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Cyanobacteria-dominated biofilms: a high quality food resource for intertidal grazers

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Abstract

Hong Kong rocky shores are dominated by cyanobacterial biofilms composed of a diversity of species. Thirteen common species, belonging to seven genera, were isolated in pure culture in MN+ and MN− media under defined growth conditions from a semi-exposed shore in Hong Kong. The nutritional values (i.e., protein, carbohydrate and calorific value) of these 13 species were determined. All species showed high nutritional quality in terms of protein, carbohydrate and calorific value, however, overall nutritional value varied between the species. Species of *Spirulina* and *Phormidium* were most nutritious (highest nutritional values) whereas species of *Calothrix* and *Lyngbya* were the least nutritious. Microphagous molluscan grazer density and diversity were relatively high at the study site, despite the seemingly low biomass (as assessed by chlorophyll *a* concentration) of the biofilm. It is suggested that the high nutritional quality of cyanobacteria, together with their fast turnover rates can support high levels of secondary production (biomass of grazers). The high nutritional quality of cyanobacteria on tropical, cyanobacteria-dominated, rocky shores is therefore of great importance in the benthic food web.

Introduction

Intertidal, epilithic biofilms are 3-dimensional structures, mainly composed of bacteria, cyanobacteria, diatoms, microalgae, protozoa and spores and sporelings of macroalgae embedded in a mucopolysaccharide matrix (Anderson, 1995). Rocky shores around the world are typically covered with these biofilms throughout the year. Temperate rocky shore biofilms are dominated by diatoms or cyanobacteria (MacLulich, 1987; Hill & Hawkins, 1991; Thompson et al., 1996). On tropical rocky shores, in contrast, the biofilms are mainly composed of cyanobacteria with only sporadic patches of bacteria, diatoms, protozoa and juvenile stages of macroalgae (Potts, 1980; Whitton & Potts, 1982; Nagarkar & Williams, 1999). Cyanobacteria are important primary producers, many species of which are able to fix atmospheric nitrogen (Stewart, 1973; Whitton & Potts, 1982). Cyanobacterial biofilms, therefore, form the energy base of the benthic food web and are very important in terms of overall productivity and community organization on tropical rocky shores (Nagarkar, 1996).

Although the importance of cyanobacteria as a food source for microphagous grazers such as intertidal molluscs (Foster, 1964; Raffaelli, 1985; Quinn, 1988) and zooplankton (Schmidt & Jónasdóttir, 1997; Repka, 1998) has long been realized, little attention has been paid to the nutritional quality of cyanobacteria (but see Ahlgren et al., 1992; Kaehler & Kennish, 1996). Many studies have found planktonic and benthic cyanobacteria to be a poor food source due to their nutritional inadequacy and toxicity (Lampert, 1987; Ahlgren et al., 1990; Thacker et al., 1997), but no such information is available for intertidal, epilithic cyanobacteria (but see Nicotri, 1977). The main reason for this nutritional inadequacy of cyanobacteria is the absence of long-chain, polyunsaturated, fatty acids (PUFA), which are known to be essential components of zooplankton diets and are an indicator of

high nutritional quality (Jónasdóttir & Kiørboe, 1996). In contrast, no information is available on the nutritional requirements of intertidal molluscs' diet. The importance of cyanobacteria as a high quality food source, however, is mainly based on their protein content and the presence of essential amino acids, which has become a focus of biotechnological exploitation of cyanobacteria (Venkataraman, 1993). Chemical screening of many laboratory grown, commercially viable, marine cyanobacteria has revealed that they have a high nutritional value, especially in terms of protein (Venkataraman, 1993). The nutritional value of ecologically significant cyanobacteria has, however, received little attention.

Hong Kong rocky shores are dominated by cyanobacterial biofilms throughout the year (Nagarkar & Williams, 1999). During winter, macroalgal growth is common on Hong Kong shores (Kaehler & Williams, 1996). Several rocky shores, however, remain free of macroalgae and are only covered with a cyanobacterial biofilm (Nagarkar, pers. obs.). On visual observation, these shores appear bare, but chlorophyll *a* and microscopic analyses reveal the presence of these cyanobacteria-dominated biofilms (Williams, 1994; Nagarkar & Williams, 1997). Cyanobacteria are the only available food source on these shores which support a wide variety of microphagous grazers (Williams, 1993; Nagarkar, 1996). No information is available, however, on the nutritional quality of these cyanobacteria (except for one species, *Kyrtuthrix maculans*, Umezaki, 1996; Kaehler & Kennish, 1996) to their molluscan grazers. This paper, therefore, presents preliminary work on nutritional quality (i.e., protein, carbohydrate and calorific value) of cyanobacteria present naturally in the intertidal, epilithic biofilm on rocky shores in Hong Kong.

Materials and methods

Study site

The study site was an ~100 m long, gently slopping granodiorite heterogeneous rock platform, with few crevices, on a semi-exposed rocky shore at Mo Tat Wan (22° 13′ N, 114° 10′ E), Lamma Island, Hong Kong. This site was located in a remote area far from anthropogenic activities and was relatively clean compared to other polluted shores. Absence of visually conspicuous macroalgae and thick biofilm at this site resulted in a bare appearance of the shore. This site, however, supports a wide variety and high density of intertidal grazers such as limpets, *Cellana grata* Gould, *Cellana toreuma* Reeve, the chiton *Acanthopleura japonica* Lischke and topshell *Monodonta labio* Linné (Nagarkar, pers. obs.).

Isolation and purification of cyanobacteria

Samples were collected during March 1999, from the intertidal zone between 1.25 m and 2.00 m above Chart Datum. Rock chips (~2 cm^2) with firmly attached cyanobacterial growth were removed using a hammer and chisel and thin biofilms were scraped from the rock with a single-sided razor blade. All the samples were transferred into plastic vials with a few drops of seawater. In the laboratory, within 6 h, each sample was divided into four parts which were inoculated into liquid MN+, MN−, ASN III+ and ASN III− media (Rippka et al., 1979). To support the growth of nitrogen fixing cyanobacteria, $NaNO_3$ was omitted from the MN− and ASN III− media. In all the media, 50 $\mu g\ ml^{-1}$ cycloheximide (antibiotic) was added to inhibit eukaryotic growth especially macroalgal spores and microalgae. Bacterial growth was reduced by adding 25 $\mu g\ ml^{-1}$ ampicillin and 15 $\mu g\ ml^{-1}$ tetracycline. Samples were incubated at 25 °C in continuous low white fluorescent, diffuse light (<1000 Lux) for about seven days. Later, light intensity was gradually increased to 1200–1500 Lux. After 3 weeks, cyanobacterial growth was observed in the respective media. Individual filaments or cells were isolated using a long neck glass capillary pipette under a dissecting microscope (Leitz Diaplan, U.S.A.) and inoculated into respective fresh liquid MN and ASN III (+ or −) media; incubated again for another 3–4 weeks. Pure cultures were obtained by repeating this procedure several times.

Nutritional quality

Thirteen ecologically common cyanobacterial species which were isolated in pure culture were selected for nutritional analysis (i.e., protein, carbohydrate and calorific value). Qualitative microscopic observations, based on the number of filaments or cells under each microscopic field of view at a fixed magnification (×40), revealed that these 13 cyanobacterial species were abundant at the study site. To investigate the nutritional quality of laboratory cultured cyanobacteria, all the non-heterocystous species (absence of heterocyst, a special cell which performs nitrogen fixation)

were maintained in MN+ media and heterocystous species (presence of heterocyst) in MN− media. Cyanobacterial cells were harvested at the log growth phase (rapid cell multiplication stage) by centrifuging at 10 000 rpm for 10 min. The resultant cyanobacterial pellet was rinsed with distilled water several times and blotted on blotting paper, air dried over night, ground into a powder and then extracted for analysis.

Protein estimation

Protein was estimated by the Coomassie Brilliant Blue (Bio-Rad Laboratories) dye-binding method of Bradford (1976). One millilitre of 0.5 M NaOH was added to 20 mg of dried powder of each species, incubated for 10 min in a water bath at 80 °C and then centrifuged at 3000 rpm for 10 min. The supernatant was removed, to which 4 ml of distilled water was added and then 1 ml of the reagent Coomassie Brilliant Blue and finally mixed with a vortex mixer. The absorbence of the sample was measured immediately using a Pye Unicam spectrophotometer at 595 nm. A similar procedure was repeated for the solid residue and the absorbance of the supernatant was again measured at 595 nm. Protein was estimated by comparison with a standard bovine serum-albumin curve (Kochert, 1978).

Carbohydrate estimation

Carbohydrate was estimated using the phenol sulphuric acid method (Dubois et al., 1956). Ten milligrams of sample were mixed with 3 ml of distilled water, added to 50 μl of 90% phenol, mixed and then finally 5 ml of concentrated H_2SO_4 was added onto the surface of the liquid. Treated samples were placed in a water bath at 60 °C for 30 min and then centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was measured spectrophotometrically at 485 nm. Carbohydrate was measured by comparison with a standard curve prepared using glycogen (Sigma Chemicals).

Calorific value

To estimate calorific value of each cyanobacterial species, dry powder (800 mg) of each species was combusted in a Parr 1261 semi-micro isoperibol bomb calorimeter, calibrated using benzoic acid.

Data analysis

To investigate trends in the nutritional value of the 13 cyanobacterial species, Principal Component Analysis (PCA) was performed on the appropriate correlation matrix (MVSP, Ver. 3.01, 1998, Kovach Computing Services, Wales).

Results

Thriteen cyanobacterial species (*Calothrix contarenii* (Zanard.) Born. *ex* Flah., *Calothrix crustacea* Thuret, *Gloeocapsa crepidinum* Thuret, *Lyngbya martensiana* Menegh. *ex* Gomont, *Lyngbya semiplena* (C. Ag.) J. Ag. *ex* Gomont, *Oscillatoria formosa* Bory ex Gomont, *Oscillatoria salina* Biswas, *Oscillatoria subbrevis* Schmidle, *Phormidium corium* (Ag.) Gomont, *Phormidium tenue* (Menegh.) Gomont, *Spirulina labyrinthiformis* (Menegh.) Gomont, *Spirulina subsalsa* Oerst. *ex* Gomont and *Synechococcus* sp.) were isolated in pure culture. Of the 13 species, only two were unicellular forms (spherical or ellipsoidal shape, i.e., *G. crepidinum* and *Synechococcus* sp.) and of the remaining 11 filamentous forms (unbranched filament shape), two were heterocystous (i.e., *C. crustacea* and *C. contarenii*) and the other nine were non-heterocystous species (Table 1).

Protein

Most of the cyanobacteria species contained a high percentage of protein (total dry weight). Protein values ranged from 18.9% for *L. martensiana* to a maximum of 70.8% for *S. subsalsa* (Table 1). Cyanobacteria belonging to *Lyngbya* and *Calothrix* genera contained the lowest protein concentrations (<28%) as compared to other species which contained >40% protein (Table 1).

Carbohydrate

Carbohydrate values ranged from a minimum of 5.4% for *L. martensiana* to a maximum of 16.6% of the total dry weight for *S. subsalsa* (Table 1). Cyanobacterial species belonging to *Phormidium* and *Spirulina* genera contained the highest carbohydrate concentrations (>14%) as compared to species belonging to other genera (<11.5%; Table 1).

Table 1. Mean (± S.D) protein, carbohydrate and calorific values of 13 cyanobacterial species isolated from a Hong Kong rocky shore (n = 3). (HF) Heterocystous filamentous cyanobacteria; (F) Non-heterocystous filamentous cyanobacteria; (C) Unicellular cyanobacteria

Species	Protein (% DW)	Carbohydrate (% DW)	Calorific value (kJ 10 g^{-1} DW)
Calothrix crustacea (HF)	21.50 ± 0.40	7.60 ± 0.50	25.16 ± 0.30
Calothrix contarenii (HF)	27.43 ± 0.47	8.23 ± 0.65	29.00 ± 0.45
Gloeocapsa crepidinum (C)	56.46 ± 0.25	7.63 ± 0.55	20.53 ± 0.55
Lyngbya martensiana (F)	18.86 ± 0.65	5.43 ± 0.41	21.73 ± 0.56
Lyngbya semiplena (F)	27.50 ± 0.45	8.93 ± 0.15	23.30 ± 0.36
Phormidium corium (F)	49.56 ± 0.55	16.46 ± 0.45	32.56 ± 0.41
Phormidium tenue (F)	62.96 ± 0.55	15.46 ± 0.40	31.33 ± 0.20
Spirulina subsalsa (F)	70.76 ± 0.90	16.63 ± 0.56	34.83 ± 0.20
Spirulina labyrinthiformis (F)	68.03 ± 0.85	14.73 ± 0.66	34.16 ± 0.37
Synechococcus sp. (C)	63.56 ± 0.60	8.56 ± 0.56	27.60 ± 0.45
Oscillatoria formosa (F)	50.85 ± 0.79	9.46 ± 0.45	15.30 ± 0.36
Oscillatoria salina (F)	41.80 ± 0.81	11.20 ± 0.36	19.30 ± 0.20
Oscillatoria subbrevis (F)	45.16 ± 0.41	11.53 ± 0.68	21.43 ± 0.30

Calorific values

Calorific values for cyanobacteria ranged from 15.30 kJ 10 g^{-1} DW for *O. formosa* to a maximum of 34.8 kJ 10 g^{-1} DW for *S. subsalsa* (Table 1). Species belonging to *Phormidium* and *Spirulina* genera had the highest calorific values (>31.3 kJ 10 g^{-1} DW) as compared to species belonging to other genera (<29 kJ 10 g^{-1} DW).

Overall nutritional value

Principal Component Analysis revealed a clear separation of three groups of species on axis 1 (Principal Component 1) which accounted for 90.11% of the total variance and axis 2 accounted only for 8.67% of the variance (Fig. 1). The first Principal Component has a high positive loading for protein and low positive loading for carbohydrate and calorific value (Fig. 1). Species on the positive side of axis one (a group containing *S. labyrinthiformis, S. subsalsa* and *P. tenue*), have the highest nutritional values, whereas species (*C. contarenii, C. crustacea, L. martensiana* and *L. semiplena*) on the negative side have the lowest nutritional values whilst the remaining species (*G. crepidinum, O. formosa, O. salina, O. subbrevis* and *P. corium*) fall in between these extremes (Fig. 1).

Discussion

A great diversity of cyanobacterial species, belonging to various morphological and functional groups, have been reported from various rocky shores around Hong Kong (Nagarkar, 1998a,b). In the present study, 13 species belonging to seven genera were isolated in pure culture from one shore, Mo Tat Wan. These 13 species were the most common cyanobacteria found in the biofilm on the study site. Most of these species are abundant components of the intertidal, epilithic biofilm in Hong Kong (Nagarkar & Williams, 1999). Similar species have also been reported on rocky shores from other geographical locations (Japan, Umezaki, 1961; Red Sea, Sinai Peninsula, Potts, 1980; India, Thajuddin & Subramanian, 1992; Red Sea, Saudi Arabia, Hussain & Khoja, 1993).

The nutritional value of a species is known to be influenced by culture media (Ben-Amotz et al., 1985), cell harvesting stage (Whyte, 1987), temperature (James et al., 1989), light intensity (Thompson et al., 1990) and pH (James et al., 1989). To compare the nutritional value of these cyanobacteria, all 13 species were grown under similar laboratory conditions and harvested at the same growth phase. Two different culture media were, however, used to support nitrogen fixing (i.e., *Calothrix* spp.) and non-nitrogen fixing cyanobacteria. Differences in protein, carbohydrate and calorific values between *Calothrix* and the

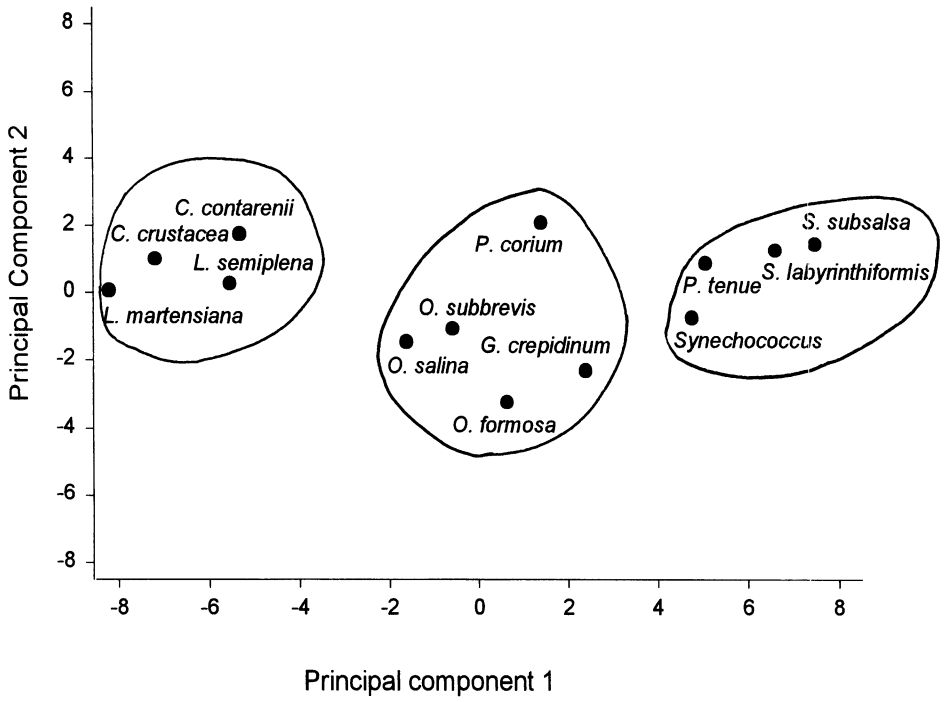

Figure 1. Principal Component Analysis (PCA) based on nutritional values (i.e., protein, carbohydrate and calorific value) of 13 isolated cyanobacterial species from a Hong Kong rocky shore.

other species may, therefore, be partially influenced by the culture media and hence this comparison should be interpreted with caution.

All the cyanobacterial species isolated from Hong Kong rocky shores showed high nutritional quality. Protein content was usually high and contributed up to 70.8% of the total dry weight. *Calothrix crustacea* had the lowest percentage of protein whereas *S. subsalsa* had the highest value. The protein content of Hong Kong species was similar to values for planktonic cyanobacteria (30–40%, Ahlgren et al., 1992) and those isolated in pure culture for biotechnology purposes from various sources such as freshwater, terrestrial or marine environments (65–71%, Venkataraman, 1993; Subramanian, 1998; Sujatha & Kaushik, 1998). Previously, *K. maculans* (one of the dominant encrusting cyanobacteria on tropical rocky shores) was, however, reported as a poor source of protein (7.0%, Kaehler & Kennish, 1996). The protein content of most of the cyanobacteria (>41%) in the present study was in contrast, much higher than planktonic microalgae (12–35%) used in mariculture except for species of *Calothrix* and *Lyngbya* which contained protein (21.5–27.5%) within the range of protein values of microalgae (Brown, 1991).

Carbohydrate values ranged from 5.4 to 16.6% of the total dry weight. Most of the isolates showed <11.5% carbohydrate, except species belonging to *Phormidium* and *Spirulina* genera. The maximum carbohydrate recorded in the present study was 16.6% for *S. subsalsa*. Carbohydrate values previously reported for *K. maculans* (>30%, Kaehler & Kennish, 1996) were much higher than the values recorded for all the 13 cyanobacterial species in the present study. Cyanobacterial species cultured for biotechnological purposes (Venkataraman, 1993) and microalgal species used in mariculture (Brown, 1991), however, contain a similar range of carbohydrate levels (6.0–16.0%) as reported in the present study.

The calorific values of the 13 cyanobacteria species ranged from 15.3 to 34.8 kJ 10 g^{-1} DW which fall within the range of values previously recorded (Venkataraman, 1993; Kaehler & Kennish, 1996). The calorific value of *K. maculans*, for example, was 17.5 kJ 10 g^{-1} DW (Kaehler & Kennish, 1996). In the present study, *G. crepidinum*, *O. formosa* and *O. salina* had calorific values (15.3–20.5 kJ 10 g^{-1} DW) close to that of *K. maculans*. The calorific values of many commercially viable species such as *Spirulina*, however, fall towards the higher side of calorific values recorded in the present study. Most of the *Spirulina* species,

for example, have calorific values between 30 and 36 kJ 10 g^{-1} DW (Venkataraman, 1993). In the present study, only *P. corium*, *P. tenue*, *S. subsalsa* and *S. labyrinthiformis* had calorific values between 31.3 and 34.8 kJ 10 g^{-1} DW.

When considering protein, carbohydrate and calorific values together, *P. tenue*, *S. labyrinthiformis* and *S. subsalsa* contained the greatest concentration as compared to the other species. These results suggest that due to their high protein content and calorific values, and in some cases high carbohydrate content, cyanobacterial species are nutritionally superior to other micro- and macroalgal food resources available on rocky shores (Dawes et al., 1974; McQuaid, 1985; Kaehler & Kennish, 1996). The importance of intertidal, epilithic cyanobacteria as a high quality food source, however, should be interpreted with caution because the present comparison is made on the basis of nutritional values of laboratory cultured cyanobacteria which may vary from the field situation. Although cyanobacteria contained high nutritional values, nutritional adequacy of cyanobacteria for intertidal grazers needs further investigation based on their PUFA and toxicity.

During winter, Hong Kong has favourable conditions for macroalgal growth (Kaehler & Williams, 1996) and shores can support a dense cover of filamentous cyanobacteria, macroalgae and diatoms (Kaehler & Williams, 1996; Nagarkar & Williams, 1999) and chlorophyll *a* values can reach 40 $\mu g\ cm^{-2}$ (Nagarkar & Williams, 1999). The study site at Mo Tat Wan did not support macroalgae in March 1999 and had very low, patchy, chlorophyll *a* values (2.6 $\mu g\ cm^{-2} \pm 1.9$ S.D., $n = 25$) resulting in the shore being visually bare (Nagarkar, pers. obs.). This patchy, sparse biofilm might appear insufficient as a food supply to support the wide variety and high density of intertidal grazers which are present at this site. The present results, however, show that all the cyanobacterial species were highly nutritious, especially in terms of protein, although these species were not tested for their nutritional adequacy and toxicity. Since cyanobacteria were the only food source available on the study site, this suggests the low biomass of the biofilm together with the known high production rate of cyanobacteria was probably able to supply enough nutrition to support the large population of grazers at this site (Nagarkar, 1996). Cyanobacteria are important primary producers and often form the energy base of the benthic food web on tropical rocky shores, therefore, the nutritional value of these species could play an important role in the energetics of intertidal coastal ecosystems.

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