Pakistan Journal of Marine Sciences, Vol. 22(1&2), 61-72, 2013.

ISOLATION AND PURIFICATION OF MARINE CYANOBACTERIA IN LABORATORY CONDITIONS

Azra Bano and Pirzada J.A. Siddiqui
Lasbela University of Agriculture Water and Marine Sciences, Uthal-90150, Baluchistan (AB); Centre of Excellence in Marine Biology, University of Karachi, Karachi-75270, Pakistan (PJAS).
email: azrabano2006@yahoo.com; phone: +92-0853-610919

ABSTRACT: Twenty four species of cyanobacteria were isolated from a variety of marine habitats collected from Karachi coast and open ocean waters. The species of cyanobacteria were isolated from mix culture using serial dilution techniques at room temperature under constant illumination. The isolated species consist of six unicellular and eighteen filamentous non-heterocystous cyanobacterial species. Heterocystous forms were not obtained during these studies. Isolates were maintained in Marine Biology Culture Collection (MBCC).

KEYWORDS: Buleji, cyanobacteria, isolation, marine habitat.

INTRODUCTION

Cyanobacteria are found abundantly in marine habitats (Caroppo *et al.,* 2012; Ohki, *et al.,* 2008). Due to their ability to tolerate high salt concentrations and preference for alkaline conditions they grow well in seawater. Many species also show resistance towards osmotic shock and extremes of temperature thus making their existence suitable in a diversity of intertidal habitats (Chauhan and Abraham, 2011). Isolation and purification of uni-algal species of cyanobacteria from ecosystems is required to obtain cultures of cyanobacteria (Nagle *et al.,* 2010; Moore *et al.,* 2007) which can be of use for laboratory studies in basic and applied research, and to advance the acquaintance about micro biota of the specified ecological unit (Rajeshwari and Rajashekhar, 2011; Sarchizian and Ardelean, 2010; Thajuddin and Subramanian, 2005). They produce several bioactive compounds and recognized as a good source for many useful products (Carmichael, 2001; Codd, 1997). In the 1990s, cyanobacteria were reported to be a significant source for vitamins, fuels, fine chemicals and several pharmaceutical products (Chacon-de-Popioici, 1994; De Vries *et al.,* 1993; Miura *et al.,* 1993; Pesando and Bouicha, 1991).

Isolation and purification of cyanobacteria is often very difficult to isolate and in some cases it is impossible to isolate an individual from rest of the community in which it normally live (Palinska *et al.,* 1999; Ferris and Hirsch, 1991). Therefore, isolation and purification procedures have been revised for different strains isolated from different habitats, for example, thermophilic, epilithic, endolithic and planktonic, epiphytic, marine, freshwater and hypersaline environments (Olsson-Francis *et al.,* 2010: Michael *et al.,* 1991: Castenholtz, 1981; Walsby, 1981; Waterbury and Stainer, 1981 and Rippka *et al.,* 1981; Newton and Herman, 1979).

The isolation method of cyanobacteria is quite different from traditional bacteriological procedures. Several attempts have been made by different workers, and they encountered various difficulties in obtaining axenic cyanobacterial cultures (Fujishiro *et al.*, 2004; Sena *et al.*, 2011). The requirements of various constituents, like vitamins, organic carbon sources, combined nitrogen and inorganic constituents differ in different cyanobacterial species cultivation (Van Baalen, 1962), as well as the physico-chemical parameters, such as pH, salt concentration, temperature, light, aeration etc. (Geider 1987; Rippka *et al.*, 1979; Brock, 1973; Allen and Stainer, 1968; Allen and Arnon, 1955). Marine cyanobacteria for optimal growth also require Na^+, Cl^-, Mg^+ and Ca^+ ions (Mc Leod, 1965; Rippka *et al.*, 1979).

Both liquid and solid enrichment media have been developed and employed for the rapid culture, isolation, purification and maintenance of marine and freshwater cyanobacteria (Allen, 1968; Waterbury and Stainer, 1981). The liquid media such ASN-III, MN and BG-11 (Shirai *et al.*, 1989*;* Rippka *et al.*, 1979), ASP-2 (Provasoli *et al.*, 1957; Van Baalen, 1962) and SAG1 (SchlÖsser, 1982) etc have been modified to be used for the propagation of marine and freshwater spp. Many techniques have been used to obtain axenic cyanobacterial culture. These procedures including variation in the media nutrients, variations in the isolation techniques, application of antibiotics for the purification of culture etc., (Leach *et al.*, 1947; Obrig *et al.*, 1971; Whitton, 1968). The cyanobacteria have been isolated from different aquatic habitat and characterized morphologically (Rippka *et al.*, 1979; Anagnostidis and Komarek, 1985,88 and Komarek and Anagnostidis, 1986, 89) as well as with respect to their salt tolerance, mode of cell division, pigment composition, optimal growth requirements and their ability to fix nitrogen (Rippka *et al.*, 1979; Kao *et al.*, 1973).

The objective of this study was to isolate and identify cyanobacteria species from various habitats and ability to culture in laboratory conditions.

MATERIALS & METHODS

Sample collection:

The marine cyanobacterial samples were collected from different habitats near Karachi coast. The samples were collected in sterile polythene bags and were immediately brought to the laboratory. For the isolation of cyanobacterial species serial dilution technique and streaking plate method were employed (Throndsen, 1969; Rippka, 1988). The dilution was made in sterilized Miquel's medium contained in test tubes. One mililitre of homogenized sample was diluted ten folds up to a level of 10^{-25}. Test tubes were thoroughly mixed before each transfer. In some cases more dilutions were required for the isolation of a single species. Dilution tubes were incubated under constant light at room temperature. As soon as uni-algal culture of cyanobacteria was obtained, they were maintained in liquid medium (e.g., ASNIII, MN, and Miquel's medium (Rippka *et al.*, 1979; Imai, 1977). For the long term storage the culture were streaked on prewashed agar slants (ASNIII medium) (Kantz and Bold, 1969; Carmichael and Gorham, 1974). Stock cultures were maintained at room temperature under diffused light. The isolated species were identified according to botanical code of classification (Rippka *et al.*, 1979; Desikachary, 1959; Anagnostidis and Komarek, 1985, 88 and Komarek and

Anagnostidis, 1986, 89). Each isolates were given a number under Marine Biology Culture Collection (MBCC) series.

RESULTS & DISCUSSION

Results:

The cyanobacterial species from different study sites were isolated and characterized by morphological characters. The serial dilution techniques resulted in the isolation of twenty four species including six unicellular and eighteen non-heterocystous filamentous cynobacteria (Plate1 & Table1). Isolated species were belong to sixteen genera. No heterocystous species was isolated in this study. The isolate were characterized and numbered in the MBCC series. Micrograph of each species were shown in plate I.

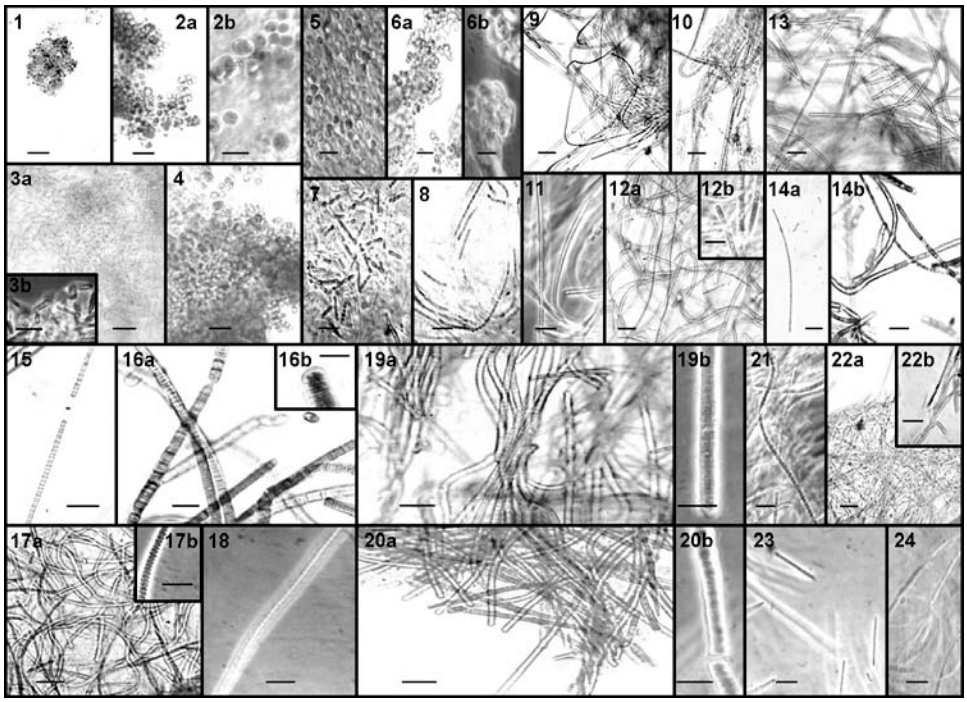

Fig. 1. *Synechocystis aquatilis*: 1. *Gloeocapsa cripidinum*: 2a, 2b. *Chlorogleopsis microcystoides*: 3a, 3b. *Myxosarcina burmensis*: 4. *Xenococcus kerneri*: 5. *X. acervatus*: 6a, 6b. *Komvophoron minutum*: 7. *K. epiphyticum*: 8. *Psuedoanabaena galeata*: 9. *P. limnetica*: 10. *P. lonchoides*: 11. *Limnothrix amphigranulata*: 12a, 12b. *Lyngbea contorta*: 13. *Hormoscilla pringsheimii*: 14a, 14b. *Oscillatoria psuedogaminata*: 15. *Katagnymen accurata*: 16a, 16b. *Spirulina major*: 17a, 17b. *Planktothrix clathrata*: 18. *P. agardhii*: 19a, 19b. *Phormidium breve*: 20a, 20b. *P. amphigranulata*: 21. *P. ambiguum*: 22a, 22b. *P. africanum*: 23. *Pseudoscytonema malayense*: 24. (Scale bar represents: a = 20 µm, b = 10 µm).

Table 1. Data presented on the unicellular and filamentous non-heterocystous species of cyanobacteria isolated during the study.

No. of Isolates	Name of the species	Habitats
MBCC0001	*Synechocystis aquatilis*	rock pool water
MBCC0002	*Gloeocapsa cripidinum*	epiphytic on *Colpomenia* thallus
MBCC0003	*Chlorogleopsis microcystoides*	edaphic
MBCC0004	*Myxosarcina burmensis*	rock pool water
MBCC0005	*Xenococcus kerneri*	epizoic on fish scale
MBCC0006	*X. acervatus*	edaphic.
MBCC0007	*Komvophoron minutum*	epiphytic on *Codium iyengarii*
MBCC0008	*K. epiphyticum*	edaphic at low tide
MBCC0009	*Psuedoanabaena galeata*	edaphic (Mangrove soil)
MBCC0010	*P. limnetica*	pool water
MBCC0011	*P. lonchoides*	epilithic at high tide
MBCC0012	*Limnothrix amphigranulata*	edaphic
MBCC0013	*Lyngbea contorta*	rock pool water
MBCC0014	*Hormoscilla pringsheimii*	rock pool water
MBCC0015	*Oscillatoria psuedogaminata*	open ocean
MBCC0016	*Katagnymen accurata*	open ocean (Manora channel).
MBCC0017	*Spirulina major*	edaphic
MBCC0018	*Planktothrix clathrata*	rock pool water
MBCC0019	*P. agardhii*	rock pool water
MBCC0020	*Phormidium breve*	rock pool water
MBCC0021	*P. amphigranulata*	edaphic
MBCC0022	*P. ambiguum*	open oceans
MBCC0023	*P. africanum*	epiphytic on *Galaxaura oblongata*
MBCC0024	*Pseudoscytonema malayense*	epizoic on sponges

Description of Isolates:

Synechocystis aquatilis Sauv **(MBCC0001)**

Bull.Soc. bot.France, 39; 121, fig. 2, 1892; Forti in De Toni, Sylloge, Algarum, 5: 26, 1907; GEITLER, Kryptogamenflora, 270, 1932. Desikachary 1959; pp. 144.

Single spherical cell, colour is yellowish green, cell content homogenous, sheath absent; cell size 2.6 µm, rotatory movement.

Habitat: Isolated from rock pool water.

Gloeocapsa cripidinum Thuret **(MBCC0002)**

In Bornet and Thuret, Algologiques, 1:1, pl . fig. 1-3, 1876; Forti in De Toni Sylloge Algarum, 5: 44, 1907; Geitler, Kryptogamenflora, 190, fig. 85, 86, 1932; Fremy, Cyano. Cotes d' Eur., 26, pl. 5, fig. 12, 1933. Desikachary 1959; pp. 117.

Cells closely arranged in colony, bright green in colour, colony oval; sheath not present; cell size 3.9 µm, colony size 32.5 µm, non-motile.

Habitat: epiphytic on *Colpomenia* thallus.

Chlorogleopsis microcystoides Geitler (**MBCC0003)**

Neue Cyano. Gruppe. Chamaesiphon., Arch. Protistenk, 51:359, fig. U, 1925; Kryptogamenflora, 310, fig. 155, 1932. Desikachary 1959; pp. 163

Cells as long as broad, dark green in colour, constricted at the cross walls; sheath absent. Cell width and length 1.3 µm, apical cell non-capitate, non-motile.

Habitat: edaphic.

Myxosarcina burmensis Skuja **(MBCC0004)**

Zur Susswasseralgenflora Burmas 21, pl. 1, fig. 12, 1949. Desikachary 1959; pp. 178.

Cells are spherical or sub spherical, closely arranged in colony, dark green or olive green in colour, cell content homogenous; sheath present, colorless; cell size 3.9 µm., colony size 22.1 µm, non-motile

Habitat: rock pool water.

Xenococcus kerneri Hansg. **(MBCC0005)**

Physiol. Algol. Stud., 111, pl. 1 fig. 19, 1887; Forti in De Toni Sylloge Algarum, 5: 134, 1907; Femy, Myxo. D' Afri. Equat. Franc., 61 fig. 67, Geitler, Kryptogamenflora, 330, fig. 163, 1932; Fremy, Cyano. Cotes d' Eur., 26, pl. 9, fig. 2, 1933. Desikachary 1959; pp. 181.

Cells yellowish green in colour, loosely arranged in colony; cell width 5.2 µm, cell length6.5 µm., non-motile

Habitat: epizoic on fish scale.

X. acervatus Setchell et Gardner. **(MBCC0006)**

In Gardner, New Pacific coast Algae, III, Uni, Calif. Publi. Bot., 6: 459, pl. 39 fig.1918. Geitler, Kryptogamenflora, 33, fig. 168, 1932. Desikachary 1959; pp. 182.

Cell oblong or oval in shape, 2-4 together in common mucilage, yellowish green; sheath colourless; cell width 3.9 µm. cell length 6.5 µm; colony size 13µm.

Habitat: edaphic.

Komvophoron minutum **(MBCC0007)**

(SKUJA) comb. N.]basionym: *Psuedoanabaena minuta* SKUJA Symb. Bot. Upsal. 9(3) 59, 1948]; type species; SKUJA 1956, KOMAREK 1974 (special species?) Anagnostidis & Komarek 1988; pp. 373, fig. 17(2)

Filament large, solitary, somewhat coiled green in colour, slightly constricted at the cross the walls, apical cell non-capitate; sheath not present; width 5.2 µm, length 2.6 µm, gliding movement.

Habitat: epiphytic on *Codium iyengarii*

***K. epiphyticum* (MBCC0008)**

(after SUJA 1948). Nomen novum [diagn. *Phormidium mucicola* f. *crassum* SKUJA Symb. Bot. Upsal. 9(3): 52, 1948 icnotype] Anagnostidis & Komarek 1988; pp. 373.

Cell longer than broad; constricted at the cross walls; apical cell non-capitate; bright green in colour; cell width and length 1.3 µm; two granules present on either side of cross walls, non-motile.

Habitat: edaphic at low tide

***Psuedoanabaena galeata* (MBCC0009)**

(after BOCHER from STARMACH 1966) Anagnostidis & Komarek 1988; pp. 384, Fig. 22(6)

Filamentous bright green in colour; distinctly constricted at the cross walls; apical cell non-capitate, round; sheath not present; width and length 1.3 µm, non-motile.

Habitat: edaphic (Mangrove soil).

***P. limnetica* (MBCC0010)**

(after KOMAREK 1958); Anagnostidis & Komarek 1988; pp. 384, fig. 22(3)

Cells green in colour; sheath not present; distinctly constricted at the cross walls; apical cell non-capitate, round width 1.3 µm, length 3.9 µm; non-motile.

Habitat: isolated from rock pool water.

***P. lonchoides* (MBCC0011)**

(after Anagnostidis 1961) Anagnostidis & Komarek 1988; pp. 384, fig. 22(10)

Cells dark green in colour; sheath not present; distinctly constricted at the cross walls; apical cell non-capitate, rounded; width 2.6 µm, length 3.9 µm., non-capitate.

Habitat: epilithic at high tide

***Limnothrix amphigranulata* (MBCC0012)**

(VAN GOOR). MEFFERT 1987 [sym. *Oscillatoria amphigranulata*VAN GOOR [1918] Anagnostidis & Komarek 1988; pp. 386.

Cells longer, slightly constricted at the cross walls, apical cell non-capitate; two granules present on either side of cross walls; cell width 2.6 µm, cell length 5.2 µm; bright green in colour; sheath not present; gliding movement.

Habitat: edaphic.

Lyngbea contorta Lemm. **(MBCC0013)**

Phytoplanct. Sachteiche. In Ploner Forscher., 6: 202, pl. Figs. 10-13. 1898; Forti De Toni, Sylloge Algarum, 5: 288, 1907: Fremy, Myxo. D' Afr. Equat. Frac., 202, fig. Fig. 172, 1929; Krytogamenflora, 1043, figs. 660a, b, 1932 Fremy, Cyano. Cotesd' Eur., 109, pl. 29 fig. 2, 1933. Desikachary 1959; pp. 290.

Filamentous straight or regularly coiled , slightly constricted at the cross walls apical cell non-capitate, rounded; cell width 2.6 µm cell length 3.9 µm, bright green in colour; sheath absent, gliding and oscillating movement.

Habitat: isolated from rock pool water.

***Hormoscilla pringsheimii* (MBCC0014)**

spec. nova (PRINGHEIM Arch. Microbiol. 63: 331-335, 1968); Anagnostidis *et al.*1983; iconotype: Fig. 32e. Anagnostidis & Komarek 1988; pp. 425, fig. 32c.

Cell dark brown or violet in colour; filament solitary; broader than long; slightly constricted at the cross walls; apical cell non-capitate, rounded; sheath present, colourless; width 3.9 µm length 2.6 µm., non-motile.

Habitat**:** rock pool water.

Oscillatoria psuedogaminata G. Schmid **(MBCC0015)**

Ber. Dtsch. Bot. Ges., 32: 124, fig.4, 1914; Geitler, Kryptogamenflora, 966, fig. 616, 1932. Desikachary 1959; pp. 228.

Filament dark green in colour; distinctly constricted at the cross walls; apical cell non-capitate, round; sheath not present; width 1.3 µm length 2.6 µm, non-motile.

Habitat: open ocean.

***Katagnymen accurata* (MBCC0016)**

(after Geitler 1982) Anagnostidis & Komarek 1988; pp. 427.

Cell wider, not constricted at the cross walls; apical cell non-capitate, rounded; sheath colourless; width 9.1 µm, length 3.9 µm; gliding movement.

Habitat: open ocean (Manora channel).

Spirulina major Kutz ex Gomont **(MBCC0017)**

Kutzing, Phyc. Gene., 183, 1843; Gomont. Monogr. Oscillariees, 251, pl. 7, fig. 29, 1892; ; Forti De Toni, Sylloge Algarum, 5: 210, 1907: Fremy, Myxo. d' Afr. Equat. Franc., 234, fig. 208, 1929; Krytogamenflora, 930, figs. 595, b, 1932. Desikachary 1959; pp. 196.

Colour bright green; spiral very close to each other; spirals width 1.3 µm to 2 µm, oscillating movement.

Habitat: edaphic.

Planktothrix clathrata (**MBCC0018)**

(SKUJA) comb.n.[basionym:Oscillatoria mougeotii var. clathrata SKUJA Nova Acta Reg. Soc. Sci. Upsal. Ser. 4, 16(3): 58, 1956]. (after SKUJA 1956). Anagnostidis & Komarek 1988; pp. 414. fig. 28(6).

Filament dark green, cell broader than long; slightly constricted at the cross walls; apical cell non-capitate, rounded; sheath not present; cell width 5.2, µm length 3.9 µm, gliding movement

Habitat: rock pool water.

P. agardhii (**MBCC0019)**

(GOM) comb. [basionym:*Oscillatoria agardhii* GOM. Ann. Sci. Nat., VII. Bot., 16: 205, [1892]; type species; SKULBERG & 1985 UTKILEN *et al.* 1985b. (After Komarek 1958-a, Geitler 1932-b, WISLOUCH from GEITLER-c, KOMAREK 1984,). Anagnostidis & Komarek 1988; pp. 416. fig. 28(1).

Filament green in colour, cell broader than long; sheath not present; constricted at the cross walls; apical cell non-capirtate, rounded; cell width 3.9 µm, length 2.6 µm, non-motile.

Habitat: rock pool water.

***Phormidium breve* (MBCC0020)**

(KUTZ., ex GOM) comb. [basionym:*Oscillatoria brevis* KUTZ., ex GOM. Ann. Sci. Nat., VII. Bot., 16: 229, 1892]. (after GOMONT and KOSINSSKAJA). Anagnostidis & Komarek 1988; pp. 397. fig. 25(1).

Filament green in colour, cell longer than broad; slightly constricted at the cross walls; apical cell non-capitate, tapering to a conical end; sheath not present; width 3.9 µm, length 2.6 µm., gliding movement.

Habitat: rock pool water.

***P. amphigranulata* (MBCC0021)**

(after HOLLEERBACH). Anagnostidis & Komarek 1988; pp. 397. fig. 25(25).

Filament dark green in colour, more or less coiled, cell content homogenous; not constricted at the cross walls; apical cell non-capitate, round; sheath present colourless, unlamellated; cell width 2.6 µm, length3 µm., non-motile.

Habitat: edaphic.

P. ambiguum Gomont **(MBCC0022)**

Monogr. Oscillariees, 178, pl., figs. 10, 1892; Forti De Toni, Sylloge Algarum, 5: 240, 1907; Fremy, Myxo. D' Afr. Equat. Franc., 156, fig. 137, 1929; Geitler, Kryptogamenflora, 1015, fig, 647e, 1932. Fremy, Cyano. Cotes d' Eur., 91, pl. 24, fig. 1, 1933. Desikachary 1959; pp. 266.

Cell wider, filament green in colour; slightly constricted at cross walls; apical cell non-capitate; gradually tapering to a pointed end; sheath present, colourless; cell width 4.5 µm, length 2.6 µm; gliding movement.

Habitat**:** open oceans.

P. africanum Lemm. **(MBCC0023)**

Deutsch. Zentr. Afr. Exped., 2: 89, 1911; Fremy, Myxo. D' Afr. Equat. Franc., 138, fig. 1929; Geitler, Kryptogamenflora, 999, 1932. Desikachary 1959; pp. 254.

Filamentous green in colour; not constricted at the cross walls; apical cell non-capitate, gradually tapering to a pointed end; sheath not present; cell width 1.3 µm, 3.9 µm, slow gliding movement. Habitat: epiphytic on *Galaxaura oblongata.*

***Pseudoscytonema malayense* (MBCC0024)**

(after Biiswas from Desikachary 1959) *P. malayense* (BISW.) ELENK. 1949; type species Anagnostidis & Komarek 1988; pp. 433.

Cell as long as broad, slightly constricted at cross walls; colour purple, violet or green; sheath absent; apical cell non-capitate, rounded; terminal cell swallowed; size2.6 µm ,cell width and length1.3 µm; non-motile.

Habitat: epizoic on sponges.

Discussion

Cynobacteria are considered as the rich source for many valuable products and bioactive compounds (Carmichael, 2001; Codd, 1997). A potentially significant source of vitamins and many other pharmaceutical products (Chacon-de-Popioici, 1994; DeVries *et al.*, 1993; Miura *et al.*, 1993; Pesando and Bouicha, 1991).

Uni-algal culture of cyanobacteria is essential for studying many aspect of the biology of organisms. All species were isolated through serial dilution technique. Agar slant and plates were used but cyanobacteria failed to grow on them probably due to the

toxicity of agar to algal species (Kantz and Bold, 1969; Carmichael and Gorham, 1974). Later pre-washed agar was used to make slants for long term storage of isolates. Although a large number of species were recorded from different habitat, the number of isolates obtained during present study was low. The serial dilution technique takes long time for growth of a single cell and therefore, only twenty four species were isolated during this research.

The inability of a particular cyanobacterial species to grow in uni-algal culture may also be as there is difference between laboratory environment and natural marine habitat (Castenholtz, 1981; Walsby, 1981; Waterbury and Stainer, 1981 and Rippka *et al.*, 1981). The biotic interactions that can exists among species may be highly important for the survival of cyanobacterial species. The heterocystous species were not isolate through serial dilution technique used in this study. Similar paucity of hetercystuos forms has been observed and attributed to the sulphide content in marine environment which may be more toxic to the heterocystous species than to the non-heterocystous species (Howsely and Pearson, 1979).

ACKNOWLEDGEMENTS

The authors are thankful to Vice Chancellor, Lasbela University of Agriculture, Water and Marine Science, for his invariable support for providing facility. We also thank to staff of faculty of Marine Science of LUAWMS for their assistance during preparation of this manuscript.

REFERENCES

Allen, M.M. 1968. Simple conditions for growth of unicellular blue-green algae on plates. *J. Phycol*. 4:1-4.

Allen, M.B. and D.I. Arnon. 1955. Studies on nitrogen-fixing blue-green algae. I. Growth and nitrogen fixation by *Anabaena cylindrica* Lemm. *Plant Physio.* 30(4), p.366.

Allen, M.M. and R.Y. Stainer. 1968. Growth and division of some unicellular blue-green algae *J. Gen. Microbiol*., 51(2): 199-202.

Anagnostidis, K. and J. Komárek. 1985. Modern approach to the classification system of cyanophytes, 1–Introduction. – *Arch. Hydrobiol. Suppl.* 71/*Algol. Stud.* 38/39: 291-302.

Anagnostidis, K. and J. Komárek. 1988. Modern approach to the classification system of cyanophytes, 3–Oscillatoriales. – *Arch. Hydrobiol.* 80/*Algol. Stud.* 50–53: 327-472.

Brock, T.D. 1973. Lower pH limit for the existence of blue-green algae: evolutionary and ecological implications. *Science* 179(4072): pp.480-483.

Carmichael, W.W. and P.R. Gorham. 1974. An improved method for obtaining axenic clones of planktonic blue-green algae. *J. Phycol.* 10: 238-240.

Carmichael, W.W. 2001. Health effects of toxin-producing cyanobacteria: "The cyanoHABs" *Hum. Ecol. Risk Assess.* 7(5): 1393-1407.

Caroppo, C., P. Albertano, L. Bruno, M. Montinari, M. Rizzi, G. Vigliotta and P. Pagliara. 2012. Identification and characterization of a new Halomicronema species

(Cyanobacteria) isolated from the Mediterranean marine sponge *Petrosia ficiformis* (Porifera). *Fottea*, Olomouc 12(2): 315-326.

Castenholz, R.W. 1981. Isolation and cultivation of thermophilic cyanobacteria. In *The prokaryotes* (pp. 236-246). Springer Berlin Heidelberg.

Chacon-de-Popoici, G. 1994. Plankton blooms, their effects on the marine organisms for the quantitative analysis of microcystins in cyanobacterial cells. *Phycol.* 35(6): 57-61.

Chauhan, R. and J. Abraham. 2011. IJPI's *J. Biotechnol. Biotherap.* 1(4): 47-57.

Codd, G.A. 1997. Cyanobacterial blooms and toxins in fresh, brackish and marine waters. *Harmful algae*. *Proceedings of the* VIII *International Conference on Harmful Algae.* (Eds.). Reguera, B., J. Blanco, M. L. Fernandez and T. Wyatt. p. 13-17.

De Varies, S.E., F.D. Galey, M. Namikoshi and J.C. Woo. 1993. Clinical and pathological findings of blue green algae (*Microcystis aeruginosa*) in toxication in a dog. *J. Vet. Diagn. Investig*. 5(3): 403-408.

Desikachary, T.V. 1959. Cyanophyta. Monographs in algae. New Delhi. Indian council of Agricultural Research.

Ferris, M.J. and C.F. Hirsch. 1991. Method for isolation and purification of cyanobacteria. *Appl. environ. microbiol*. 57(5): 1448-1452.

Fujishiro, T., T. Ogawa, M. Matsuoka, K. Nagahama, Y. Takeshima and H. Hagiwara. 2004. Establishment of a pure culture of the hitherto uncultured unicellular cyanobacterium *Aphanothece sacrum*, and phylogenetic position of the organism. *Appl. environ. microbiol.* 70(6): 3338-3345.

Geider, R.J. 1987. Light and temperature dependence of the carbon to chlorophyll a ratio in microalgae and cyanobacteria: implications for physiology and growth of phytoplankton. *New Phytologist* 106(1): 1-34.

Howsley, R. and H.W. Pearson. 1979. pH dependent sulphide toxicity to oxygenic photosynthesis in cyanobacteria. *FEMS Microbiol. Letters* 6(5): 287-292.

Imai, T. 1977. The culture of microorganisms used as feed. Pp. 55-578. In: Aquaculture shallow seas. Progress in shallow sea culture. (Ed.) Takeo Imai Translted from Japanese Oxford and IBH Publishing Company, New Delhi, Bombay, Calcutta.

Kantz, T. and H.C. Bold. 1969. Phycological Studies. IX. Morphological and taxonomic investigations of *Nostoc* and *Anabaena* in culture. University of Texas Publication no. 6924.

Kao, O.H., D.S. Bern and W.R. Town. 1973. The characterization of C-phycocyanin from an extremely halotolerant blue-green alga *Coccochloris elabans*. *Biochem. J.* 131: 39-50.

Komárek, J. and K. Anagnostidis. 1986. Modernapproach to the classification system of cyanophytes, 2–Chroococcales. – *Arch. Hydrobiol.* 73/*Algol. Stud.* 43:157-226.

Komárek, J. and K. Anagnostidis. 1989. Modern approach to the classification system of Cyanophytes, 4–Nostocales. – *Arch. Hydrobiol.* 82/*Algol. Stud.* 56: 247-345.

Leach, B.E., J.H. Ford and A.J. Whiffen. 1947. Actidione, an antibiotic from *Streptomyces griseus*. *J. Amer. chem. Soc.* 69(2): 474-474.

Luukkainen, R., K. Sivonen, M. Namikoshi, M. Färdig, K.L. Rinehart and S.I. Niemelä. 1993. Isolation and identification of eight microcystins from thirteen *Oscillatoria*

agardhii strains and structure of a new microcystin. *Appl. environ. microbiol.* 59(7): 2204-2209.

MacLeod, R.A. 1965. The question of the existence of specific marine bacteria. *Bacteriol. rev. 29*(1): 9.

Michael J. Ferris and C. F. Hirsch. 1991. Method for Isolation and Purification of Cyanobacteria *Appl. Environ. Microbiol*. 57(5): 1448.

Miura, Y., K. Sode, Y. Narasaki and T. Matsungaga. 1993. Light induced antimicrobial activity of extracts from marine *Chlorella*. *J. Mar. Biotechnol.* 1(3): 143-146.

Moore, L.R., A. Coe, E.R. Zinser, M.A. Saito, M.B. Sullivan, D. Lindell, K. Frois-Moniz, J. Waterbury and S.W. Chisholm. 2007. Culturing the marine cyanobacterium *Prochlorococcus*. *Limnol Oceanogr Methods*, *5*(10): 353-362.

Nagle, V.L., N.M. Mhalsekar and T.G. Jagtap. 2010. Isolation, optimization and characterization of selected Cyanophycean members. *Ind. J. Mar. Sci*. 39(2): 212-218.

Newton, J.W. and Herman, A.I., 1979. Isolation of cyanobacteria from the aquatic fern, *Azolla*. *Archi. Microbiol.* 120(2): 161-165.

Obrig, T.G., W.J. Culp, W.L. McKeehan and B. Hardesty. 1971. The mechanism by which cycloheximide and related glutarimide antibiotics inhibit peptide synthesis on reticulocyte ribosomes. *J. Biol. Chem.* 246(1): 174-181.

Ohki, K., M. Kamiya, D. Honda, S. Kumazawa and K.K. Ho. 2008. Morphological and phylogenetic studies on unicellular diazotrophic cyanobacteria (cyanophytes) isolated from the coastal waters around singapore1. *J. phycol.* 44(1): 142-151.

Olsson-Francis, K., R. de la Torre and C.S. Cockell. 2010. Isolation of novel extreme-tolerant cyanobacteria from a rock-dwelling microbial community by using exposure to low Earth orbit. *Appl. environ. microbiol.* 76(7): 2115-2121.

Palinska, K.A., C. Becker and W.E. Krumbein. 1999. Isolation and purification techniques for benthic marine cyanobacteria: biotechnology potential. *Bulletin de l'Institut océanographique*, pp.585-592.

Pesando, D. and N. Bouicha. 1991. Antifungal compounds from marine algae; recent data and prospective programme and abstracts. Second IMBC.-91. pp. 75.

Provasoli, L., J.J.A. McLaughlin and M.R. Droop. 1957. The development of artificial media for marine algae. *Archiv für Mikrobiologie* 25(4): 392-428.

Rajeshwari, K.R. and M. Rajashekhar. 2011. Biochemical composition of seven species of cyanobacteria isolated from different aquatic habitats of Western Ghats, Southern India. *Brazil. archives biol. technol.* 54(5): 849-857.

Rippka, R. 1988. [1] Isolation and purification of cyanobacteria. *Methods in enzymology* 167: 3-27.

Rippka, R., J. Deruelles, J. B. Waterbury, M. Herdman and R.Y. Stanier. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* 111**:** 1-61.

Rippka, R., J.B. Waterbury and R.Y. Stanier. 1981. Isolation and purification of cyanobacteria: some general principles. In *The prokaryotes* (pp. 212-220). Springer Berlin Heidelberg.

Sarchizian, I. and I.I. Ardelean. 2010. Axenic culture of a diazotrophic filamentous cyanobacterium isolated from mesothermal sulphurous springs (Obanul mare–Mangalia). *Rom. J. Biol. Plant Biol*. 55: 47-53.

Schlösser, U.G. 1982. Sammlung von Algenkulturen. *Ber. Deutsch. Bot. Ges.* 95:181-276.3.

Sena, L., D. Rojas, E. Montiel, H. González, J. Moret and L. Naranjo. 2011. A strategy to obtain axenic cultures of *Arthrospira* spp. cyanobacteria. *World J. Microbiol. Biotechnol.* 27(5): 1045-1053.

Shirai, M., K. Matumaru, A. Ohotake, Y. Takamura, T. Aida and M. Nakano. 1989. Development of a solid medium for growth and isolation of axenic *Microcystis* strains (cyanobacteria). *Appl. environ. microbiol.* 55(10): 2569-2571.

Thajuddin, N. and G. Subramanian. 2005. Cyanobacterial biodiversity and potential applications in biotechnology. *Curr. Sci*. 89 (1): 47-57.

Throndsen, J. 1969. Flagellates of Norwegian coastal waters. *Nytt. Mag. Bot*. 16(3-4): 161-216.

Van Baalen, C.H.A.S.E. 1962. Studies on marine blue-green algae. *Botanica Marina* 4(1-2): 129-139.

Walsby, A.E. 1981. Cyanobacteria: planktonic gas-vaculated forms. *In*: M. Star, H. Stolp, Waterburey, J.B.R.Y. Stainer, 1981. Isolation and growth of cyanobacteria from marine and hypersaline environments. *In*: MP Starr, H Stolp, HG Truper, A Balows, HG Schlegel, (eds), The Prokaryotes: A handbook of habitats, Isolation and Identifications of Bacteria. Berlin-Springer-Verlage. p. 221-223.

Waterbury, J.B. and R.Y. Stanier. 1981. Isolation and Growth of Cyanobacteria from Marine and Hypersaline Environments. In: The Prokaryotes: A handbook on Habitats, Isolation and Identification of Bacteria. M.P. Starr, H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel, (Eds.). Springer-Verlag, Berlin. 1: 221-223.

Whitton, B.A. 1968. Effect of light on toxicity of various substances to *Anacystis nidulans. Plant Cell Physiol.* 9(1): 23-26.