

EFFECT OF SALINITY AND GROWTH MEDIUM ON *SYMBIODINIUM* SP ISOLATED FROM GIANT CLAM

Maria Goretti Lily Panggabean[1], Rory A. Hutagalung[2] and Eveline Ayu[2]
[1]Marine Resources Division, Research Centre for Oceanography, Jl. Pasir Putih 1 Ancol Timur, Jakarta 14430
[2]Faculty of Biotechnology, Atma Jaya Catholic University, Jl. Jendral Sudirman 51, Jakarta 12930
E-mail: mglilyp@yahoo.com
Received: 13 October 2008 Revised and Accepted: 24 November 2009

ABSTRACT

An experiment on isolate *Symbiodinium* sp. from *Hippopus hippopus* was carried out to determine growth medium and optimum salinity for in vitro culture of the symbiont. The split-plot factorial design with two factors was applied with 3 replications. The first factor was medium with 2 levels, namely f/2 and modified GPM. The second factor was salinity with five levels i.e. 20, 25, 30, 35 and 40 PSU. All treatments were incubated under continuous light (2600 lux) and 25 °C. The maximum growth was reached at day 16 and there was significant interaction ($p<0.05$) between medium and salinity. The maximum density (64.8 x 10^4 cell/ml) was found in media f/2 and salinity 35 PSU.

Keywords: *Symbiodinium* sp., *Hippopus hippopus*, growth medium, f/2, modified GPM

INTRODUCTION

Symbiodinium are dinoflagellate symbiont (commonly known as zooxanthellae) that form mutualistic relationship with various marine invertebrates and protists, including Cnidaria, Platyhelminthes, Mollusca, Porifera and Foraminefera (Trench, 1997; Stat *et al.*, 2006). Among tropical reef community, zooxanthellae are the most important producer which estimated to range from 1 to 10% of total benthic productivity (Muscatine in Kuhl *et al.*, 1995). In tridacnid clams, zooxanthellae live in tubules the emanating from the host stomach in close proximity to the haemal-sinuses (Norton *et al.*, 1992). The phototrophic algae directly absorb nutrient (mostly ammonia) from the haemolymph of the clam's blood supply and on the other hand release their metabolites such as glycerols to be absorbed by the host.

Various strains of zooxanthellae have been cultured in laboratories. In vitro culture zooxanthellae has been used to investigate their physiological characteristics, such as response to photoperiod (Lerch and Cook, 1984), photoadaptation (Chang *et al.*, 1983; Iglesias-Prieto and Trench *et al.*, 1994, 1997), thermal adaptation (Iglesias-Prieto *et al.*, 1992), etc.

One strain isolated from *Hippopus hippopus* from Pari Island in 2008 has been kept in f/2 media in the laboratory of Research Centre for Oceanography, LIPI. To further maintenance, medium used is first interest of study. It was questioned if a medium contained soil extract could replace f/2 media for the dinoflagellate symbiont, following Tompkins *et al.* (1995) who stated that medium with soil extract was a good medium for maintenance culturing of many marine species.

This is a preliminary study on the culture condition favouring a new *Symbiodinium* sp. strain isolated from horse's hoof clam, *Hippopus hippopus*. The experiment was carried out to determine growth medium and optimum salinity to culture the symbiont *in vitro*.

MATERIALS AND METHODS

A culture of *Symbiodinium* sp was obtained from the Research Centre for Oceanography Labobatory of Microalgae Cullture. This strain was originally isolated from giant clam *Hippopus hippopus* cultured in Kepulauan Seribu, Indonesia. They were grown in 125 ml Erlenmeyer flask containing 50 ml filtered seawater enriched with media f/2 and GPM according to Guillard (1975) and Loeblich (1975) *respectively*. After inoculation with the algae, containers were placed under 3 fluorescence 40 watt lamps (2600 lux) at 25°C.

DOI: 10.14203/mri.v34i2.513

For experimental purpose, 'starter' in media f/2 and GPM were acclimatized in five different salinities (20, 25, 30, 35 and 40 PSU) until reaching stationary phase before being used as inocula. Factorial experiment with three replicates was carried out to assess salinity tolerance of *Symbiodinium* sp in different culture condition. Factor one was culture medium, i.e. f/2 and modified GPM. Factor two was five levels of salinity, i.e. 20, 25, 30, 35 and 40 PSU. Starter cultures were inoculated into each treatment medium to make uniform initial density of 10^4 cells/ml. Cell densities of each treatments were counted using Improved Neubauer Haemacytometer. Cells counting were conducted at day 7, 10, 13, 16 and 19 after inoculation respectively. Before sampling, cells attached on Erlenmeyer flasks surface were scraped off by sterilized ose needle and stirred to distribute the particle evenly.

Statistical analysis was performed using MINITAB software program. A two-way analysis of variance was performed and specific differences in mean density for each sample were identified.

RESULTS

Cell counts of *Symbiodinium* sp. grown in f/2 medium were significantly higher ($p<0.05$) than that of in modified GPM medium (Fig. 1). The mean density observed in each treatment is summarized in Table 1. Interaction between media and salinity was not obvious at day 7 after inoculation, but interaction was more obvious afterwards. The highest cell density was obtained from *Symbiodinium* sp. grown in f/2 at 35 PSU. At day 7, cell density grown in 35 PSU was significantly ($p<0.05$) higher than those grown in others salinity except 30 PSU (Figure 1A). At day 10, cell densities grown in 30, 35 and 40 PSU were significantly ($p<0.05$) higher than those grown in 20 and 25 PSU (Fig. 1B). At day 13, cell densities grown in 30, 35 and 40 PSU were significantly higher than those grown in 20 and 25 PSU (Fig. 1C). The maximum density was reached at day 16. At this day the interaction between medium and salinity was significant ($p<0.05$) and the high densities were found in 30 (5.87×10^5 cell/ml) and 35 PSU (6.48×10^5 cell/ml) which were significantly higher than those grown in 20 and 40 PSU (Fig. 1D). At day 19 the growth decreased but the cell density in medium f/2 with salinity 35 PSU was still the highest and significantly higher than other groups (Figure 1E).

DISCUSSION

In vitro culture of *Symbiodinium* sp. was grown best in f/2 at 35 PSU based on this experiment. Optimum salinity for the *Symbiodinium* growth was 35 PSU, demonstrating the true marine species. This was in agreement with McLachlan (1973) who stated that 35 PSU was 'normal' salinity for the oceanic microalgae.

Symbiodinium sp. grown in modified GPM medium in our experiment was not responded well. We employed modified GPM to grow *Symbiodinium* in reduced salinity in our experiment, following Blackburn *et al.* (1989) and Loeblich (1975) who have been used the medium for growing dinoflagellates. The dinoflagellate symbiont was expected to grow better in modified GPM than f/2, for the medium contain higher concentra-

Table 1. Cell density of *Symbiodinium* sp. ($\times 10^4 \pm$ se) grown in f/2 dan GPM media at different salinities (temperature 25 °C, continuous light intensity 2600 lux)

	Day 7	Day 10	Day 13	Day 16	Day 19
F/2 - 20	2.00 ± 0.50	6.08 ± 1.45	6.13 ± 0.35	14.70 ± 3.44	11.53 ± 1.77
F/2 - 25	4.43 ± 1.18	6.53 ± 1.39	11.80 ± 4.54	39.30 ± 7.45	23.23 ± 7.23
F/2 - 30	7.30 ± 2.86	26.65 ± 1.88	34.43 ± 3.85	58.73 ± 5.06	38.52 ± 1.78
F/2 - 35	9.20 ± 1.18	25.40 ± 2.10	42.28 ± 8.92	64.80 ± 7.07	59.17 ± 7.02
F/2 - 40	3.77 ± 1.29	21.85 ± 1.74	25.20 ± 4.31	40.90 ± 1.08	32.27 ± 7.27
GPM - 20	1.73 ± 0.48	2.77 ± 0.19	3.58 ± 0.39	6.17 ± 0.55	1.80 ± 0.12
GPM - 25	2.03 ± 0.45	3.43 ± 0.55	11.17 ± 1.88	6.57 ± 1.47	6.20 ± 3.76
GPM - 30	2.50 ± 0.49	3.10 ± 0.42	13.63 ± 4.56	4.47 ± 0.63	2.87 ± 0.50
GPM - 35	3.93 ± 0.67	4.05 ± 0.56	7.95 ± 1.58	4.97 ± 1.34	2.70 ± 0.91
GPM - 40	1.73 ± 0.18	2.70 ± 0.46	8.77 ± 0.56	5.13 ± 0.90	4.13 ± 1.87

tion of nitrogen (1.98 X 10^{-3} vs. 8.82 X 10^{-4} mol N) and phosphate (2.01 X 10^{-4} vs. 3.62 X 10^{-5} mol P). Modified GPM also contain soil extract which is more natural. Theoretically soil extract provides various elements and vitamins needed for plant growth, complexing toxic metals and keep iron in solution (Harrison, 2005).

However there was no change in cell densities of *Symbiodinium* sp. grown in modified GPM medium. The cell densities of *Symbiodinium* sp. grown in f/2 were higher than those grown in modified GPM medium (Fig. 1). Belda *et al.* (1998) and Belda & Yellowlees (1995) observed consistent N:P ratio in zooxanthellae from giant clam, which is >30:1. Belda & Yellowlees (1995) observed no significant difference in phosphate uptake between zooxanthellae treated in elevated phosphate or control. Zooxanthellae hosted in giant clam were use to live in limited nutrient in situ, particularly in phosphate starved condition (N:P ratio >30:1).

Medium f/2 was probably more suitable for culturing zooxanthellae isolated from giant clam in vitro. This medium has N:P ratio 24:1 close to the natural ratio of zooxanthellae (N:P ratio >30:1) if comparing to modified GPM (N:P ratio 10:1). From the historical-list of growth media for culturing zooxanthellae, medium f/2 was commonly used to culture zooxanthellae in vitro (Anonymous, 2009).

CONCLUSION

The best growth of *Symbiodinium* sp. isolated from *Hippopus hippopus* was found in f/2 medium with salinity 35 PSU in maximum density of 64.8 x 10^4 cell/ml at day 16. Medium f/2 was suitable for growing the symbbiont dinoflagellate.

REFERENCES

Anonymous. A historical –list of growth media that has been employed in zooxanthellae culturing.http://www.nsm.buffalo.edu/Bio/burr/BURR%20Cultures/Growth%20Media.pdf. Acessed on .March 2009.

Belda, C.A. 1999. Growth and metabolic responses of the giant clam-zooxanthellae symbiosis in reef-fertilisation experiment. *Mar.Ecol.Prog.Ser.* 170: 131-141.

Belda, C.A., W. Leggat and D. Yellowless. 1998. Growth and metabolic responses of the giant clam zooxanthellae symbiosis in a reef-fertilization experiments. *Mar.Ecol.Prog.Ser.* 170: 131–141.

Belda, C.A. and D. Yellowlees. 1995. Phosphate acquisition in the giant clam-zooxanthellae symbiosis. *Marine Biology* 123: 261–266.

Blackburn, S., G. Hallegraeff and C.J.S. Bolch 1985. Vegetative reproduction and sexual life cycle of the toxic dinoflagellates *Symbiodinium catenatum* from Tasmania, Australia. *J. Phycol.* 25: 577–590.

Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. In: *Culture of Marine Invertebrate Animals* (Smith, W.L. & M.H. Chanley Eds.). Plenum Press, New York, pp 26-60.

Harrison, P.J. 2005. Marine culture media. In: *Algal Culturing Techniques* (Andersen,R.A. ed.). Elsevier Acad.Press. Pp 21–33.

Iglesias-Prieto, R. and R.K. Trench. 1997. Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I, Responsees to chlorophyll-protein complexes to different photon flux densities. *Mar.Biol.* 130: 23–33.

Iglesias-Prieto, R. and R.K. Trench. 1994. Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I, Responsees to the photosynthetic unit to changes in photon flux density. *Mar.Biol.* 130: 23–33.

Kuhl, M., Y. Cohen, T. Dalsgaard, B. Jorgensen and N.P. Revsbech 1995. Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O2, pH and light. *Mar.Ecol.Prog.Ser.* 117: 159–172.

Lerch, K.A. and C.B. Cook, 1984. Some effects of photoperiod on the motility rhytym of cultured zooxanthellae. *Bull.Mar.Sci.*, Vol. 34: 477–483

Loeblich, A. 1975. A seawater medium for dinoflagellates and the nutrition of *Cachonina niei. J.Phycol.* 11: 80–86

Norton, J.H., M.A. Shepherd, H.M.Long and W.K. Fitt 1992. The zooxanthellae tubular system in giant clam. *Biol.Bull. mar.biol. lab., Wood Hole* 183: 503–5–6.

McLachlan, J. 1973. Growth media: Marine In: *Culture Methods and Growth Measurements* (Stein, J.R. ed.). Cambridge Univ.Press. pp 25–51.

Stat, M., D. Carter and O.H. Guldberg 2006. The evoliutionary history of Symbiodinium and scleractinian hosts-symbiosis, diversity and the effect of climate change. *Perspectice in Plant Ecology, Evolution and Systematics* 8:23-43.

Tompkins,J., M.M. De Ville, J.G. Day and M.F. Turner 1995. Culture collection of Algae and Protozoa. Catalog of Strains, Ambleside, U.K. 204 p.