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Contribution of the Thraustochytrid *Corallochytrium limacisporum* Raghu-kumar to Microbial Biomass in Coral Reef Lagoons

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ATP and organic carbon (OC) contents of cells of 5 isolates of thraustochytrids, including one of *C. limacisporum* were estimated from cultures. OC:ATP ratio in these isolates averaged 99. Based on these and the data collected on the number of *C. limacisporum*, total viable bacteria (saprophyte numbers), chlorophyll *a* content and total ATP in the lagoon waters, the contribution of *C. limacisporum* to the microbial biomass in coral reef lagoons of the Lakshadweep islands was calculated. The thraustochytrid contributed 2.88 to 213.57% ATP and 1.15 to 85.43% OC relative to total viable bacteria and 0.024 to 3.09% ATP and 0.002 to 1.24% OC to total microbial biomass. *C. limacisporum* and total viable bacteria together contributed only 0.844 to 6.02% ATP and 0.822 to 4.97% OC to the biomass. About 90% of the microbial biomass in the lagoons appeared to be from organisms other than the thraustochytrid, total viable bacteria and phytoplankton.

Little is known about the role of the marine microorganisms, the thraustochytrids, although these fungi-like protists have been reported to occur in substantial numbers from the sea in many parts of the world[1-6]. A thraustochytrid, *Corallochytrium limacisporum* has been found to occur in great numbers in the waters of the coral reef lagoons of the Lakshadweep islands in the Arabian Sea[7]. However, the extent of its contribution to the total biomass in these waters has not been investigated so far. An attempt has been made to estimate the possible contribution of this thraustochytrid to the total microbial biomass in these lagoons based on ATP and organic carbon content per cell. This demands the knowledge of ATP content of these cells and its ratio to organic carbon. Hence, in the present study, ATP and organic carbon contents of cells of 5 isolates of thraustochytrids including *C. limacisporum* have been estimated. On the basis of data on bacterial (saprophyte[12]) numbers, chl *a* and total ATP levels in waters of these lagoons, the contribution of this thraustochytrid to total microbial biomass has been assessed.

Materials and Methods

Five isolates of thraustochytrids from NIO culture collection were examined: *Thraustochytrium striatum* Schneider (NIOCC 66), *T. motivum* Goldstein (NIOCC 40), *Thraustochytrium* sp. (NIOCC 90), *Ulkenia visurgensis* (Bahnweg & Sparrow) Gaertner (NIOCC 89) and *Corallochytrium limacisporum* Raghu-kumar (NIOCC 69).

These isolates were grown for 1 week in liquid medium in stationary cultures[7]. All estimations were made with 4 replicates. ATP and organic carbon per cell were estimated as follows:

The number of cells in 1 ml of culture medium was estimated by means of a counting cell, after homogenising the culture on a magnetic stirrer to break up cell clumps. The diameters of 100 cells were measured under the microscope and the percentage was calculated under 5 different diameter (μm) ranges viz. (i) 1.5-3.5 (av. 2.6), (ii) 3.6-5.5 (av. 4.6), (iii) 5.6-7.5 (av. 6.6), (iv) 7.6-9.5 (av. 8.6) and (v) cells larger than 9.6. However, very few cells fell into the last category. From this percentage, cell no. ml^{-1} in each size range was calculated. The total volume of cells in each range was calculated by

$$\text{Cell no. ml}^{-1} \times \frac{4}{3}\pi r^3.$$

From this, the percentage volume of cells in each size range in relation to the sum total volume of cells per ml was estimated. It was assumed that under given cultural conditions, ATP per cell can be correlated to the volume of the cell. ATP content of 5 ml of each culture was estimated according to the method of Holm-Hansen and Booth[8] using firefly luciferase (Sigma, USA) in a Turner Luminometer TD-20. Organic carbon content of 5 ml of each culture was estimated according to Parsons *et al.*[9] involving the wet oxidation of carbon by acid dichromate. ATP and organic carbon values per ml were then distributed for each size range based on the percentage volume. ATP or organic carbon per cell was derived from this.

Total ATP content of water in the Lakshadweep lagoons was obtained using Holm-Hansen and Booth's[8] method; the total viable counts of bacteria by filtering the sample through membrane fil-

ter (Millipore, 0.22 μm), placing the filters on Zo-Bell's marine agar medium and counting the colonies; and the chl. *a* values by fluorometric method according to Parsons *et al.*[9]. The number of *C. limacisporum* in the lagoons was estimated by filtering the water through a glass-fibre filter (Whatman, GF/F with a retention size of 1.1 μm), placing the filter on thraustochytrid medium I[10] and counting the colonies. Factors used to convert total ATP, chl. *a* and bacterial ATP to biomass (organic C) are presented.

Results

Values of ATP and organic carbon (OC) per cell obtained for 3 different size ranges, namely, 2.6, 4.6 and 6.6 μm average diam. of 5 isolates of thraustochytrids are presented in Table 1. *T. striatum* exhibited lowest ATP and OC values, whereas *U. visurgensis* yielded the highest values. Average ATP content per cell for the 3 sizes were 0.04, 0.23 and 0.69 pg respectively. Average OC content per cell for the 3 sizes were 4, 21 and 72.7 pg respectively. OC:ATP ratio averaged 99.4 for all 3 size ranges.

Contributions of *C. limacisporum*, total viable bacteria and phytoplankton to the total biomass in the coral lagoons of the Lakshadweep islands are presented in Table 2. The cell ATP and OC

Table 1— ATP (A), Organic Carbon (B) and A:B Ratio per Cell in Species of *Thraustochytrium, Ulkenia* and *Corallochytrium*

Isolates		Size ranges, μm*		
		1	2	3
T. striatum	A	0.02	0.08	0.3
	B	1.4	7.6	21.6
	A:B	73	100	72
T.motivum	A	0.02	0.11	0.3
	B	1.9	11.1	33.0
	A:B	95	101	100
Thraustochytrium sp.	A	0.05	0.23	0.95
	B	6,5	27.6	114.04
	A:B	130	120	120
U. visurgensis	A	0.07	0.44	1.32
	B	7.4	43.53	131.2
	A:B	100	99	99
C. limacisporum	A	0.04	0.26	0.59
	B	3.9	20.0	63.9
	A:B	97.5	77	108
Mean (± SD):	ATP	0.04 (±0.02)	0.23 (± 0.14)	0.69 (± 0.44)
	OC	4.22 (± 2.68)	21.97 (±14.36)	72.75 (±48.47)
	OC:ATP	99.1 (±20.3)	99.4 (±18.69)	99.8 (±17.67)

*1 = 1.5-3.5 (av. 2.6); 2 = 3.6-5.5 (av. 4.6); 3 = 5.6-7.5 (av. 6.6).
ATP and OC values in pg.

Table 2— Contribution of *C. limacisporum*, Total Viable Bacteria and Phytoplankton to ATP and Biomass in Lagoons

Station[1]	*C. limacisporum*			Bacteria			Phytoplankton		Total	
	Cells (no.l^{-1})	ATP[2] (μg.l^{-1}) $\times 10^{-3}$	Biomass[3] (μg C.l^{-1})	Count (no.l^{-1}) $\times 10^{3}$	ATP[4] (μg.l^{-1}) $\times 10^{-3}$	Biomass[5] (μg C.l^{-1})	Chl. *a* (μg.l^{-1})	Biomass[6] (μg C.l^{-1})	ATP[7] (μg.l^{-1})	Biomass[8] (μg C.l^{-1})
Agatti 1	100	0.026	0.0026	602	0.903	0.226	0.0308	3.08	0.11	27.5
Agatti 7	1500	0.390	0.039	654	0.981	0.245	0.0243	2.43	0.13	32.5
Bingaram 2	740	0.192	0.0192	766	1.149	0.287	0.0064	0.64	0.038	9.5
Kavaratti 4	1600	0.416	0.0416	686	1.029	0.257	—	—	0.024	6.0
Kavaratti 6	3450	0.897	0.0897	280	0.420	0.105	—	—	0.029	7.25
Kavaratti 11	965	0.251	0.0251	335	0.503	0.126	—	—	0.016	4.0

1 = See Raghu-kumar[7] for exact location of stations; 2 = Conversion: 0.26 pg ATP. $cell^{-1}$; 3 = ATP × 100; 4 = 0.0015 pg ATP. $cell^{-1}$; 5 = ATP × 250; 6 = Chl. *a* × 100; 7 = Total microbial ATP as estimated independently; 8 = ATP × 250

for *C. limacisporum* presented in Table 2 are for the size ranges of 3.6-5.5 μm (av. 4.6 μm), which is most frequent in cultures. This organism contributed 26-897 pg ATP and 0.0026-0.0897 μg OC to the biomass per litre of water in the lagoons. This corresponded to 2.88-213.57% of ATP and 1.15-85.43% of OC contributed by the total viable bacteria (Table 3).

C. limacisporum contributed 0.024-3.09% ATP and 0.009-1.24% OC to the total microbial biomass (Table 4). In sts AG1, AG7 and B2, *C. limacisporum*, total viable bacteria and phytoplankton together contributed only 12.03, 8.35 and 9.96% of the total OC respectively. Phytoplankton contribution was not estimated in the other stations, where thraustochytrid and total viable bacteria together contributed only 4.98, 2.69 and 3.78% to the total OC (Table 4). ATP contribution by *C. limacisporum* and total viable bacteria amounted to only 0.844-6.02% of the total ATP.

Discussion

Reliability of techniques employed will have to be considered in a study of this sort. Although isolation by the glass-fibre filtration technique yields a variety of species[10], only *C. limacisporum* has been isolated from the lagoons using this technique. Hence, this appears to be the predominant species in these waters. For enumeration of bacteria, epifluorescence technique[11] could have been used. However, in the present study, only the viable bacterial counts (saprophyte numbers[12]) and their biomass, and not the total number of bacteria which can be estimated by epifluorescence, were studied. The term 'saprophytic bacteria' has been applied to those which are able to grow on agar media and which can be counted by means of plate method[12]. Possessing high metabolic activity and nutrient requirements, such bacteria may reflect the content of easily degradable matter. Their proportion may amount to 1:5 to 1:100 of the total number of bacteria in extremely nutrient rich waters to only1:100 to 1:10000 in nutrient poor oceanic waters[13]. Since the coral reef lagoons are rich in nutrients, the saprophytic bacteria may constitute the major proportion of the total bacteria. Saprophyte numbers similar to that given here, ranging from 200 to 1000 $\cdot$ ml^{-1} are common in many coastal waters[12,13].

For biomass conversions, average values for bacteria and chl. *a* have been employed. However, ATP per cell and OC:ATP ratio may vary considerably depending on the species and growth conditions[14]. As for the thraustochytrids, since only one species is involved in this study whose ATP and cell carbon have been estimated in the laboratory, this problem may not be acute. As for the growth conditions, the waters of the coral reef lagoons are nutrient-rich and may not cause stress changes in OC:ATP ratio. We have used the above conversions in place of the cell volume, density and OC biomass conversions from epifluorescence counts for reasons given earlier.

For biomass estimations, values for the size range of 3.9-5.5 μm could be taken as representative for the thraustochytrids, since normally most of the cells of these organisms fall within this size range in culture. ATP per cell for this size range varied from 0.08 to 0.44 pg, which is 67 to 360 times more than that of an average bacterial cell. The marine bacteria are known to have 0.0005 to 0.006 pg ATP cell^{-1}, with an average[15] of 0.0015.

Table 3—Percentage Contribution of *C. limacisporum* to ATP and Organic Carbon in Lagoon Waters, Relative to Total Viable Bacteria

Station*	ATP	Organic carbon
Agatti 1	2.88	1.15
Agatti 7	39.76	15.92
Bingaram 2	16.71	6.69
Kavaratti 4	40.43	16.19
Kavaratti 6	213.57	85.43
Kavaratti 11	49.9	19.96

*See Raghu-kumar[7] for location of stations

Table 4—Percentage Contribution of *C. limacisporum*, Total Viable Bacteria and Phytoplankton to Total ATP and Biomass in Lagoons of Agatti (A), Bingaram (B) and Kavaratti (K) Islands*

	ATP						Biomass (organic carbon)					
	Ag 1	Ag 7	B 2	K 4	K 6	K 11	Ag 1	Ag 7	B 2	K 4	K 6	K 11
C. limacisporum	0.024	0.3	0.51	1.73	3.09	1.57	0.009	0.12	0.2	0.69	1.24	0.63
Bacteria	0.82	0.75	3.02	4.29	1.45	3.14	0.82	0.75	3.02	4.29	1.45	3.15
Phytoplankton	—	—	—	—	—	—	11.2	7.48	6.74	—	—	—
Total	0.844	1.05	3.53	6.02	4.54	4.71	12.03	8.35	9.96	4.98	2.69	3.78
Other organisms	99.155	98.95	96.47	93.98	95.46	95.29	87.97	91.65	90.04	95.02	97.31	96.22

*See Raghu-kumar[7] for location of stations

The conversion factor for ATP to OC of 72 to 130, obtained for the thraustochytrids in the present study, is lower than the average value of 250 obtained for many other organisms[14,15]. Sakshaug and Holm-Hansen[14] have, however, also reported values of 70 for some brackish water diatoms. We suggest that an average value of 0.23 pg ATP $cell^{-1}$ for 3.6 to 5.5 μm size range and a conversion factor of 100 for ATP to OC could be used for the thraustochytrids.

Since this study deals particularly with the thraustochytrid *C. limacisporum* in the lagoons, the value of 0.26 pg ATP $cell^{-1}$ and a conversion factor of 100 for ATP to cell carbon has been used (Table 1). For the bacteria, the average value of 0.0015 pg per cell has been used to convert the bactrial numbers to bacterial ATP. To convert the bacterial ATP as well as the total microbial ATP to OC, a factor of 250 has been used[2,14,15]. A factor of 100 has been used to convert chl. *a* to organic carbon[16,17].

In sts Ag1 and K6, *C. limacisporum* contributed 2.88 and 213.57% ATP and 1.15 and 85.43% OC (Table 3) relative to the bacteria (saprophytes). These may be extreme cases. In other stations, 16 to 50% ATP and about 6 to 20% of OC were contributed by *C. limacisporum* relative to the total viable bacteria (Table 3). These bacteria contributed 0.75 to 4.29% of ATP as well as organic carbon to the total biomass (Table 4). Similar range (9.2-4.8%) has been reported earlier[18]. Although their contribution to the biomass is small (5-15%), bacteria, with their high rate of turnover, are known to make up 90% of the heterotrophic activity[19]. Species of thraustochytrids, particularly organisms like *C. limacisporum* which is remarkably fast growing and contributes substantially to the biomass compared to the total viable bacteria as judged by the present estimations, could play an extremely important role in the marine ecosystem, even when their contribution to the total biomass is low, as in the case of bacteria.

In the 3 stations (Ag1, Ag7, B2, Table 4) where chl *a* was estimated, 87-92% of the microbial biomass could not be accounted for by *C. limacisporum*, total viable bacteria and phytoplankton. It could be expected that in the other 3 stations (K4, K6 and K11) too a similar situation existed. This balance in the biomass could be from other microorganisms such as yeasts, higher fungi, picoplankton or microzooplankton. Whenever the former 2 are present in high numbers as in coastal waters, they seriously hamper isolation of thraustochytrids in Petri plates due to rapid growth and this was not the case in the present study. It is quite possible that picoplankton, smaller than the 1.2 μm size retained by the filter (GF/C Whatman glass fibre) employed for chlorophyll estimations and the microflagellates might contribute significantly to the biomass.

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