



Cyanobacterial diversity of Bhitarkanika mangrove forests, Odisha

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ABSTRACT

Bhitarkanika National Park in the district of Kendrapara is represented by the mangrove forests of Brahmani and Baitarani Delta of the Odisha coast. Cyanobacteria diversity was assessed in this mangrove forest in five major areas viz. Bhitarkanika, Dangamal, Kalibhanjidian, Gupti and Ekakula. A total of 29 cyanobacteria were isolated and identified from these sites and grouped in to eleven genera. The genus *Lyngbya* was found to be most abundant in this mangrove forest. Among these five areas, Kalibhanjidian showed maximum species richness (11) and dominance (0.44) whereas, minimum richness was recorded for Ekakula (4) and minimum dominance at Bhitarkanika (0.22). Both Shannon index and Simpson's diversity index indicated that Bhitarkanika is having the highest cyanobacteria diversity and Kalibhanjidian, the lowest. The diversity and distribution of cyanobacteria in the study sites will throw light on the health and functioning of the mangrove ecosystem.

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1. Introduction

Mangrove ecosystems are the most unique environment and are considered as one of the highly productive natural ecosystems of the world. They serve as the base of an elaborate and productive food web in the tropical and subtropical coastal marine environments. The exceptional diversity and distribution of the flora and fauna in an estuary is mainly controlled by the fluctuations in the physico-chemical characteristics of water and active participation of the microorganisms which perform various bio-geochemical and nutrient cycles by degradation of foliage litters. Dynamic mangrove ecosystem supports the growth of many microbes such as phosphate solubilizing, nitrogen fixing, sulphate reducing and methanogenic bacteria, wood degrading fungi as well as photosynthetic microbes like cyanobacteria and microalgae, which perform complex interactions for maintaining nutrient cycle and ecological balances in this ecosystem (Rao and Rao, 2015). The knowledge on microbial diversity and distribution in a

mangrove forests would improve the understanding of their functionality, interaction and the role they play in such an ecosystem. These microorganisms, due to their characteristic adaptation and active participation for sustainable development of mangrove ecosystem, could be utilized as industrially important microbes for different value-added products. Several studies on microbial diversity and physico-chemical constituents has been reported for various mangroves ecosystems of Pichavaram (Tamilnadu) and Sundarban (West Bengal), which explains the physiology and stability of this ecosystem (Silambarasan *et al.*, 2011; Ramanathan *et al.*, 2008; Satpati *et al.*, 2013).

Cyanobacteria and microalgae are microscopic photosynthetic organisms, which constitute the world's phytoplankton. These are available in diverse kind of aquatic environments ranging from fresh water to very saline water, very cold arctic region to hot springs. Most cyanobacteria are cosmopolitan species which can grow and sustain in adverse environmental conditions. These cyanobacteria play

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a major role as primary producers and constitute the integral component of the microbiota and their food web in mangrove ecosystem along the tropical coasts (Kathiresan and Bingham, 2001; Sakthivel, 2004). Further, the delta being the slowest stretch of river flow accumulates the major portion of effluents and nutrient carried over by the river system and thus creates a typical ecological environment suitable for growth of cyanobacteria. These organisms can produce various complex compounds such as lipid, carbohydrates and proteins using simple inorganic substances from the ecosystem. Few of them even synthesize bioactive compounds such as polyhydroxyalkanoates, pigments, oils, proteins, polysaccharides, vitamins, antioxidants, UV-protectants, health products etc. (Blackburn and Volkman, 2012). Therefore, exploration of diversity of these bioresources from different ecosystems will not only be helpful for further bioprospecting and biotechnological applications but also to understand the ecosystem functioning as a whole.

Geographically, Bhitarkanika is located between 20°4'-20°82' N Latitudes and 86°45'-87°50' E longitudes in the

Kendrapara district of Odisha. It is the second largest mangrove ecosystem of India which consists of mangrove forests, rivers, river deltas, creeks, estuaries, backwater, accreted land and mud flats. These areas exhibit bidirectional tidal fluxes and thus form extensive, low gradient inter-tidal zones available for mangrove colonization (Chadha and Kar, 1999). Bhitarkanika is one of the richest and diverse mangrove ecosystems in terms of mangrove plants as well as microbial community. Most of the literatures of this region are focused on the flora and the fauna. But in Bhitarkanika mangrove region, due to vast expanse of water and inaccessible forests, the microbial diversity remains unexplored. Only a few reports about the microbial diversity with special emphasis to soil bacteria are available (Mishra *et al.*, 1995; Gupta *et al.*, 2007; Thatoi *et al.*, 2012). Preliminary surveys on microalgae and cyanobacteria were reported by Rath and Adhikary (2006) and Thatoi *et al.* (2012) from estuaries and mangrove soils of Bhitarkanika. Hence, this study was attempted to assess the detailed cyanobacteria diversity of Bhitarkanika to understand their ecological importance in particular and for further biotechnological applications, in general.

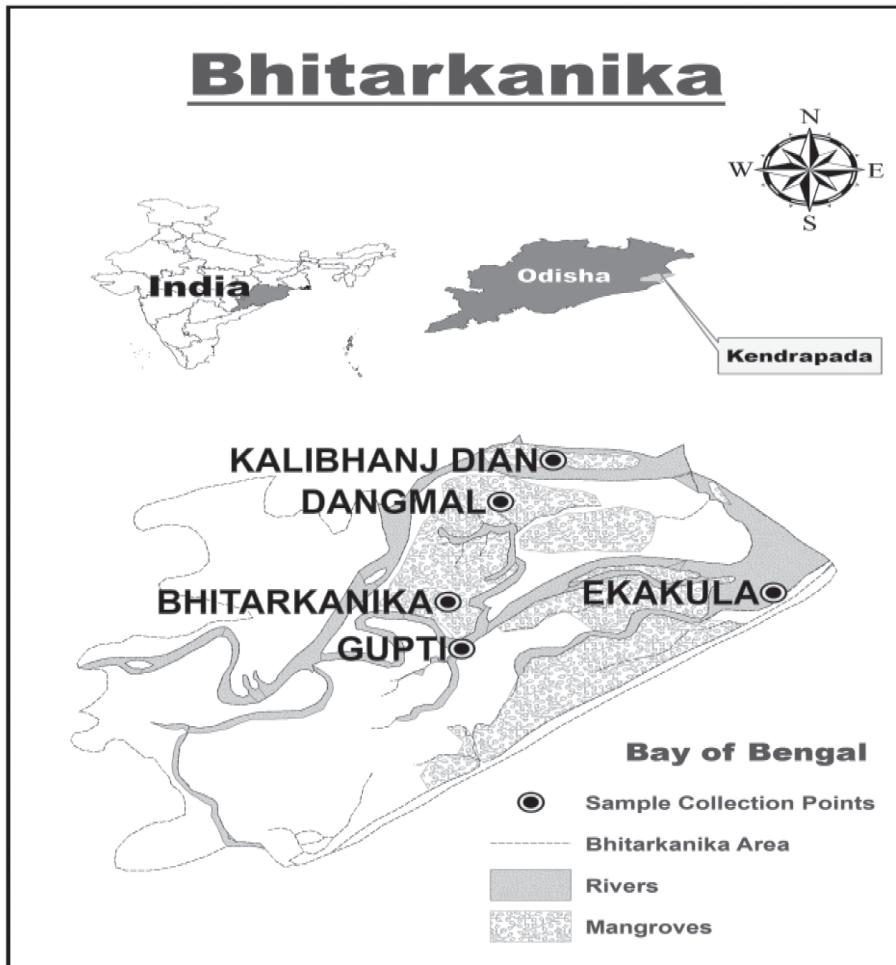


Fig. 1. Sample collection sites of Bhitarkanika mangrove forest

2. Material and methods

2.1. Sample collection

The study area was divided into five major areas of Bhitarkanika as shown in the Fig 1. Samples were collected during 2017-19 from different niches of mangrove sediments, estuarine water, pneumatophores and from the surfaces of woods and shells of the study area by random sampling method. Approximately fifty samples were collected from different sampling sites in autoclaved plastic bags, glass vessels and water storage bottles for further analysis in laboratory.

2.2. Isolation and identification of cyanobacteria

Isolation of cyanobacteria was done by successive serial dilution and repeated spread plate and streak plate method on BG-11 agar plates. Cyanobacteria filaments that grew in the culture plates were isolated and subsequently transferred to BG-11 media in cotton stopped conical flask.

The purified cyanobacteria were observed under microscope for morphological characterization and identified following manual by Desikechary (1959).

2.3. Cyanobacterial culture and maintenance

Each cyanobacteria isolate was cultured in Erlenmeyer flasks with complete BG-11 medium or BG₀-11 (without nitrate) as per their nitrogen requirement (Ripka *et al.*, 1979). The cultures were maintained in a temperature-controlled incubator at 27 ± 2°C under illumination with white fluorescent light (photon) with photoperiod of 14:10 hours. The isolates were sub-cultured at regular intervals to maintain their growth.

2.4. Cyanobacteria diversity index calculation:

The cyanobacteria isolated under each genus were counted for estimating diversity, richness and evenness of cyanobacteria in study area. Generic diversity was estimated by Shannon-Weiner Index (H) (Shannon and Weaver, 1949) and Simpson's diversity and Simpson's dominance (Simpson 1949) using the following formulae.

$$\text{Shannon-Weiner Index, } H = \Sigma p_i \ln p_i$$

$$\text{Species evenness} = \frac{H}{H_{\max}}$$

$$\text{Simpson's Dominance } D = \Sigma (p_i)^2$$

$$\text{Simpson's Diversity Index, } 1 - D = 1 - \Sigma (p_i)^2$$

Species Richness (S)= Total no of isolates present in the areae

where pi = Total number of isolates of genus i / total number of all isolates

$H_{\max} = \ln(S)$ i.e maximum diversity possible where

D= Simpson's Dominance

3. Results

3.1. Isolation and identification of cyanobacteria strains

About ten to fifteen samples were collected from different micro-habitats of each five major areas of Bhitarkanika forest (Fig. 1) with GPS locations for possible isolation of cyanobacteria strains. After the initial growth of cyanobacteria in BG-11 media and agar plates, they were separated by repeated streaking on fresh plates with microscopic observation till the pure cultures were obtained. Then each pure culture was grown again with BG₀-11 (without nitrate) to check the presence of heterocyst in

them. Those isolates who can sustain their growth in BG₀-11 medium were further cultured in that medium and those which could not sustain their growth without nitrate for more than 15 days were cultured in complete BG-11 medium. A total of 29 cyanobacteria strains were isolated from five different areas having six isolates from Dangamal and Bhitarkanika area, eight isolates from Gupti, eleven isolates from Kalibhanjadian and four isolates from Ekakula area. The cyanobacterial samples isolated from soil and water samples and from other sources from different areas were mentioned in Table 1. The isolates belonged to both unicellular and filamentous forms and filaments with or without heterocysts. Morphologically branched and unbranched filaments were also observed. On the basis of microscopic observations and morphological characterization, the isolates were identified and grouped under the genera *Lyngbya*, *Aphanocapsa*, *Gloeocapsa*, *Anabaena*, *Oscillatoria*, *Phormidium*, *Scytonema*, *Calothrix*, *Trichodesmium*, *Leptolyngbya* and *Fristchiella* (Plate 1).

3.2. Genetic diversity and abundance of cyanobacteria samples

Kalibhanjadian recorded highest abundance of cyanobacteria genera followed by Gupti, Bhitarkanika & Dangamal and lowest was recorded from Ekakula as presented in Table 2. Percent abundance of cyanobacterial genera is presented in Fig. 2. The most abundant genera among all isolates were found to be *Lyngbya* (45%) followed by *Aphanocapsa* (10%), *Anabaena* (10%) and *Oscillatoria* (7%). The occurrence of other genera was sporadic. Species richness was found to be maximum in Kalibhanjadia. However, evenness index was highest in Ekakula (0.99) followed by Bhitarkanika (0.87) and lowest in Kalibhanjadian (0.48). All the diversity indices for various areas are graphically represented in Fig 3. Shannon index and Simpson's diversity index revealed the sequence of Bhitarkanika as Dangamala>Ekakula>Gupti>Kalibhanjadian. Simpson's dominance index was maximum in Kalibhanjadian and minimum in Bhitarkanika and Dangamal area.

4. Discussion

Availability of fresh water from Brahmani, Baitarani, Dhamara and Bhitarkanika (Maipura river) rivers and saline water from sea in core area of Bhitarkanika, provide a wide range of niches for different cyanobacteria species to grow. In the present study, 29 cyanobacteria species have been isolated from the mangrove soil, water and from surface of other hard substrates like tree bark, pneumatophores and submerged woods (Table 1, Plate 1). Most of the cyanobacteria isolated were filamentous forms which were found attached to any substratum or soil surface than that

Table 1

Cyanobacteria isolates from Bhitarkanika forest region.

Area	Sampling site	Isolated from	Code of isolate	Name
Bhitarkanika and Dangamal	BD-1	Boat surface and water	BD-1a	<i>Lyngbya</i> sp.
	BD-2	Water	BD-2a	<i>Fristchiella</i> sp.
			BD-2b	<i>Apahnocapsa</i> sp.
	BD-3	Soil and stem	BD-3a	<i>Trichodesmium</i> sp.
	BD-4	soil	BD-4	<i>Oscillatoria pseudogeminata</i> v. <i>unigranulata</i>
	BD-7	Bark	BD-7	<i>Lyngbya</i> sp.
Gupti	G-1	Soil	G-1	<i>Lyngbya keutzinigii</i>
	G-2	Water	G-2	<i>Apahnocapsa koordersi</i>
	G-3	Water	G-3	<i>Gloeocapsa</i> sp.
	G-4	Soil	G-4	<i>Anabaena sphaerica</i>
	G-5	Soil	G-5	<i>Lyngbya mesotricha</i>
	G-7	Soil	G-7	<i>Lyngbya</i> sp.
	G-13	Water	G-13	<i>Anabaena fertilissima</i>
	G-18	Pneumatophore of <i>Rhizophora</i> <i>apiculata</i>	G-18	<i>Lyngbyasp.</i>
Kalibhanjadian	K-3	Bark of <i>Tamarix ericoides</i>	K-3	<i>Lyngbya</i> sp.
	K-5	Soil	K-5	<i>Lyngbya</i> sp.
	K-8	Water	K-8	<i>Lyngbya</i> sp.
	K-9	water	K-9a	<i>Lyngbya</i> sp.
			K-9b	<i>Phormidium tenue</i>
	K-10	Water	K-10a	<i>Chlorococcus</i> sp.
			K-10b	<i>Leptolyngbya lignicola</i>
	K-12	Pneumatophore of <i>Heritiera littoralis</i>	K-12	<i>Aphanocapsa</i> sp.
	K-13	Bark of <i>Excoecaria agallocha</i>	K-13	<i>Microcoleus</i> sp.
	K-14	Pneumatophore of <i>Avicennia officinalis</i>	K-14	<i>Lyngbya</i> sp.
Ekakula	K-15	Root of <i>Phoenix</i> sp.	K-15	<i>Lyngbyasp.</i>
	EK-6	water	EK-6	<i>Anabaena vaginicola</i>
	EK-8	Dried Stem	EK-8	<i>Scytonema</i> sp.
	EK-9	Dried Stem	EK-9	<i>Oscillatoria pseudogeminata</i>
	EK-12	Soil	EK-12	<i>Calothrix</i> sp.

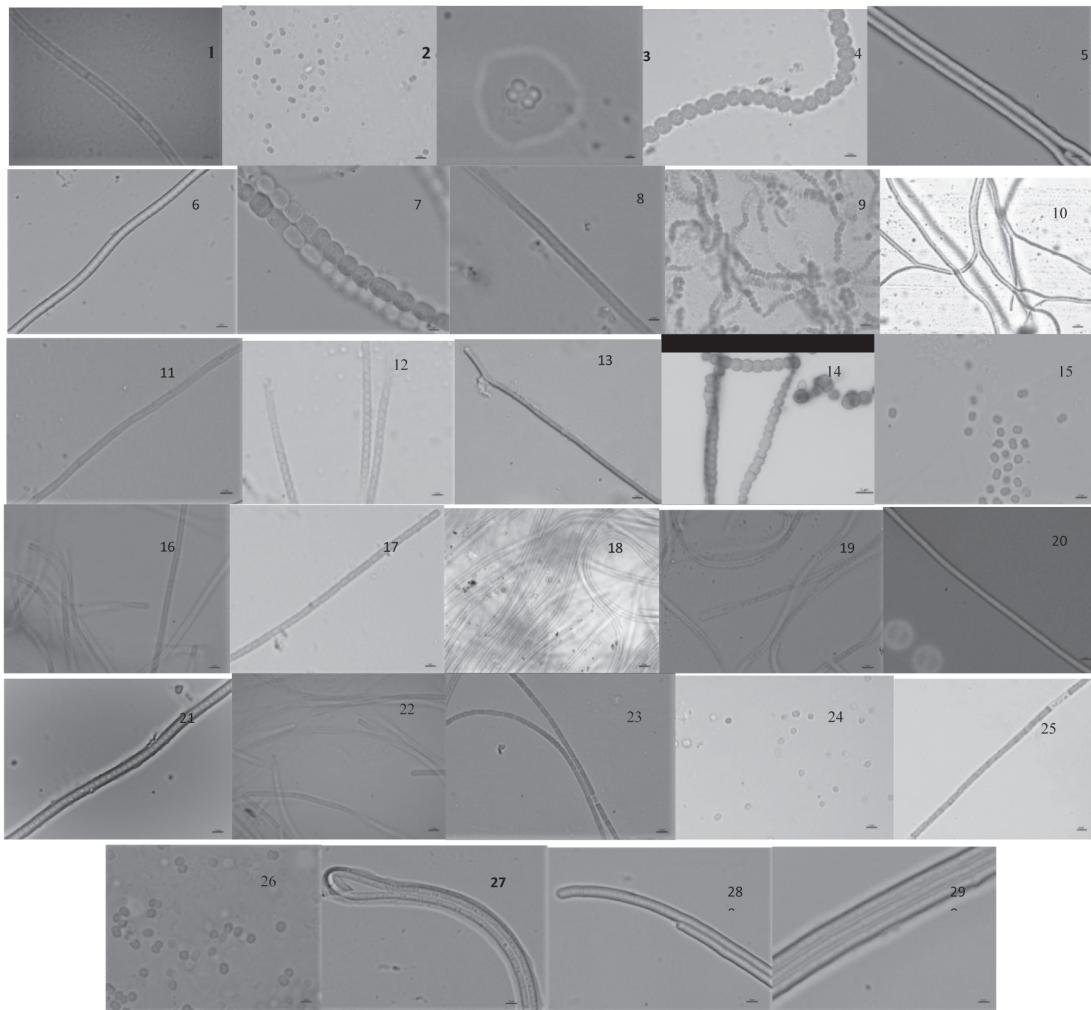


Plate 1 Photographs of cyanobacterial isolates: 1) *Lyngbya keutzinii* (G-1) 2) *Aphanocapsa koordersi* (G-2) 3) *Gloeocapsa* sp. (G-3) 4) *Anabaena sphaerico* (G-4) 5) *Lyngbya mesotricha* (G-5) 6) *Lyngbya* sp. (G-7) 7) *Anabaena fertilissima* (G-13) 8) *Lyngbya* sp. (G-18) 9) *Anabaena vaginicola* (EK-6) 10) *Scytonema* sp. (EK-8) 11) *Oscillatoria pseudogeminata* (EK-9) 12) *Calothrix* sp. (EK-12) 13) *Lyngbya* sp. (BD-1a) 14) *Fritschella* sp. (BD-2a) 15) *Aphanocapsa* sp. (BD-2b) 16) *Trichodesmium* sp. (BD-3a) 17) *Oscillatoria pseudogeminata* v. *unigranulata*. (BD-4b) 18) *Lyngbya* sp. (BD-7) 19) *Lyngbya* sp. (K-3) 20) *Lyngbya* sp. (K-5) 21) *Lyngbya* sp. (K-8) 22) *Lyngbya* sp. (K-9a) 23) *Phormidium tenue* (K-9b) 24) *Chlorococcus* sp. (K-10a) 25) *Leptolyngbya lignicola* (K-10b) 26) *Aphanocapsa* sp. (K-12) 27) *Microcoleus* sp. (K-13) 28) *Lyngbya* sp. (K-14) 29) *Lyngbya* sp. (K-15)

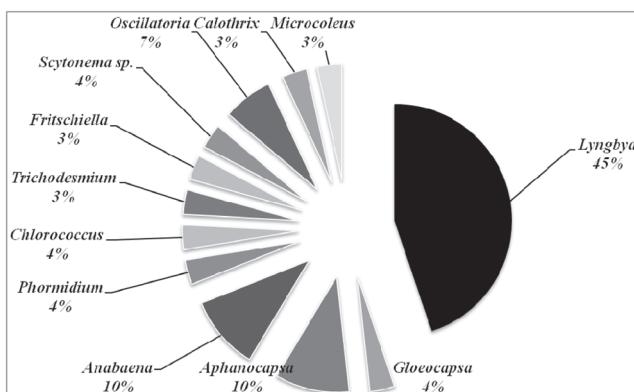


Fig.2. Percent abundance of cyanobacterial genera in Bhitarkanika forest.

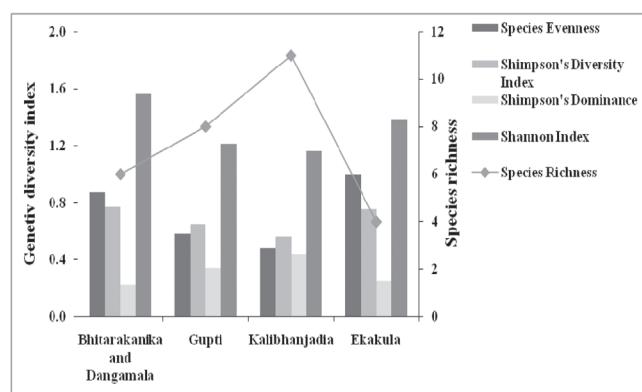


Fig. 3. Genetic diversity of cyanobacterial population from Bhitarkanika forest.

Table 2

Abundance of cyanobacteria sample collected from different areas of Bhitarkanika.

Genus	Total no. of isolates	Bhitarkanika and Dangamal	Gupti	Kalibhanjadian	Ekakula
<i>Lyngbya</i>	13	2	4	7	-
<i>Gloeocapsa</i>	1		1	-	-
<i>Aphanocapsa</i>	3	1	1	1	-
<i>Anabaena</i>	3		2	-	1
<i>Phormidium</i>	1	-	-	1	-
<i>Chlorococcus</i>	1	-	-	1	-
<i>Trichodesmium</i>	1	1	-	-	-
<i>Fritscheilla</i>	1	1	-	-	-
<i>Scytonema sp.</i>	1	-	-	-	1
<i>Oscillatoria</i>	2	1	-	-	1
<i>Calothrix</i>	1	-	-	-	1
<i>Microcoleus</i>	1	-	-	1	-
Total	29	6	8	11	4

of planktonic forms. The species richness, dominance and diversity of the cyanobacteria were analyzed to predict how well the species are distributed within an area. Kalibhanjadian area showed maximum abundance (Table 2, Fig.2) of cyanobacteria which might be due to the geographical position and physico-chemical properties of soil and water sample of the area (Ahad *et al.*, 2015). It is a dense mangrove forest surrounded by two rivers in both the sides and other side is opening to Dhamra port. Therefore, it might have a wide range of variation in pH, salinity, chloride content and effluents from the rivers which influence the cyanobacterial growth and species richness. Moreover, the mangrove forest with diverse type of plant roots and pneumatophores give much surfaces to the filamentous cyanobacteria to grow. However, Bhitarkanika and Dangamal area showed maximum diversity of cyanobacteria with minimum dominance (Fig. 3), which might be due to the existence of a number of cricks in this area that divides the total area in to various micro-niches thus allowing diverse cyanobacteria to grow. Further analysis of physico-chemical parameters of these areas will explain more about the possible cause of such diversity distribution of cyanobacteria.

5. Conclusion

Biotic and abiotic factors influence the distribution of cyanobacteria in any environment. Basic knowledge of ecological factors is important for understanding the ecology and biodiversity of cyanobacteria. Total 29 species belonging to 11 genera were isolated from the mangrove

forest samples from which *Lyngbya* spp. is showing maximum abundance and the maximum cyanobacterial diversity was found in Bhitarkanika and Dangamal region. This shows the rich diversity of cyanobacteria in the ecosystem which may possibly be explored further for biotechnological applications.

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