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DISTRIBUTION OF EPIPHYTIC CYANOBACTERIA ON GREEN MACROALGAL SPECIES OCCURRING AT A ROCKY SHORE (BULEJI), KARACHI, PAKISTAN

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ABSTRACT

The present study is the part of research concerning identification of cyanobacteria from coastal water of Karachi. As report on the distribution cyanobacterial epiphytes in coastal area of Pakistan is scarce. In this study sixty eight species of cyanobacteria were identified from eight marine green macroalgal species collected from Buleji, a rocky coast of Karachi. The majority of cyanobacteria were from the order Chroococcales and Nostocales and only a small number of species were found to belong to Chamaesiphonales and Pleurocapsales. The epiphytic species consists of 27 unicellular and 41 filamentous non-heterocystous cyanobacterial species. Heterocystous forms were not observed during these studies. Cyanobacterial species exhibited a variable capability to grow in a variety of media. Also, some cyanobacteria were observed specifically associated on particular macroalgal species, while other were widespread on all species.

Key-words: Buleji, Cyanobacteria, Epphytic, Marine habitat.

INTRODUCTION

Cyanobacteria occur in a variety of habitats of tropical, sub-tropical and temperate areas in both marine and freshwater environments (Sukenik *et al*., 2012; Murrell and Lores, 2004; Saker and Griffiths 2001; Hoffmann, 1999; Desikachary 1959). Endophytic cyanobacteria are known from terrestrial and freshwater environments. For example, endosymbiosis relationship has been established for water fern *Azolla* (Baker, *et al*., 2003; Stewart *et al*., 1980; Singh and Singh, 1988). Cycads (Ow *et al*.,1999; Peter *et al*., 1986; Millbank, 1974), *lichens* (Wirtz *et al*., 2003; Fogg *et al*., 1973; Rai, 1990; Desikachary, 1959) and an angiosperm *Gunnera* (Bergman and Osborne, 2002; Silvester, 1976). An endophytic cyanobacteria *Richellia intracellularis* is also reported in a microalgal species *Rhizosolenia styloformis* inhabiting in Tropical Ocean. *Richellia-Rhizosolenia* association is probably the only known endosymbiosis established from marine environment. (Madhu *et al*., 2013; Bergman *et al*., 1992). The epiphytic cyanobacterial species on marine plants are comparatively better described. Cyanbacteria has been shown associated with seagrass, *Zostera marina* (Hamisi *et al*., 2013; Pinckney and Micheli, 1998; Penhale, 1977; Penhale and Smith, 1977; Sand- Jansen, 1977), mangrove barks and pneumatophores (Naidoo *et al*., 2008; Potts, 1980), macroalgae (Carpenter, 1972; Cattaeno and Kalff, 1979, 1980; Desikachary, 1959) etc. Most of these species were from terrestrial and freshwater habitats. Comparatively a little work has been done on the occurrence of cyanobacteria associated particularly with marine plants. Most epiphytic cyanobacteria are briefly mentioned in the literature. There has been no dedicated work as regard to epiphytic cyanobacteria associated with marine algae, particularly from Pakistan. Therefore, present study was undertaken to report on the species diversity of cyanobacteria occurring as epiphytes on six species of macroalgae.

MATERIALS AND METHODS

Cyanobacterial association with marine flora

A total of 8 species of seaweeds were randomly collected from Buleji, a rocky coast of Karachi. List of the species is set out in Table (1). Seaweeds species were kept separately in polythene bags and brought to the laboratory. The seaweeds were gently washed with sterilized seawater to remove loosely attached epiphytic species and other organisms. The seaweeds were divided into different portions, for example, frond, utricle and rhizoids, each portions was separately inoculated in tubes containing three different types of media ASNIII, MN and Miquel's media and incubated under cool florescent light (12L/ 12D) at 30 ± 2°C.

All seaweeds were inoculated in triplicates and in some cases where growth was either low or unobservable experiments were repeated more rigorously. The field materials and growth obtained in culture tubes were observed under the light microscope. The taxonomic assessments were done with the help of literature (Rippka *et al*., 1979; Desikachary, 1959; Anagnostidis and Komarek, 1985, 1988, Komarek and Anagnostidis, 1986, 1989).

RESULTS

Cyanobacterial associates of macroalgal species

The study on the association of cyanobacteria with marine algae revealed that a number of species are living attached to macrophytes in nature. The study was performed on 8 green algal species (Chlorophytes)(Table 1).

However, 41 species were exclusively associated with green seaweed species. The epiphytic cyanobacteria were either unicellular form or non-heterocystous filamentous species. The heterocystous form was not observed. This research constitutes first detail report on the epiphytic cyanobacteria from Pakistani waters.

In total, 68 cyanobacteria species were identified on the green seaweeds. They were predominantly Chroococcalean (18 species) and Nostocalean (41species) (Tables 1 and 2). The highest number of species were recorded in the genus *Phormidium* (23 species), followed in the descending order of the number of representing species by genera: *Oscillatoria* (9 species), Chroococcus (8 species), *Dermocarpa* (5 species), *Spirulina* (3 species), *Lyngbea* (3 species), *Merismopedia* (3 species), *Synechocystis* (2 species), *Aphanocapsa* (2 species), and *Myxosarcina* (2 species). The genera with only one representative species were *Planktothrix, Komvophoron, Pseudoanabaena, Gloeocapsa, Synchococcus, Gloeothece, Chroococcidiopsis and Chamaecalyx* (Table 2).

Considering the individual green algal species, it may be deduce that there are some (15 species) more common cyanobacteria which associated with three or more algal species. It is also observed that group of cyanobacteria belonging to Chroococcles, Chamaesiphonales and Pleurocapsales were not associated with *Ch. Prostrata, E. intestinales, V. pachynema, C. taxifolia ,C. peltata* and *U. indica* (Table 2 and 3).

Some species of cyanobacteria appeared to have associated with only one algal species, i.e., algal species-specific association. Out of 68 species, 41 species had macroalgal species-specific association most of which observed on *E. intestinales* and *C. peltata* (9 species each). *U. indica, U. rigida, Ch. Prostrate, Bryiopsis pennata, V. pachynema* and *C. taxifolia* had 8, 5, 5, 4, 3, 2 and 2, species of cyanobacteria attached with them, respectively (Fig.1. and Table 1).

Three types of media (ASNIII, MN and Miquel's) were used to improve the chances of obtaining obtained in above three media media (Table 2). The highest numbers of species (40 species) were observed in Miquel's medium. Thirty six species were able to grow in ASNII medium and only 25 species were obtained from culture in MN medium (Table 1). Although some cyanobacteria (8 species) were able to grow in all three media (Table 1, Fig. 2.), there were many species which were observed in one type of medium. ASNIII, MN and Miquel's media showed 15, 11 and 17 species that grew in the media, respectively (Table 2 and Fig. 2.).

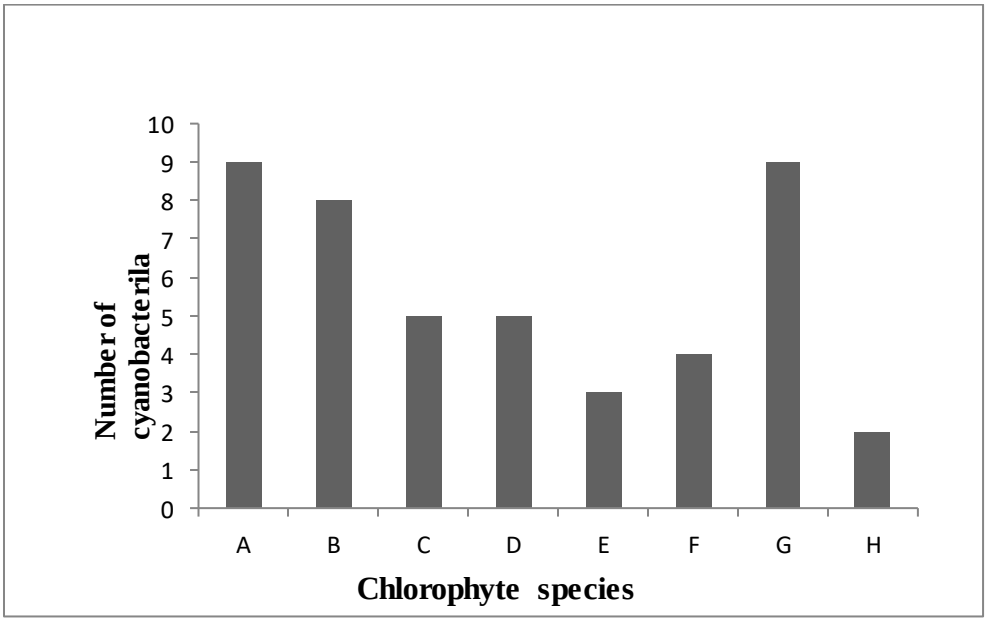

Fig. 1. Species-specific cyanobacteria associated with A- *Enteromorpha intestinalis*, B- *Ulva indica*, C- *Ulva rigida*, D- *Chaetomorpha prostata*, E- *Valoniopsis pachynema*, F- *Bryopsis pennata*, G- *Caulerpa peltata*, H- *Caulerpa taxifolia*.

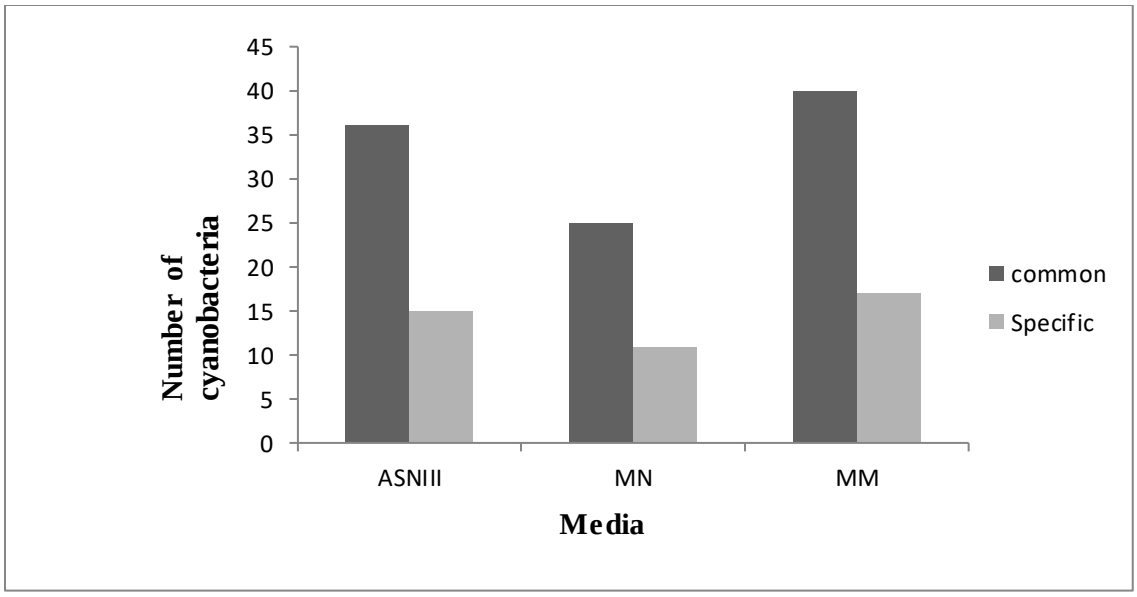

Fig. 2. Cyanobacterial species were common in all three media but several species showed up only in one specific medium.

Table 1. List of chlorophyte species studied for the epiphytic species of cyanobacteria.

Bryopsidophyceae	**Ulvophyceae**
Caulerpa peltata	*Enteromorpha intestinalis*
Caulerpa taxifolia	*Ulva indica*
	Ulva rigida
	Chaetomorpha prostata
	Valoniopsis pachynema
	Bryopsis pennata

DISCUSSION

The data presented here contributes to the regional diversity data pool, and at the same time confirms the presence of many species cyanobacteria reported from the other part of the world in the northern Arabian sea bordering Pakistan.

Only a few reports are available on epiphytic cyanobacteria from coastal zones of Pakistan. These study reports only a few species of macroalgae (Nizamuddin and Gessner, 1970; Shameel, 1987; Bano and Siddiqui, 2003) and on mangrove plants (Saifullah and Taj, 1995; Siddiqui and Bano, 2001).

Although its importance in the aquatic ecosystem, marine cyanobacteria has not been studied in detail. The growth of cyanobacteria on the group of macroalgae may suggest its possible role in the physiology of the algal species (Sellner, 1997; Phlips and Zeman, 1990). The epiphytic cyanobacterial relationship may be symbiotic relationship. It has previously been reported that an exchange of carbon and nitrogen compounds exist between epiphytic cyanobacteria and the marine plants (Mohamed and Shehri, 2010; Vahtera, *et al*., 2007; Murakami *et al*., 2004; Toledo *et al*., 1995; Moisander *et al*., 2005; Harlin and Craigie, 1975; Wetzel and Penhale, 1979; Penhale and Thayer, 1980; Harlin, 1973; McRoy and Georing 1974). For example, diazotrophic epiphytes have been reported from a few marine algae (Gómez, *et al*., 2005; Toledo *et al*., 1995; Capone, 1977; Capone *et al*., 1977; Carpenter, 1972; Hansen, 1977; Head and Carpenter, 1975).

Some cyanobacterial species encountered in the study as epiphytes are also present in many other niches of the rocky shore and are also reported from other habitats as well (Desikachary, 1959; Thajuddin and Subramanian, 1992, 1994; Santra and Pal 1988; Santra *et al*., 1988). These organisms therefore, appeared to be cosmopolitan and

marine plant surface may simply be a substrate for attachment (Sukenik *et al*., 2012; Verspagen *et al*., 2005; Furey, 2003). However, there are some species of cyanobacteria found exclusively on macroalgae, and hence suggested a possibility of the existence of a symbiotic relationship (Lesser *et al*., 2004). Some variation in the diversity of species associated with different seaweed species, suggest that there may be a specific relationship between some macroalgae and cyanobacteria (Thacker and Starnes, 2003; Thacker *et al*., 2001).

Table 2. Distribution of cyanobacterial species on eight different species of chlorophytes inhabiting a rocky shore of Buleji near Karachi.

		Macroalgal Species							
Cyanobacterial species	**Media***	***Enteromorpha intestinalis***	***Ulva indica***	***Ulva rigida***	***Chaetomorpha prostata***	***Valoniopsis pachynema***	***Bryopsis pennata***	***Caulerpa peltata***	***Caulerpa taxifolia***
Chroococcales									
Synechocystis pevalekii	ASNIII, MN	-	+	+	-	-	-	+	-
S. aquatilis	MM	-	-	-	-	-	-	+	-
Gloeocapsa cripidinum	ASNIII	-	-	-	-	-	+	-	-
Chroococcus cohaerence	ASNIII, MN, MM	+	-	+		+	-	-	-
C. turgidus	ASNIII, MM	-	-	-	-	-	-	+	+
C. minor	MM	-	+	+	-	-	-	-	-
C. montanus	ASNIII	+	-	-	-	-	-	-	-
C. tenax	ASNIII, MM	-	-	-	-	-	-	+	+
C. hansgirgi	ASNIII	-	-	-	-	-	-	+	-
C. dispersus	MM	-	-	-	-	-	-	-	+
C. pallidus	ASNIII	+	-	-	-	-	-	-	-
Synechococcus elongatus	ASNIII, MM	-	+	+	-	-	-	-	+
Aphanocapsa littoralis	MN	-	+	-	-	-	-	-	-
A. rivulais	MN	-	+	-	-	-	-	-	-
Gloeothece rhodochlmys	ASNIII, MN	-		+	-	-	-	-	-
Merismopedia elegans	ASNIII, MN	-	+	+	-	-	+	+	-
M. punctata	MN	-	+	-	-	-	-	-	-
M. tenuissima	ASNIII	+	-	-	-	-	-	-	-
Chamaesiphonales									
Chrroococidiopsis indica	MM	-	-	-	-	-	-	-	+
Dermocarpa leibleiniae	ASNIII, MN, MM	-	+	+	+	-	+	-	-
D. olivacea	MM	-	-	+	-	-	-	-	-
D. versicolor	MN	-	+	-	-	-	-	-	-
D. clavata	MN	-	-	+	-	-	+	-	-
D. sphaerica	MN	-	-	+	-	-	-	-	-
Chaemaecalyx swirrenkoi	MN	-	-	+	-	-	-	-	-
Pleurocapsales		-	-	-	-	-	-	-	-
Myxosarcina spectabilis	MN, MM	+	-	+	-	-	+	-	-

M. burmensis	ASNIII, MM	-	-	-	-	-	+	-	-
Nostocales									
Komvophoron minutum	ASNIII	-	+	-	-	-	-	-	-
Psuedoanabaena catenata	ASNIII, MN, MM	-	+	+	-	-	+	-	-
Lyngbea nordgarddhii	ASNIII, MM	+	-	-	-	-	-	-	-
L. lagerheimi	MN	-	+	-	-	-	-	-	-
L. chlorospira	ASNIII, MM	-	+	-	-	+	-	-	-
Oscillatoria limnetica	ASNIII, MM	+	-	-	-	-	-	-	-
O.schultzii	MM	-	-	-	+	-	-	-	-
O. fremyii	MM	+	-	-	-	-	-	-	-
O. guttulata	MM	+	-	-	-	-	-	-	-
O. splendida	MM	+	-	-	-	-	-	-	-
O. pseudogaminata	ASNIII, MN, MM	-	+	+	+	+	+	-	-
O.wellei	MN	-	-	-	+	-	-	-	-
O. proteus	ASNIII	-	-	-	+	-	-	-	-
O. deflexa	MM	-	-	-	+	-	-	-	-
Spirulina labyrinthiformis	ASNIII, MN, MM	+	+	-	-	-	+	+	-
S. subsalsa	ASNIII	-	-	-	-	-	-	+	-
S. major	MN	-	-	-	-	-	-	+	-
Planktothrix clathrata	MM	+	-	-	-	-	-	-	-
Phormidiumpurpurascence	ASNIII, MN, MM	-	-	-	-	-	+	+	+
P.laminosum	ASNIII, MM	-	-	-	-	+		+	+
P. tenue	ASNIII, MN, MM	-	-	-	-	+	+	+	-
P. insigne	ASNIII	-	-	+	+	-	-	-	-
P. angustissimum	ASNIII, MM	-	-	-	+	+	-	+	-
P. acutissimum	MM	-	-	-	-	+	-		-
P. ambiguum	ASNIII	-	-	-	+	-	-	+	-
P. africanum	MN, MM	-	+	-	-	-	-	+	-
P. amplivaginatum	MM	-	-	-	-	+	-	-	-
P. fragile	ASNIII, MN, MM	+	-	+	-	+	+	-	-
P. inudatum	ASNIII, MM	-	-	-	-	-	-	+	-
P. incrustatum	ASNIII	-	+	-	-	-	-	-	-
P. retzii	MM	-	-	-	-	-	-	+	+
P. mucicola	MM	-	-	+	-	-	-	-	-
P. luteum	ASNIII	-	+	-	-	-	-	-	-
P. papyraceum	MN, MM	-	-	-	-	+	+	-	-
P. valdrrianum	MN, MM	-	-	+	-	+	-	+	-
P. jadianianum	MM	-	-	-	-	-	-	+	-
P. subincrustatum	ASNIII	-	-	-	-	-	-	+	-
P. anamola	ASNIII	-	-	-	-	-	-	+	-
P. luridum	ASNIII	-	-	-	-	-	-	+	-
P. laetevirens	MM	-	-	-	+	-	-	-	-
P. rimosum	MN	-	-	-	-	-	+	-	-

*ASNIII, MN (Rippka et al 1979), MM (Imai 1977)

Table 3. Total number of species observed on surface of chlorophyte species. Species are also grouped according to their taxonomic orders.

Cyanobacterial orders	*Enteromorpha intestinalis*	*Ulva indica*	*Ulva rigida*	*Chaetomorpha prostate*	*Valoniopsis pachynema*	*Bryopsis pennata*	*Caulerpa peltata*	*Caulerpa taxifolia*
Chroococcales	4	7	6	-	1	2	6	4
Chamaesiphonales	-	2	5	1	-	2	-	1
Pleurocapsales	1	-	1	-	-	2	-	-
Nostocales	8	9	6	9	10	8	16	3
Total	13	18	18	10	11	14	22	8

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