

A Preliminary Identification of Cyanophyta/Cyanobacteria in the brackish milkfish ponds of Marakei, Nikunau and Kiritimati Atolls, Republic of Kiribati

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ABSTRACT

Common cyanophyta/cyanobacteria from the milkfish ponds of Marakei, Nikunau and Kiritimati atolls are species of *Lyngbya* and *Spirulina*. Those found on Marakei and Nikunau only include *Chroococcus minutus*, and species of *Microcystis*, *Oscillatoria* and *Spirulina*. *Chroococcus minor* and *Chroococcus sp.* were identified from Marakei, Nikunau and Kiritimati, respectively. *Phormidium muricola* (associated with *Microcystis aeruginosa*) and *Phormidium sp.* are found on Marakei and Kiritimati, respectively. Those found on Marakei only include *Anabaena*, *Coelosphaerium kuetzingianum* (dominant), *Microcystis aeruginosa* (co-dominant) and *Merismopedia*. The taxa found on Nikunau only include *Dactylococcopsis*, *Nodularia* and *Raphidiopsis*. Those found on Kiritimati only include *Chlorogloea* (dominant) with co-dominants *Aphanothece*, *Johannesbaptistia*, *Chroococcidiopsis*, *Dunaliella*, *Coccochloris* and *Leptolyngbya*.

1 INTRODUCTION

'*Te Bokaboka*', literally 'mud' was researched in 2003 (Maata, *et al.*, 2003) for various cyanobacterial species presence and nutritional components. Follow-up visits were made in 2005 by Tebano (2005) to Beru and Marakei atolls to determine the potential of 'micro-algae' culture in the Gilbert Group. In late 2006, Tebano revisited Marakei, then Nikunau and Kiritimati to quantify the biomass of detrital scum in the ponds and determine which cyanophyta/cyanobacteria have potential for culture in atoll conditions.

A clear distinction between '*te bokaboka*' and '*meritaua*' needs to be made. The former refers to the remains of all dead plants and animals blended with sand and mud giving off a strong sulphide odour. The latter refers to living cyanobacteria on which milkfish and other organisms feed. This paper discusses the types of '*meritaua*' or cyanophyta/cyanobacteria collected and identified from the Gilbert Group atolls of Marakei (northern island) and Nikunau (southern island), and Kiritimati in the Line and Phoenix Group (Figs. 1a, b, c and d).

Because they are photosynthetic and aquatic, cyanobacteria are often called 'blue-green algae', but this does not reflect any relationship between the cyanobacteria and algae. Cyanobacteria are prokaryotic relatives of the bacteria and it is only the chloroplast in eukaryotic algae to which the cyanobacteria are linked (ucmp.berkeley.edu/bacteria/cyanointro.html). Many strains of cyanobacteria exist world-wide, even in extreme habitats such as hot and cold desert conditions. A classical example is *Chroococcidiopsis sp.* (Pentecost, 2003). Some cyanobacteria, for example, *Lyngbya* C.A. Agardh ex Gormont, are considered toxic in some areas but not in others; they also have medicinal uses and research for the cure of HIV/AIDS using specific strains of *Lyngbya* is ongoing (Burja, 2004).

Most water based poisonings of cyanobacteria occur when heavy surface growths or scums accumulate near shorelines of lakes, ponds and reservoirs where animals have easy access to toxic levels of cells (Campbell, *et al.*, 1997). *Spirulina* Turpin ex Gormont and *Phormidium* Kuetzing ex Gormont are very high in nutritional value and with the less nutritious *Calothrix*, *Chlorella* and

Lyngbya, are being cultured in many parts of the world for human food, animal feed, medicine and cosmetics (Liang, *et al.*, 2004; Nakargar, *et al.*, 2004; Shimamatsu, 2004).

The Republic of Kiribati comprises three island groups, the Gilbert Islands, the Phoenix Islands and the Line Islands (Fig. 1a). The islands stretch for about 4,000 km from east to west and span both the equator and 108th meridian, extending from 8°N to 13°30'S, and from 168°E to 147°W. The Gilbert Islands consists of sixteen atolls or limestone islets extending 640 km from north to south and located 700 km to east of Nauru, they lie astride the Equator between approximately between 172°E and 173°E longitude (Thaman and Tebano, 1994) to include Marakei and Nikunau atolls (Figs. 1b and c). Kiritimati is in the Line Islands (Fig. 1d). Pond names as study sites for each island are given in the Tables of Results.

2 MATERIALS AND METHODS

2.1 MICRO-ALGAL SAMPLES COLLECTION

The cyanobacteria samples were collected from ponds and placed in plastic bags, the first samples were collected from the undisturbed top layer of the scum, second samples were collected from the middle layer; all were taken to the laboratory, and transferred to small vials and fixed with a 10% formalin in seawater. Duplicates of fresh samples were used for further observations. Formalin fixed samples were used for later work and safe keeping. Permanent slides were made with the application of iodine solution for fixing, cover slips put on top of slides and sealed with clear nail varnish. Sun-dried samples were collected in plastic bags for further analysis.

2.2 MICROSCOPY

Fresh specimens were observed under both stereo and compound microscopes. Where possible the highest resolution was used to determine the identification to genus or species level where physical characteristics were visibly distinct. Permanent mounts on slides were sent to algal taxonomists for confirmation.

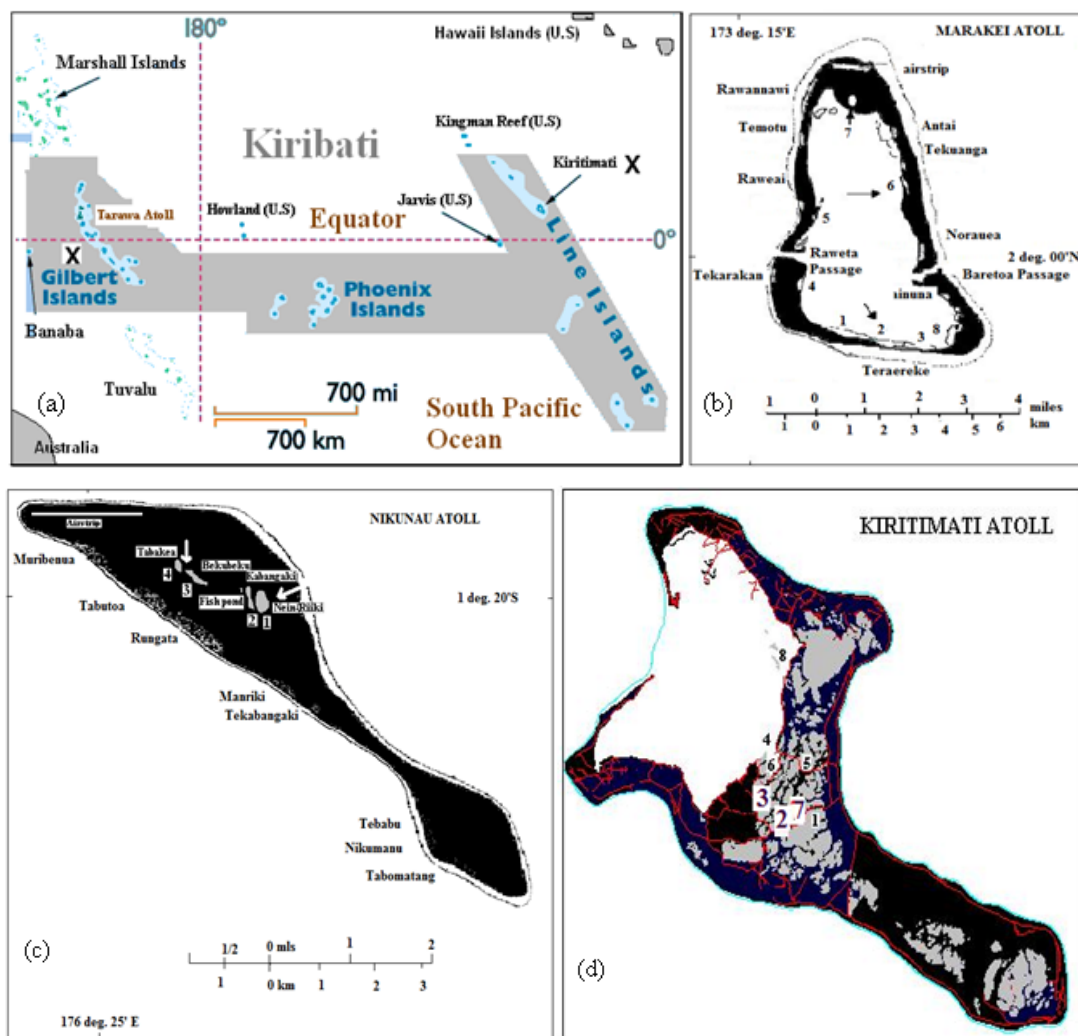


Figure 1 Sampling sites

2.3 SALINITY AND TEMPERATURE

Two water samples were taken from each pond; one sample was taken from mid-pond water about 50 cm below the surface, the second sample was taken below the water surface from the edge of a pond; three salinity readings were taken from each water sample with pure water washings on the ATAGO S-28 Hand Refractometer sample face after each reading. A mean reading for the two samples was calculated. Temperature was taken from the most accessible inner portions of a pond of approximately one meter deep. Three readings were made each time using a portable spectrophotometer and double checked with a hand thermometer. A mean reading was computed.

2.4 MAPS

Large clear maps of each island were obtained from the Lands Department in Tarawa and Kiritimati islands. Study and sampling sites are indicated as shown on the maps above (Figs. 1 b, c, and d).

2.5 IDENTIFICATION SOURCES

1. Etheredge, M.K and Pridmore, R.D., 1987. The freshwater planktonic Blue-Green (Cyanophyta/Cyanobacteria) of New Zealand: A taxonomic guide. Water & Soil Miscellaneous Publication No. 111, Wellington.
2. Mr. Karl Safi MSc, a microalgal taxonomist, Auckland University, New Zealand.
3. Numerous sources from Internet searches.

3 RESULTS

The cyanobacteria found in the brackish water ponds of Marakei Atoll include *Chroococcus minutus* (Kützinger) Nageli, *Chroococcus minor* (Kützinger) Nageli, *Lyngbya* C.A Agardh ex Gormont, *Microcystis* Lermmermann (dominant), *Oscillatoria* Vaucher ex Gormont, *Phormidium muricola* Hüber-Pestalozzi (associated with *Microcystis aeruginosa*) (Kützinger) Kuzing and *Spirulina* Turpin ex Gormont (dominant). No cyanobacteria were found in the freshwater pond of Rokinaba and at the seawater pond of Bainuna. Those taxa found on Nikunau Atoll include

Chroococcus minutus, *Chroococcus* Nageli, *Dactylococcopsis* Hansgirg, *Lyngbya*, *Microcystis*, *Nodularia* Martens ex Bornet and Flahault, *Oscillatoria*, *Raphidiopsis* Fritsch and Rich and *Spirulina*, all equally distributed. The species from Kiritimati include *Aphanothece* Nageli (co-dominant), *Chlorogloea* (dominant), *Chroococcidiopsis*, *Chroococcus*, *Johannesbaptistia*, *Leptolyngbya perelegans*, Lemmermann, *Lyngbya*, *Spirulina* and *Phormidium* Kuetzing ex Gormont.

Results show that species number is salinity-dependent. Very high concentration of salt in water allows few species of micro-algae to survive. Very low concentration of salt is tolerated by few species. Many species thrive in brackish to moderately salty ponds. A few eukaryote Chlorophyceae, e.g. *Dunaliella* sp. thrives in high-salinity environments but most do not tolerate the high acidic nature of coral atoll lakes (Ben-Amotz A. 2004).

Where salinity approaches sea salt and fresh water the species diversity was sharply reduced as in the case of Kiritimati. But for Marakei and Nikunau where ponds are filled with brackish water through gravel and coarse sand seepage (around 10 ppt) the species are quite diverse. The inference could be that a gelatinous scum of dead cyanobacteria, that can be more than one metre thick, may act as a partitioning interface separating fresh water at the surface from the brackish water below.

Both Marakei and Nikunau (Tables 1, 2) share more species compared with either one pooled with Kiritimati (Table 3). Pond salt concentrations in the former two atolls are far less than Kiritimati ponds. In Kiritimati, *Chlorogloea* and *Aphanothece* are dominant and co-dominant, respectively. The former is found in most ponds in either green or red coloration. It is assumed that it also exists in small amounts in the milkfish ponds at Marakei and Nikunau atolls.

Table 1 Sampling and study sites on Marakei Island (see Fig. 1b for locations)

Pond #	Length (m)	Width (m)	Water temperature (°C)	Water depth middle(cm)	Salinity (ppt)	Algal taxa identified
1. Tamwaiti	1,200	250	29	30	13	<i>Oscillatoria</i> sp., <i>Microcystis aeruginosa</i> (Kützing) Kützing, <i>Spirulina</i> sp., <i>Phormidium muricola</i> Hüber-Pestalozzi associated with <i>Microcystis</i> sp.
2. Tamwaiti	800	250	26	35	15	<i>Microcystis aeruginosa</i>
3. Teraereke (unknown)	800	400	27	10	24	<i>Oscillatoria</i> sp., <i>Lyngbya bipunctata</i> Lemmermann, <i>Anabaena</i> sp., <i>Coelosphaerium kuetzingianum</i> Nageli (dominant), <i>Microcystis aeruginosa</i> , <i>Merismopedia</i> sp., <i>Chroococcus minutus</i> (Kützing) Nageli, <i>Chroococcus minor</i> (Kützing) Nageli, <i>Spirulina major</i> Kützing, unidentified blue-green alga
4. Uabong	50 m (diameter)		40	60 (thin water on top of mud)	13	<i>Oscillatoria</i> sp., <i>Anabaena</i> sp., <i>Spirulina major</i> Kützing
5. Tekuanga (Bareatau)	800	00	35	20	24	<i>Microcystis elabens</i> – <i>Aphanothece elabens</i> (Brébisson) Elenkin (dominant), <i>Lyngbya</i> sp., <i>Chroococcus</i> sp., <i>Oscillatoria</i> sp.
6. Rokinaba	200	60	35	40	0 fresh-water	None
7. Bainuna	1,100	100	35	40	32	None

Table 2 Sampling and study sites on Nikunau Island (see Fig. 1c for locations).

Pond #	Length (m)	Width (m)	Water temperature (°C)	Water depth middle(cm)	Salinity (ppt)	Algal taxa identified
1. Nein Riiki	500	425	35	40+	12	<i>Chroococcus minutus</i> , <i>Chroococcus minor</i> , <i>C. dispersus</i> (Keissler) Lemmermann, <i>Chroococcus</i> sp., <i>Lyngbya limnetica</i> , <i>Microcystis aeruginosa</i> , <i>Nodularia</i> sp., <i>Raphidiopsis</i> sp., <i>Spirulina major</i> , unidentified
2. Kabangaki	600	100	36	35	12	<i>Chroococcus minutus</i> , <i>Chroococcus</i> sp., <i>Lyngbya limnetica</i> , <i>Microcystis aeruginosa</i> , <i>Nodularia</i> sp., <i>Raphidiopsis</i> sp., <i>Spirulina major</i> (dominant).
3. Bekubeku	700	250	35	1.5+	6	<i>Chroococcus</i> sp., <i>Dactylococcopsis</i> sp., <i>Lyngbya</i> sp., <i>Microcystis</i> sp., <i>Oscillatoria</i> sp., <i>Raphidiopsis</i> sp., many unidentified
4. Tabakea	330	250	35	1.5+	6	<i>Chroococcus</i> sp., <i>Dactylococcopsis</i> sp., <i>Lyngbya</i> sp., <i>Microcystis</i> sp., <i>Oscillatoria</i> sp., <i>Raphidiopsis</i> sp.

Table 3 Sampling and study sites on Kiritimati Island (refer to Fig. 1d for pond locations)

Pond #	Length (m)	Width (m)	Water temperature (°C)	Water depth middle(cm)	Salinity (ppt)	Algal taxa identified
1. Te Nii Uakai	4,000	1,250	30.9	4.1	105	Green gel-like mats with <i>Chlorogloea</i> (dominant) or <i>Chroococciopsis</i> sp.; <i>Chroococcus</i> sp. (co-dominant), <i>Aphanothece</i> sp. less dominant). <i>Phormidium</i> and <i>Spirulina</i> sp. (occasional); clear sheats of <i>Lyngbya</i> sp. present in all samples.
2. Te Tang Uoua	1,750	750	32.6	2.1	190	Green gel-like mats with <i>Chlorogloea</i> (dominant) or <i>Chroococciopsis</i> sp.; <i>Chroococcus</i> sp. (co-dominant), <i>Aphanothece</i> sp. less dominant). <i>Phormidium</i> and <i>Spirulina</i> sp. (occasional); clear sheats of <i>Lyngbya</i> sp. present in all samples.
3. Tonga	1,250	750	30.2	2.4	115	<i>Chlorogloea</i> sp. (dominant); <i>Aphanothece</i> sp. (co-dominant); <i>Leptolyngbya</i> , <i>Chroococcus</i> sp. (rare); <i>Lyngbya</i> sp., <i>Phormidium</i> (occasional).
4. Fred	750	500	31	2.2	125	Green gel-like mats with <i>Chlorogloea</i> (dominant) or <i>Chroococciopsis</i> sp.; <i>Chroococcus</i> sp. (co-dominant), <i>Aphanothece</i> sp. less dominant). <i>Phormidium</i> and <i>Spirulina</i> sp. (occasional); clear sheats of <i>Lyngbya</i> sp. present in all samples.
5. Y-Side	2,000	500	30.8	3	45	<i>Chlorogloea</i> sp. (dominant); <i>Aphanothece</i> sp. (co-dominant); <i>Leptolyngbya</i> , <i>Chroococcus</i> sp. (rare); <i>Lyngbya</i> sp., <i>Phormidium</i> sp., <i>Johannesbaptista</i> sp. (occasional).

4 DISCUSSION

Preliminary work on cyanobacteria from the ponds of Kiritimati Atoll indicated that high and supersaturated salt water prohibits the growth of many organisms. *Chlorogloea* is dominant while *Aphanothece* is co-dominant in these ponds. Conversely, the ponds in Marakei and Nikunau are brackish and less salty than

lagoon water. The latter is around 34 parts per thousand (ppt). About half a dozen of cyanobacteria strains were common amongst these islands, including Beru, more are yet to be identified. Salt concentration determines the number of organisms growing in such environments. For example, Pentecost (2003) described some species of *Oscillatoria* and other cyanobacteria that flourish in hot spring travertines. Similarly, Billi, *et al.* (2001) researched

on gene transfer from a desiccation tolerant cyanobacterium *Chroococcidiopsis* that dominates microbial communities in the most extreme arid and cold deserts around the world. These support the fact that mass production of some selected strains of cyanobacteria suitable for a given environmental condition is possible, for example, *Spirulina* production in Chiba, Japan (Shimamatsu, 2004).

Toxic cyanobacteria such as *Lyngbya*, one of the neurotoxic anatoxins producing cyanobacteria, are present in the ponds of Marakei and Nikunau, as well as *L. perelegans* in the Beru ponds (Maata, *et al.*, 2003), yet the locals of the latter island have been eating cyanobacteria for a long time without any reported ill effects. *Lyngbya*, however, appears to be occurring worldwide as Maata, *et al.* (2003) postulated. Listed with *Lyngbya* as neurotoxin producers are *Anabaena* and *Oscillatoria*, both are present in the ponds of Marakei and Nikunau but were not among those species identified from Beru Atoll. Swimmers' itch from seawater or brackish or super saturated salt water is caused by cyanobacteria such as these and many others. Symptoms include burning and itching skin, red lips, sore eyes, sore throat, headache and dizziness (Codd, *et al.*, 1989).

Similarly, *Microcystis* sp., but *M. aeruginosa* in particular, together with *Nodularia* sp. are known to synthesize hepatocytes destructive to liver cells (ucmp.berkeley.edu/bacteria/cyanointro.html), were identified from Marakei and Nikunau atolls. *Microcystis orissica* was reported to exist in Beru ponds (Maata, *et al.*, 2003). More work is needed to fully determine the chemical composition of these organisms in light of the ongoing consumption of cyanobacteria from the ponds of Beru Atoll. A health survey on the effect of cyanobacteria consumption would be a crucial component of any further research.

Spirulina and *Phormidium*, both found on Marakei and Nikunau, are very high in nutritional value with the lesser nutritious of *Calothrix* (Liang, *et al.*, 2004; Nakargar, *et al.*, 2004; Shimamatsu, 2004). The former is present on Beru as well (Maata, *et al.*, 2003). These are being cultured to improve nutrition for humans in many parts of the world, for example, Japan and India. Substantive consideration needs to be taken for their culture and ultimate utilization in light of poor nutrition epidemics affecting the populace of Kiribati and some neighboring countries.

One of the cyanobacteria species identified from guts of milkfish caught from the Fisheries ponds on Kiritimati Atoll is *Coccochloris* sp. Maata, *et al.* (2003) reported two species from Beru as *C. stagnina* and *C. peniocystis*. These were not identified or overlooked in the samples collected from Marakei and Nikunau ponds. Milkfish samples from the two islands were not available either; assuming that milkfish were plentiful before the introduction of tilapia in those ponds, at least one of the cyanobacteria species must also be present.

Kiritimati residents claimed that the freshly consumed cyanobacteria found in the guts of milkfish sampled, presumably *Coccochloris*, is edible. The processed and partly processed gut contents are exuded and the fresh ones are retained, cooked with the fish, or consumed separately. No ill effects have been reported from Kiritimati or Beru.

The light detrital scum on the surface is dark to light greenish in color. The bright pinkish-brown layer occupies the middle layer. More work is required in identifying the organisms as well as proper analysis on the toxins and nutritional components of the detrital scum from these islands.

All ponds surveyed are filling up with scum, most probably from decades ago, with little water column for fish to swim around. For Kiritimati large ponds, there is a vast water body with thick scum along the pond perimeter and shallow areas. The potential of the scum in terms of agricultural, medicinal and animal feed needs to be explored further in light of the ailing economy of Kiribati and its neighboring countries in the South Pacific. There is obviously a need to confirm the identity of all taxa reported here.

5 CONCLUSIONS

The micro-algae studied showed that very high or very low salt concentrations are tolerated by few cyanophyta/cyanobacteria present in the islands studied. There is potential for culture of some commonly cultured species as in other parts of the world however this requires more thorough studies.

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