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An investigation of the mycoflora in marine soil from Andaman Islands

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ABSTRACT

The mycoflora of the marine soil was investigated from four different localities of Andaman Islands. Physico – chemical characters of the soil were also analyzed to find out their impact on fungal population. The fungi were identified and assigned to twenty five genera and fifty four species. Greater populations as well as a wide spectrum range of fungal genera and species were recorded in Chidiya Tapu while Ross Island was the poorest one. Most of the genera detected belonged to the form class Deuteromycetes (21genus and 50 species) with less populations belonging to the Ascomycetes ((3 genus and 3 species) and Phycomycetes (1genus and 1 species). Aspergillus and Penicillium were seems to be the predominant genera with 17 and 7 species respectively. The soil characters such as pH (7.26 to 7.87), electrical conductivity (0.29 to 0.56 dsm⁻¹), organic carbon (0.17 to 0.32), available nitrogen (79.8 to 116 kg/ac), available iron (2.30 to 4.52 ppm), calcium (5.20 to 6.65 mg/kg) and potassium (0.03 to 0.06) recorded also showed variation.

Key Words: Mycoflora, Physico-chemical characters, Deuteromycetes, *Aspergillus, Penicillium.*

INTRODUCTION

Biodiversity in extreme habitats attract great attention among researchers because the study of these systems can increase our understanding of the relationship between organisms and their environment, and the unraveling of mechanisms of their adaptation to extreme conditions (Horikoshi and Grant, 1998; Oren, 1999).

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Fungi are one of the important microbial components of the soil. Since 1860's, research have been carried out on the fungi of different soil types, such as soils of forest (Domsch *et al.*, 1980; Chowdhury and Rai,1980; Joshi and Chauhan, 1981) driftwood (Figueira and Barata, 2007), grasslands,(Ray and Dwivedi, 1962, Dutta and Ghose, 1960; Jabbar Miah *et al.*, 1980) polar region(Cooke and Fournelle, 1960), desert (Durrell and Shield, 1960) marine and mangrove habitats (Sparrow,1937; Matondkar *et al.*,1980; Gibert and Sousa,2002) and coastal sand (Upadhyay, 1978) from various parts of the world. All these studies revealed that the fungi might reside permanently, temporarily for a period in the soil. Their number and species composition in the soil habitat differs from place to place depending upon the physical, chemical and biological factors of the particular habitat.

Among the three major habitat of the biosphere, the marine realm which covers 70% of the earth's surface provides the largest inhabitable space for living organisms, particularly microbes. The best working definition for a marine fungus is that proposed by Kohlmeyer and Kohlmeyer (1979): Obligate marine fungi are those that grow and sporulate exclusively in a marine or estuarine habitat; facultative marine fungi are those from fresh water and terrestrial milieus able to grow and possibly sporulate in the marine environment.

Importance of Marine Fungi

- Play an important link in the biogeochemical cycling
- Decomposers of dead and decaying organic matter
- Clean up of the environment from the pollution

✤ Recently marine fungi have proved to be a rich source of bioactive natural products such as, novel anticancer, antibacterial, antiplasmodial, anti-inflammatory and antiviral agents.

The distribution of fungi in the marine environment has not been well studied as compared with the studies on the fungi in freshwater and terrestrial ecosystems. They are poorly represented in the sea since the marine fungi account for only 5% of the total fungal flora. So in the present study fungal distribution was documented from South Andaman Islands such as Chidiya Tapu, Ross, Red skin and North bay Island, respectively.

MATERIALS AND METHODS

Study site

The present study was carried out in the marine environment of South Andaman Island starting from Chidiya Tapu, the southern most tip of the south Andaman (Lat 11° 27' N and Long 92° 44'E) and few small islands nearby South Andaman such as Ross, Red skin and North bay island.

Sample Collection

The soil samples were collected manually by wearing hand gloves then transferred to sterile polythene bags, sealed properly and transferred to the laboratory. For the analysis of soil nutrients, one kg of soil was separately collected in polythene bags from each station.

Isolation of soil Mycoflora

Dilution plating technique described by Warcup (1950) was used to isolate the fungi from soils. Soil sample weighing 1g was diluted in 10 ml of 50% seawater (1:1 v/v seawater (30 ppt): distilled water). One ml of the diluted sample was poured and spread on petri plates containing sterilized PDA medium (Extract from 250 g of potato (boiled and filtered), dextrose, 20 g; agar, 15 g and distilled water, 1000 ml; pH, 7.0) in replicates. The inoculated plates were incubated in a dust free cubourd at the room temperature $(24\pm2^{\circ}C)$ for 7 days. One per cent streptomycin solution was added to the medium before pouring into petriplates for preventing bacterial growth. After the incubation the development of fungal colonies were observed. The fungal cultures were then transferred, subcultured and pure cultures were maintained. The semi permanent slides were prepared using lacto phenol cotton blue staining method. The slide was observed and microphotography of the individual fungal species was also taken using Nikon phase contrast microscope (Nikon, Japan).

Identification

Colony colour and morphology were noted besides hyphal structure, spore size, shapes and spore bearing structures. They were compared with the standard works of Manual of Soil fungi (Gillman, 1957); Hyphomycetes (Subramanian, 1971); A Manual of Penicillia (Raper and Thome, 1949); Manual of Aspergillus (Raper and Fennell, 1965); Dematiaceous Hyphomycetes (Ellis, 1971, 1976); Higher fungi (Kohlmeyer and Kohlmeyer, 1979) and Soil fungi (Domsch *et al.*, 1980).

Presentation of data

Number of species is referred as species diversity. Population Density is expressed in terms of colony forming unit (CFU) per gram of soil with dilution factor. In order to assess the dominance of individual species in each site percentage contribution was worked out as follows.

No. of colonies of fungus in a sample

% contribution =-

Total number of colonies of all the species in a sample

Frequency occurrence was calculated as follows in order to identify their existence in the soils collected from different areas.

Number of samples in which a particular fungus occurred

% frequency =

Total number of samples examined

x 100

- x 100

Based on the frequency occurrence the fungi were grouped as rare (0-25% frequency), Occasional (26-50%. frequency), Frequent (51-75% frequency) and common (76-100% frequency) species.

Analysis of physico-chemical characteristics of the soil

Soil samples after removing the debris were suspended in distilled water (1:2 w/v) and allowed to settle down the sand particles. The pH of the suspension was read using pH meter (Systronics, India), to find out the soil pH.

Electrical conductivity of soil was determined in the filtrate of the water extract using conductivity bridge as described by Jackson (1973), Cation exchange capacity (CEC) of the soil was determined by using 1 N ammonium acetate solution as described by Jackson (1973).

Organic carbon content was determined by adopting chromic acid wet digestion method as described by Walkley and Black (1934), available nitrogen was estimated by alkaline permanganate method as described by Subbiah and Asija (1956) and available phosphorus by Brayl method as described by Bray and Kutz (1945). Available potassium was extracted from soil with neutral 1 N ammonium acetate (1:5) and the potassium content in the extract was determined by using flame photometer (Standfold and English, 1949), calcium (Neutral 1 N NH4 OAC extractable 1:5) was extracted with neutral 1 N ammonium acetate and the available calcium in the extract was determined by versenate method (Jackson, 1973). Available micronutrients such as Zn, Cu and Mn were determined in the diethylene triamine pentaacetic extract of soil using Perkin-Elmer model 2280 Atomic Absorption Spectrophotometer (Lindsay and Norvell, 1978). Other nutrients such as magnesium, sodium and available iron were analysed following the method of Barnes (1959) and Muthuvel and Udayasoorian (1999).

RESULTS

Fungal diversity

Totally 54 fungal species belonging to 25 genera were isolated from Andaman Islands (Table 1). Besides the above, maximum number of species diversity was encounted with the fungal species belonging to the class Deuteromycetes (21genus and 25 species), followed by Phycomycetes (3genus and 3 species) and Ascomycetes (1genus and 1 species).

Species composition

Among the 25 genera recorded, the genus *Aspergillus* (17 species) was dominant followed by *Penicillium* (7 species), *Fusarium* (5 species), *Cladosporium, Geotrichum* and *Verticillium* (2 species each). All other genera were represented by one species each.

Station wise occurrence

Station I

In different stations, the fungal diversity showed variations. The highest number of 34 species was recorded in Chidiya Tapu followed by 32 species in Red Skin Island, 28 species in North Bay and 27 species in Ross Island (Table 2,3,4 & 5).

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42. P. expansum (Link) Thom		
43. P. granulatum Biourge		
	43.	P. granulatum Biourge
44. <i>P. janthinellum</i> Biourge		5
45. <i>P. luteum</i> Zuka l		
46. <i>Penicillium</i> sp.		-
47. <i>Scopulariopsis</i> sp.		
48. Spicaria elegans (Corda)	48.	Spicaria elegans (Corda)
49. Sporotrichum sp.	49.	
50. Torula sp.	50.	Torula sp.
51. Trametes hirsuta	51.	-
52. Tubercularia sp.	52.	Tubercularia sp.
53. Verticillium terrestre (Link)Lindau	53.	
54. <i>Verticillium</i> sp.		

Table 1. Isolation of fungi from Andaman Islands

S.No	Fungal Isolates	TNC	MD	% Contribution
1.	Absidia glauca	4	1.33	2.941
2.	Aspergillus awamori	4	1.33	2.941
3.	A. flavipes	3	1	2.205
4.	A. flavus	7	2.33	5.147
5.	A. koeningii	3	1	2.205
6.	A. luchensis	4	1.33	2.941
7.	A. nidulans	5	1.66	3.676
8.	A. niger	3	1	2.205
9.	A. ochraceous	6	2	4.411
10.	A. oryzae	7	2.33	5.147
11.	A. quercinus	6	2	4.411
12.	A. terreus	2	0.66	1.470
13.	A. ustus	4	1.33	2.941
14.	Botrytis cinerea	8	2.66	5.882
15.	Chaetomium sp.	6	2	4.411
16.	Cladosporium herbarum	3	1	2.205
17.	C. lignicolum	4	1.33	2.941
18.	Curvularia lunata	3	1	2.205
19.	Fusarium oxysporum	2	0.66	1.470
20.	F. semitectum	1	0.33	0.735
21.	Geotrichum candidum	3	1	2.205
22.	Masoniella grisea	3	1	2.205
23.	Penicillium chrysogenum	3	1	2.205
24.	P. citrinum	2	0.66	1.470
25.	P. janthinellum	1	0.33	0.735
26.	P. luteum	4	1.33	2.941
27.	Penicillium sp.	3	1	2.205
28.	Scopulariopsis sp.	2	0.66	1.470
29.	Spicaria elegans	4	1.33	2.941
30.	Thamnidium sp.	2	0.66	1.470
31.	Torula sp.	3	1	2.205
32.	Trametes hirsuta	4	1.33	2.941
33.	Tubercularia sp.	8	2.66	5.882
34.	Verticillium terrestre	9	3	6.617
	Total no of colonies	136		
	Total no of species	34		

Table 2. Total number of colonies, mean density (CFU/g) and percentage contribution of fungi from Chidiya Tapu

Table 3. Total number of colonies, mean density (CFU/g) and percentage contribution of fungi from North Bay

S. No	Fungal Isolates	TNC	MD	% Contribution
1.	Acremonium sp.	3	1	2.857
2.	Acrocylindrium oryzae	5	1.66	4.761
3.	Alternaria sp.	4	1.33	3.809
4.	Aspergillus awamori	2	0.66	1.904
5.	A. chevalieri	3	1	2.857
6.	A. flavus	5	1.66	4.761
7.	A. granulosis	6	2	5.710
8.	A. koeningii	6	2	5.710
9.	A.niger	3	1	2.857
10.	A. oryzae	5	1.66	4.761
11.	A. ruber	2	0.66	1.907
12.	A. terreus	3	1	2.857
13.	A.ustus	3	1	2.857
14.	A. terricola	5	1.66	4.761
15.	Chaetomium sp.	3	1	2.857
16.	Circinella sp.	1	0.33	0.952
17.	Fusarium lactis	4	1.33	3.809

18.	F. oxysporum	3	1	2.857
19.	Geotrichum sp.	6	2	5.710
20.	Masoniella grisea	6	2	5.710
21.	Oospore lupuli	5	1.66	4.761
22.	Penicillium chrysogenum	3	1	2.857
23.	P. granulatum	2	0.66	1.904
24.	P. janthinellum	4	1.33	3.809
25.	Penicillium sp.	2	0.66	1.907
26.	Sporotrichum sp.	5	1.66	4.761
27.	Torula sp.	2	0.66	1.904
28.	Verticillium sp.	4	1.33	3.809
	Total no of colonies	105		
	Total no of species	28		

Table 4. Total number of colonies, mean density (CFU/g) and percentage contribution of fungi from Red Skin Island

S.No	Fungal Isolates	TNC	MD	% Contribution
1.	Absidia glauca	6	2	4.444
2.	Acrocylindrium oryzae	5	1.66	3.703
3.	Aspergillus awamori	1	0.33	0.740
4.	A. flavipes	3	1	2.222
5.	A.flavus	3	1	2.222
6.	A. granulosis	4	1.33	2.962
7.	A. koeningii	5	1.66	3.703
8.	A. nidulans	7	2.33	5.185
9.	A.niger	4	1.33	2.962
10.	A. ochraceous	6	2.00	4.444
11.	A. oryzae	4	1.33	2.962
12.	A. quercinus	5	1.66	3.703
13.	A. ruber	7	2.33	5.185
14.	A.terreus	3	2.55	2.222
15.	A.ustus	3	1	2.222
16.	A. versicolor	4	1.33	2.962
17.	Chaetomium sp.	4	1.33	2.962
18.	Cladosporium lignicolum	7	2.33	5.185
19.	Fusarium moniliforme	5	2.55	3.703
20.	F.oxysporum	3	1.00	2.222
21.	F. poae	4	1.33	2.962
22.	F. semitectum	1	0.33	0.740
23.	Geotrichum sp.	2	2.00	1.481
24.	Masoniella grisea	6	2.00	4.444
25.	Penicillium chrysogenum	3	1.55	2.222
26.	Penicillium expansum	7	1 1.66	3.703
27.	P. janthinellum	5	1.00	3.703 2.222
28.	Penicillium sp.	3	1.00	2.222
29.	Scopulariopsis sp.	4	1.33	2.962
30.	Thamnidium sp.	4	1.55	2.962
31.	Tubercularia sp.	5	1.00 0.66	3.703 1.481
32.	Verticillium sp.	2	0.00	1.401
	Total no of colonies	135		
	Total no of species	32		

S.No	Fungal Isolates	TNC	MD	% Contribution
1.	Absidia glauca	7	2.33	5.223
2.	Acrocylindrium oryzae	4	1.33	2.985
3.	Aspergillus chevalieri	3	1	2.238
4.	A. flavipes	7	2.33	5.223
5.	A. flavus	4	1.33	2.985
6.	A. granulosis	2	0.66	1.492
7.	A. koeningii	4	1.33	2.985
8.	A. nidulans	7	2.33	5.223
9.	A. niger	4	1.33	2.985
10.	A. oryzae	2	0.66	1.492
11.	A. terreus	4	1.33	2.985
12.	A. ustus	7	2.33	5.223
13.	A. versicolor	6	2	4.477
14.	Cephalosporium sp.	7	2.33	5.223
15.	Fusarium moniliforme	4	1.33	2.985
16.	F. oxysporum	5	1.66	3.731
17.	Helminthosporium oryzae	7	2.33	5.223
18.	Masoniella grisea	5	1.66	3.731
19.	Penicillium chrysogenum	6	2	4.477
20.	Penicillium expansum	4	1.33	2.985
21.	P. janthinellum	3	1	2.238
22.	P. luteum	5	1.66	3.736
23.	Penicillium sp.	4	1.33	2.985
24.	Spicaria elegans	8	2.66	5.223
25.	Torula sp.	3	1	2.238
26.	<i>Tubercularia</i> sp.	5	1.66	3.731
27.	Verticillium terrestre	7	2.33	5.223
	Total no of colonies	134		
	Total no of species	27		

Table 5. Total number of colonies, mean density (CFU/g) and percentage contribution of fungi from Ross Island

Table 6. Percentage frequency and frequency class of different species of fungi recorded at different stations (n=4)

S. No	Fungal Isolates	Chidyatapu	North Bay	Red Skin	Ross	% Frequency	Frequency class
1.	Absidia glauca	+	-	+	+	75	F
2.	Acremonium sp.	-	+	-	-	25	R
3.	Acrocylindrium oryzae	-	+	+	+	75	F
4.	Alternaria sp.	-	+	-	-	25	R
5.	Aspergillus awamori	+	+	+	-	75	F
6.	A. chevalieri	-	+	-	+	50	0
7.	A. flavipes	+	-	+	+	75	F
8.	A. flavus	+	+	+	+	100	С
9.	A. granulosis	-	+	+	+	75	F
10.	A. koeningii	+	+	+	+	100	С
11.	A. luchensis	+	-	-	-	25	R
12.	A. nidulans	+	-	+	+	75	F
13.	A. niger	+	+	+	+	100	С
14.	A. ochraceous	+	-	+	-	50	0
15.	A. oryzae	+	+	+	+	100	С
16.	A. quercinus	+	-	+	-	50	0
17.	A. ruber	-	+	+	-	50	0
18.	A. terreus	+	+	+	+	100	С
19.	A. terricola	-	+	-	-	25	R
20.	A. ustus	+	+	+	+	100	С
21.	A. versicolor	-	-	+	+	50	0
22.	Botrytis cinera	+	-	-	-	25	R
23.	Cephalosporium sp.	-	-	-	+	25	R
24.	Chaetomium sp.	+	+	+	-	75	F
25.	<i>Circinella</i> sp.	-	+	-	-	25	R
26.	Cladosporium herbarum	+	-	-	-	25	R

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27.	C. lignicolum	+	-	+	-	50	0
28.	Curvularia lunata	+	-	-	-	25	R
29.	Fusarium lactis	-	+	-	-	25	R
30.	F. moniliforme	-	-	+	+	50	0
31.	F. oxysporum	+	+	+	+	100	С
32.	F. poae	-	-	+	-	25	R
33.	F. semitectum	+	-	+	-	50	0
34.	Geotrichum candidum	+	-	-	-	25	R
35.	Geotrichum sp.	-	-	+	-	25	R
36.	Helminthosporium oryzae	-	-	-	+	25	R
37.	Masoniella grisea	+	+	+	+	100	С
38.	Oospore lupuli	-	+	-	-	25	R
39.	Penicillium chrysogenum	+	+	+	+	100	С
40.	P. citrinum	+	-	-	-	25	R
41.	P. expansum	-	-	+	+	50	0
42.	P. granulatum	-	+	-	-	25	R
43.	P. janthinellum	+	+	+	+	100	С
44.	P. luteum	+	-	-	+	50	0
45.	Penicillium sp.	+	+	+	+	100	С
46.	Scopulariopsis sp.	+	-	+	-	50	0
47.	Spicaria elegans	+	-	-	+	50	0
48.	Sporotrichum sp.	-	+	-	-	25	R
49.	Thamnidium sp.	+	-	+	-	50	0
50.	<i>Torula</i> sp.	+	+	-	+	75	F
51.	Trametes hirsuta	+	-	-	-	25	R
52.	Tubercularia sp.	+	-	+	+	75	F
53.	Verticillium sp.	-	+	+	-	50	0
54.	Verticillium terrestre	+	-	-	+	50	0

R - Rare (0-25%); O - Occasional (26-50%); F - Frequent (51-75%); C - Common (76-100%)

Table: 7 Physico - chemical parameters of soil samples	s

		Sampling stations					
S. No	Name of the Parameters	Chidiya Tapu	North Bay	Red Skin Island	Ross Island		
1.	pH	7.56	7.69	7.26	7.87		
2.	Electrical Conductivity (dsm ⁻¹)	0.56	0.29	0.36	0.42		
3.	Colour	Brown	Brown	Brown	Brown		
4.	Texture	Sandy	Sandy	Sandy	Sandy		
5.	Lime status	Nil	Nil	Nil	Nil		
6.	Organic Carbon (%)	0.27	0.19	0.17	0.32		
7.	Organic Matter (%)	0.54	0.38	0.38	0.64		
8.	Available Nitrogen (Kg/ ac)	84.0	91.0	79.8	116.0		
9.	Available Phosphorus (Kg/ac)	3.0	4.5	3.0	3.75		
10.	Available Potassium (Kg/ ac)	75.0	67.5	70.0	115.0		
11.	Available Zinc (ppm)	1.05	1.18	0.78	0.98		
12.	Available Copper (ppm)	0.15	0.41	0.18	0.59		
13.	Available Iron (ppm)	2.77	3.97	2.30	4.52		
14.	Available Manganese (ppm)	2.22	3.12	2.19	2.73		
15.	Fine sand (%)	25.45	23.79	23.86	23.26		
16.	Coarse sand (%)	53.19	55.28	56.19	54.10		
17.	Silt (%)	15.79	15.28	15.39	14.59		
18.	Clay (%)	05.57	05.65	05.56	8.05		
19.	Cat ion exchange capacity (C.Mole Proton ⁺ /kg)	12.30	14.50	11.40	15.60		
20.	Calcium (mg/kg)	5.60	5.30	5.20	6.65		
21.	Magnesium (mg/kg)	3.50	2.30	2.20	4.89		
22.	Sodium (mg/kg)	1.17	2.16	2.13	1.59		
23.	Potassium (mg/kg)	0.06	0.03	0.04	0.03		

Frequency class

Based on the frequency of fungi, Aspergillus flavus, A. koeningii A. niger, A. terreus, A. ustus, Fusarium oxysporum, Masoniella grisea, Penicillium chrysogenum, P. janthinellum and Penicillium sp. were classified as common; Absidia glauca, Acrocylindrium oryzae, Aspergillus awamori, A. flavipes and Torula sp. as frequent, Acremonium sp., Alternaria sp., Aspergillus luchensis, A. versicolor, Curvularia lunata, Fusarium lactis and P. citrinum as rare species in marine soils (Table 6.)

Physico - chemical properties of marine soil

Physico – chemical properties of marine soil were presented in Table 7. The results showed the variations in different stations. pH was alkaline in all the soil samples (7.56, 7.69, 7.26 & 7.87). The maximum pH was observed in Ross Island. Electrical conductivity exhibited variation from 0.29 to 0.56. The maximum organic carbon (0.32%) was observed in Ross Island and the minimum (0.17) was observed in Red skin Island. The available nitrogen content of soil was high in Ross Island. The available potassium content range was 67.5 to 115.0 kg/ac. Cation exchange capacity showed variation from 11.40 to 15.60 c.mol proton $^+$ /kg in the samples.

DISCUSSION

Marine microbes represent a potential source for commercially important bioactive compounds and their bioremediation capabilities are also remarkable. They also play a crucial role in decomposition of organic matter and cycling of nutrients. Among the 54 species recorded in the present investigation, the genus Aspergillus and Penicillium showed broad spectrum range, it represented by 17 and 7 species respectively. Dominant occurrence of Aspergillus was reported from various marine soil. Evidently Madhanraj et al., (2010) reported that Aspergillus was dominant genera among the 24 fungal species isolated from entire Tamilnadu Coast. Babu et al. (2010) also recorded Aspergilli and Penicillia were predominant genera from South East Coast of India. Aspergillus genus has been cited as one of the fungi which are present in the atmosphere (Meyer et al., 1983, Oliveira, 1993). Dominance of the genus Aspergillus and Penicillium in the present study sites may be due to their greater rate of spore production, dispersal and partly due to their resistance over extreme environmental conditions (Schimel, 1995). They have explained their suitability to grow in higher saline concentration for their dominant distribution in coastal marine habitats. In the present study, it seemed that the field of marine mycology is necessary to investigate diversity of fungi in the marine environment before we can understand their ecological significance and their distinct characters.

Rani and Panneerselvam, (2010) reported that the diversity and distribution of different organisms in the marine environment are influenced by the physico-chemical properties of soil. All the soil samples were alkaline in nature. Electrical conductivity is the indirect measure of salinity which showed range from 0.29 to 0.56 dsm⁻¹. The nutrient levels were very less in marine habitats.

Therefore it could be concluded that there is no uniformity in the diversity of marine fungi and their distribution pattern in different geographical regions. Several factors of salinity, origin, nature of substrata, pH and oceanic region affect the occurrence and diversity of marine fungi.

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