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Bio inoculation of S*igmodocia carnosa* sponge on the growth of sponge associated cyanobacterial symbionts

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ABSTRACT

Marine cyanobacteria are the largest primary producers of biomass in the marine environment. Most of the marine microorganisms are not available due to the lack of media corresponding to the microbes. Hence, the present study was attempted to find out the substrate based media development from the host sponges. The effect of Sigmodocia carnosa sponge extract on the biomass production of sponge associated cyanobacterial species was carried out. Water extract from Callyspongia fibrosa significantly enhanced the biomass (193.14%), total chlorophyll (195.09%), chlorophyll-a (99.17%), carotenoid (685%), phycocyanin (172.91%), allophycocyanin (240.67%), phycoerythrin (488.46%), protein (76.44%), total amino acid (338.35%) and carbohydrate (55.77%) than the control and found significant among the species of sponge associated cyanobacteria.

Keywords: Cyanobacteria, Biomass, S*igmodocia carnosa*, Media formulation, Pigments, Sponge, Waste utilization.

INTRODUCTION

Sponges have been recognized as a rich source of novel compounds that are of potential interest to mankind [1]. Over exploitation of sponge species leads to mass extinction of the important species. Hence, recent focus is on the exploration of sponge associated microorganisms for the bioactive compounds discovery. However, the cultivation of sponge associated microorganisms by using conventional media is difficult. Identification of new methods for the mass scale production of target compound is the immediate concern to meet the global demand. For instance, *Oscillatoria spongeliae* a cyanobacterial symbiont of *Dysidea herbaceae* failed to culture under laboratory conditions by using the existing media. Hence, new media might be developed for symbionts that are not easily culturable [2]. Hinde [3] reported that, the growth media must resemble as much as possible with the conditions that prevail inside the sponge

mesohyl. Webster [4] conducted an experiment and reported that, the extract from sponge tissues enhanced the growth of associated actinomycetes. But no attempt has been undertaken with the cyanobacterial symbionts. Hence, the present study has been mooted out to find out the effect of sponge extract on the growth, pigments and biochemical constituents in identified species of sponge associated cyanobacteria.

MATERIALS AND METHODS

Isolation and identification of sponge and associated cyanobacteria

S*igmodocia carnosa s*ponge species was collected from Kuttankuzhi (Lat. 8^0 16'45" N, Long. 77^0 54'06" E) coast located along South East coast of India, and identified by using standard methodology [5]. A portion of S*igmodocia carnosa* sponge tissue was transferred to 250 ml Erlen Meyers flask containing sterilized BG11 medium. 1 ml of solution II ($g.l^{-1}$) – (H_3BO_3: 2.86; $MnCl_2.4H_2O$: 1.81; $NaMoO_4.2H_2O$: 0.39; $CuSO_4.5H_2O$: 0.079; Co $(NO)_3.6H_2O$: 0.0494;) and 5 ml of solution I ($g.l^{-1}$) – ($NaNO_3$: 300.0; K_2HPO_4: 8.0; $MgSO_4.7H_2O$: 15.0; $CaCl_2.2H_2O$: 7.2; $Na_2EDTA.2H_2O$: 0.2; Na_2CO_3: 4.0) were mixed and made up to 1 litre [6]. Inoculated flask was kept at 29 ± 2^0C with light intensity of 3000 lux (12 hrs light/12 hrs dark photoperiod) for a period of 30 days. The axenic culture of cyanobacterial symbionts were identified at species level and deposited at National Facility for Marine Cyanobacteria (NFMC), Bharathidasan University, Tiruchirapalli, Tamil Nadu, India by following the method of Deshikachary [7].

Extract preparation from sponge

Extract preparation was carried out by following the method [4]. Extract 1 (E1) sponge soaked in cold distilled water, extract 2 (E2) sponge soaked in sponge homogenate and extract 3 (E3) sponge soaked in boiled water. 1 ml of each extracts was added separately into sterilized BG11 medium. The axenic culture of 3 cyanobacterial species *viz. Ph.angustissmum, C. minor* and *O. amphibia* were inoculated and kept at 29 ± 2^0C. Control was maintained without the addition of sponge extracts.

Analysis of growth parameters

After incubation, the cultures from each treatment and control were filtered using fine cheese cloth and the wet weight was determined by using electronic balance (Schimadzu, Japan). The samples were used further for the determination of chlorophyll pigments [8], carotenoids [9], phycobilins [10], total aminoacids [11] and Carbohydrates [12].

RESULTS

The effect of different extracts from S*igmodocia carnosa* on the growth of associated cynobacterial species was carried out. It reveals that, the sponge homogenate extract (E2) was noticed maximum growth by 193.14%, 159.81% and 154.70% in *Ph.angustisissimum, C.minor* and *O.amphibia* respectively over control and other treatments (E1 and E2). The maximum content of total chlorophyll was noticed in *Ph.angustisissimum* by 195.09%, *C.minor* by 156.60% and *O.amphibia* by 190% grown in the extract of sponge homogenate (E2) than in the control and other treatments (E1 and E3). The content of chlorophyll-a increased by 99.17%, 55.90% and 70.58% in *Ph.angustisissimum*, *C.minor* and *O.amphibia* respectively over control and other treatments. The content of carotenoid increased by 685%, 364.70% and 247.82% in *Ph.angustisissimum*, *O.amphibia* and *C.minor* respectively over control and other treatments. The content of Phycocyanin was noticed by 172.91%, 81.32% and 153.63% in *Ph.angustisissimum*, *C.minor* and *O.amphibia*, respectively over control and other treatments (E1). The maximum content of allophycocyanin by 240.67%, 142% and 125.39% in

Ph.angustisissimum, *O.amphibia* and *C.minor* respectively was noticed in the extract where the sponge tissue boiled in distilled water (E3) when compared with the control and treatment (E1). The content of Phycoerythrin increased by 266.36%, 217.24% and 488.46% in *O.amphibia*, *C.minor* and *Ph.angustisissimum*, respectively over control. The protein content was increased by 76.44%, 70.88% and 51.93% in *Ph.angustisissimum*, *C.minor* and *O.amphibia*, respectively over control. The aminoacids was noticed by 338.35%, 287.01% and 131.46% in *Ph.angustisissimum*, *C.minor* and *O.amphibia* respectively over control and other treatments (E1 and E2). The content of carbohydrate was noticed by 55.77% in *Ph.angustisissimum*, 44.09% in *C.minor* and 29.94% in *O.amphibia* when compared with control and other treatments.

DISCUSSION

Cyanobacteria and sponges have a very long evolutionary history, little is known about the identity and phylogeny of cyanobacterial sponge symbionts [13, 14]. Sponge symbiosis with cyanobacteria can be obligate or non obligate depending upon the sponge species [15]. The biology of bacterium sponge relationship has elucidated considerable interest among the researchers. Approximately 0.1% of the total bacterial community was amenable to culture under lab conditions. It is not useful for less than 1% of cells in marine samples to be culturable. However, Santavy [16] reported, culturable counts of 3 – 11% of the total bacterial population for the sponge *Ceratoporella nicholsoni*, indicating that there may be considerable variability in the proportion of sponge bacteria that are readily cultivated. Innovative culture techniques should be explored in order to obtain additional sponge symbionts. These potentially novel and diverse isolates would be a useful resource for screening for bioactive compounds. Schmidt [17] succeeded in propagating the filamentous bacterial symbionts isolated from the sponge *Theonella swinhoei* in a mixed culture on agar plates containing aqueous sponge extracts. Webster [4] reported that, the addition of aqueous sponge extract in marine agar 2216E, starch-casein agar and MSN agar resulted in an increase in the number of novel cultivated morphotypes. Based on this, the present study was carried out and the results reveals that, the extracts prepared from sponge species showed considerable promontory effect on the growth, pigments and biochemical constituents [18, 19] in cyanobacterial symbionts. The maximum number of growth parameters also observed in the BG11 medium supplemented with sponge homogenate (E_2) than the other two extracts. The presence of growth promoting substances synthesized from the cyanobacterial symbionts might be stored in the sponge tissue, which in turn used by the cyanobacterial symbionts as the growth promoters [18, 19, 20,]. Ravikumar [20] reported that, the IAA, IBA at 0.1 ppm concentration enhanced the growth, pigment and carbohydrates content in *Phormidium fragile* isolated from solar saltpans.

The present findings support the view of Webster [4] that either the sponge extract prepared with cold distilled water or the sponge extract prepared in boiled distilled water enhanced the bacterial growth. The present study inferred that, the sponge homogenate from the sponge species particularly S*igmodocia carnosa* from South East coast of India, could be used as a supplement in BG11 medium for the cultivation of different morpho taxonomic sponge associated symbionts. So as to enable to reduce the accumulation of sponge wastes and to reduce the unpleasant odour along the coast. Further elaborate studies on the development of new media for the cultivation of unculturable sponge associated symbionts are highly warranted.

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Table 1. Effect of S*igmodocia carnosa* sponge extracts on the level of pigments and biochemical constituents in sponge associated cyanonobacterial species *Phormidium angutissimum*

Treatments	Biomass (gm)	Total chlorophyll (mg.g^{-1})	Chlorophyll-a (mg.g^{-1})	Carotenoid (mg.g^{-1})	Phycocyanin (mg.g^{-1})	Allphycocyanin (mg.g^{-1})	Phycoerythrin (mg.g^{-1})	Protein (mg.g^{-1})	Aminoacid (mg.g^{-1})	Carbohydrate (mg.g^{-1})
E1	2.919 (186.17)	0.0263 (195.09)	0.0193 (59.50)	0.0117 (485)	0.1022 (108.15)	0.0799 (35.42)	0.049 (88.49)	9.06 (59.22)	0.209 (186.30)	20.43 (35.65)
E2	2.99 (193.14)	0.0301 (195.09)	0.0241 (99.17)	0.0157 (685)	0.1340 (172.91)	0.201 (240.67)	0.153 (488.46)	10.04 (76.44)	0.320 (338.35)	23.46 (55.77)
E3	2.04 (100)	0.0162 (58.82)	0.0145 (19.83)	0.0099 (395)	0.0790 (60.89)	0.163 (176.27)	0.100 (284.61)	9.00 (58.19)	0.129 (76.71)	20.03 (33.00)
Control	1.02	0.0102	0.0121	0.0020	0.0491	0.059	0.026	5.69	0.073	15.06

Values in parenthesis showed percentage increase or decrease over control
E1- sponge soaked in cold distilled water, E2- sponge soaked in sponge homogenate and E3- sponge soaked in boiled water

Table 2. Effect of S*igmodocia carnosa* sponge extract on the level of pigments and biochemical constituents in sponge associated cyanonobacterial species *Chroococcus minor*

Treatments	Biomass (gm)	Total chlorophyll (mg.g^{-1})	Chlorophyll-a (mg.g^{-1})	Carotinoid (mg.g^{-1})	Phycocyanin (mg.g^{-1})	Allophycocyanin (mg.g^{-1})	Phycoerythrin (mg.g^{-1})	Protein(mg.g^{-1})	Aminoacid (mg.g^{-1})	Carbohydrate (mg.g^{-1})
E1	2.455 (128.97)	0.0239 (125.47)	0.0167 (31.49)	0.0076 (230.4)	0.0903 (81.32)	0.109 (73.01)	0.0701 (141.72)	8.42 (40.09)	0.276 (258.44)	21.68 (36.24)
E2	2.78 (159.81)	0.0272 (156.60)	0.0198 (55.90)	0.0055 (139.13)	0.0631 (26.70)	0.099 (57.14)	0.082 (182.75)	10.27 (70.88)	0.298 (287.01)	22.99 (44.09)
E3	2.31 (115.88)	0.0149 40.56	0.0124 (2.36)	0.0080 (247.82)	0.0601 (20.68)	0.142 (125.39)	0.092 (217.24)	7.67 (27.62)	0.106 (37.66)	21.02 (32.03)
Control	1.07	0.0106	0.0127	0.0023	0.0498	0.063	0.029	6.01	0.077	15.92

Values in parenthesis showed percentage increase or decrease over control
E1- sponge soaked in cold distilled water, E2- sponge soaked in sponge homogenate and E3- sponge soaked in boiled water

Table 3. Effect of S*igmodocia carnosa* sponge extracts on the level of pigments and biochemical constituents in sponge associated cyanonobacterial species *Oscillatoria amphibian*

Treatments	Biomass (gm)	Total chlorophyll (mg.g^{-1})	Chlorophyll-a (mg.g^{-1})	Carotinoid (mg.g^{-1})	Phycocyanin (mg.g^{-1})	Allophycocyanin (mg.g^{-1})	Phycoerythrin (mg.g^{-1})	Protein (mg.g^{-1})	Aminoacid (mg.g^{-1})	Carbohydrate (mg.g^{-1})
E1	2.21 (88.88)	0.0169 (0.0290)	0.0192 (61.34)	0.0067 (294.11)	0.1012 (153.63)	0.116 (132)	0.0806 (266.36)	10.62 (51.93)	0.186 (106.74)	20.96 (29.14)
E2	2.98 (154.70)	0.0290 (190)	0.0203 (70.58)	0.0023 (35.29)	0.0712 (78.44)	0.121 (142)	0.079 (259.09)	9.89 (41.48)	0.206 (131.46)	21.09 (29.94)
E3	2.90 (147.86)	0.0134 (34)	0.0136 (14.28)	0.0079 (364.70)	0.0499 (25.06)	0.0109 (118)	0.076 (245.45)	8.09 (15.73)	0.097 (8.98)	19.99 (23.16)
control	1.17	0.0100	0.0119	0.0017	0.0399	0.050	0.022	6.99	0.089	16.23

Values in parenthesis showed percentage increase or decrease over control

E1- sponge soaked in cold distilled water, E2- sponge soaked in sponge homogenate and E3- sponge soaked in boiled water

REFERENCES

[1] D.J. Faulkner. Marine natural products. *Nat Prod Rep.* **2002,** 19: 1-48.
[2] R. Osinga, E. Armstrong, J. Grant Burges, F. Hoffmann, J. Reitner and D. Schumann Kindel. *Hydrobiologia.* **2001,** 461: 55-62.
[3] R. Hinde, E. Pironet and M.A. Borowitzka. *Mar. Biol.*, **1994,** 119: 99-104.
[4] N.S. Webster, R.T. Hill and C.T. Marat. *Mar. Biol.* **2001,** 138: 843 – 851.
[5] J.N.A. Hooper. Guide to sponge collection and identification. Sponguide, Queensland Museum, Australia. **2000.**
[6] R.V. Stainer, R. Kunisawa, M. Mandel and G. Cohen Bazire. Purification and properties for unicellular BGA Bact. Rev. **1971,** 35: 171-205.
[7] T.V. Desikachary. Cyanophyta, a monograph – I.C.A. R. Pubs, New Delhi. **1959.**
[8] J.D. Hiscox, G.F. Israiel Stan. *Can. J. Bot.* **1979,** 57: 1332 – 1334.
[9] A. Jensen. Chlorophyll and carotenoids. In Handbook of physiological and biochemical methods (Eds). T.A. Hellibest & I.S. Graise, Gambridge Univ. Press. **1978.** Pp. 59 – 7.
[10] G. Subramanian. L. Uma, N. Thajuddin, D. Prabaharan, S. Sekar, M. Sundararaman, V. Sophia ranjini. Dept. of Biochemistry, Govt.of India, New Delhi. Bharathidasan Univ. Trichirapalli, **1992,** pp 1-100.
[11] S. Moore, W.H. Stein. *J Biol Chem.* **1948,** 176: 367–388.
[12] M. Dubios, K.A. Gills, J.K. Hamilton, P.A. Reser and F. Smith. *Anal Chem.* **1956,** 28: 350–356.
[13] T. Adell, V.A. Grebenjuk and M. Wiens, W.E.G. Muller. *Dev. Genes Evol.* **2003,** 213: 421–434.
[14] S.M. Awramik. The oldest records of photosynthesis. *Photosynth. Res*. **1992,** 33: 75–89.
[15] A. Arillo, G. Bavestrello, B. Burlando and M. Sara. *Mar. Biol.* **1993,** 117: 159–162.
[16] D.L. Santavy, P. Willenz and R.R. Colwell. *Appl. Env. Microbiol.* **1990,** 56: 1750 – 1762.
[17] E.W. Schmidt, A.V. Obraztsova, S.K. Davidson, D.J. Faulkner, and M.G. Haygood. *Mar. Biol.* **2000, 136:** 969 – 977.
[18] S. Ravikumar, G. Ramanathan, N. Suba, L. Jeyaseeli and M. Sukumaran. *Ind. J. Mar. Sci.* **2002,** 31(2): 157 – 160.

[19] S. Ravikumar, K. Kathiresan, S. Thadedus Maria Ignatiammal, M. Babuselvam and S. Shanthy. *J. Exp. Mar. Biol. Ecol.* **2004,** 312(1): 5 – 17.
[20] S. Ravikumar, A. Nural Shiefa and K.S. Nanitha. *J.Environ. Biol.* **2005,** 26(1): 55-59.