

ENUMERATION OF THRAUSTOCHYTRIDS (HETEROTROPHIC MICROORGANISMS) FROM THE ARABIAN SEA

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ABSTRACT

The number of thraustochytrids in seawater has been estimated in several samples from the Arabian Sea by concentrating the sample on a glass-fibre filter and plating on a nutrient medium. The number ranged from 1 to 69/liter in the oceanic water samples and up to 11,000/l in samples from the lagoons of the Lakshadweep coral islands. The advantages and disadvantages of plating techniques to isolate thraustochytrids and the overall problems in enumerating these protists are discussed.

Key-words : Thraustochytrids, plating technique, enumeration, Arabian Sea.

INTRODUCTION

Among the marine heterotrophic microorganisms, the thraustochytrids are relatively little studied. These eukaryotic, unicellular organisms, which possess an osmotrophic mode of nutrition, have been placed among the lower fungi in the order Thraustochytriales of the Oomycetes (Sparrow, 1976). Olive (1975) has classified them in the family Thraustochytriidae of the Class Labyrinthulea among the Phylum Gymnomyxia in the Kingdom Protista. Only a little is known about their ecology in the marine environment and their role (Gaertner and Raghu-kumar, 1980; Raghu-kumar and Gaertner, 1980 and Gaertner, 1980). However, even the few investigations that have been carried out till now, mainly from the North Sea, have revealed significant numbers of these organisms in seawater and sediments. Thraustochytrids have also been isolated from several parts of the world (Olive, 1975), including Antarctic waters (Bahnweg and Sparrow, 1974). These organisms possibly play an important role in biodegradation and turnover of nutrients in the sea.

Research on the ecology of thraustochytrids has been partly hampered by the lack of a suitable technique to rapidly quantify them in the marine samples. Gaertner (1968) devised a modification of the MPN technique, commonly employed in bacteriology, to quantify them. In his technique, various dilutions of the seawater or sediment samples are baited with sterile pine pollen in sterile flasks. The thraustochytrids, when present, grow upon the pollen

grains. Plating the samples on a suitable medium has been employed by some. Vishniac (1956) suggested a medium, on which bits of algae and small quantities of seawater could be plated to isolate lower fungi. Where Phycomycetous fungi are fewer in seawater samples. Fuller and Poyton (see Jones, 1971) suggested continuous flow centrifugation of seawater and plating it on a nutrient medium. Miller (1967) isolated Phycomycetes by filtering water through a Millipore filter and plating the filter on a nutrient medium. Bahnweg and Sparrow (1974) estimated the number of thraustochytrids in Antarctic waters by filtering a given volume of seawater through glassfibre filters and plating this on a nutrient medium. However, he found difficulties in using this technique for coastal water samples due to heavy contamination by mold fungi.

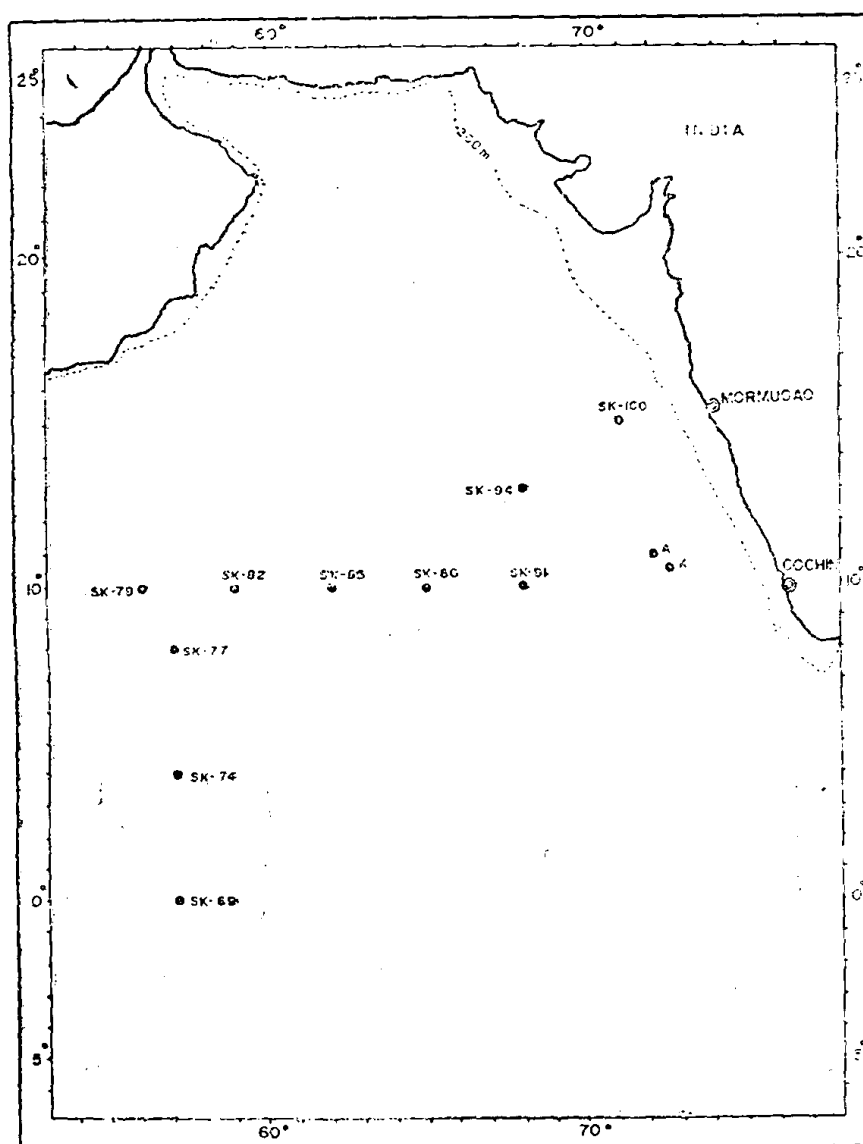


Fig 1. Map showing location of sampling stations. (See Table I for details.)
A—Agatti Island; K—Kavaratti Island.

This paper reports the numbers of thraustochytrids estimated by using a plating technique, from various water samples in the Arabian Sea.

MATERIALS AND METHODS

The following method was employed to estimate the number of thraustochytrids in seawater samples from the Arabian Sea during cruise 1 of ORV *Sagar Kanya* (12.7. — 2.9.1983) and from the coral reef lagoon waters of Agatti and Kavaratti islands of the Lakshadweep during cruises 136 (22.4. — 3.5.1984) and 147 (7.1. — 25.1.1985) of the RV *Gaveshani*. The sampling stations are shown in Fig. 1.

A known volume of seawater sample was filtered through a GF/F Whatman glassfibre filter. This volume ranged from 500 ml for oceanic waters to 10 ml for the lagoon and estuarine waters. This was followed by washing the filter with 2 ml of seawater of a salinity corresponding to that used in the medium and containing 400 units of Penicillin and 0.001 g of Streptomycin per ml. This antibiotic solution was prepared from a stock solution, made by adding preweighed antibiotic powders to appropriate amount of sterile seawater of required salinity. After concentrating the sample on the glassfibre filters, the filter was removed with sterile forceps and placed on any one of the following two media.

<u>Medium 1</u>		<u>Medium 2</u>	
Peptone	0.25 g	Peptone	0.25 g
Yeast extract	0.05 g	Yeast Extract	0.05 g
Glucose	0.5 g	*Tween 80	1.0 ml
Thiamine	0.2 mg	Vitamins	As in Medium 1
Biotin	0.5 mg	Agar	0.4 g
Nicotinic acid	0.1 mg	35‰ Seawater	80 ml
Pyridoxin HCl	40 µg	Streptomycin	0.1 g
Folic acid	2.5 µg	Penicillin	40000 units
p-Aminobenzoic acid	10 µg	Nystatin	500 µg
Calcium pantothenate	0.1 mg	*Autoclaved separately and added later.	
Cebalamin	0.01 µg		
Agar (Hi-Media)	0.4 g		
20 ‰ Seawater	80 ml		
Penicillin	40000 units		
Streptomycin	0.1 g		
pH adjusted to 8.0			

Table I Number and species of thraustochytrids isolated from different water samples using the plating technique.

Cruise No. and date	Station No.	Position	Depth (m)	Number of thraustochytrids/l	Species isolated
<i>Sagar Kanya</i> 12.7 — 2.9.1983	69	0°00.1 S 56°59.3 E	5	< 1	—
			55	< 1	—
			125	< 1	—
	74	4°01.3 N 57°00.3 E	5	< 1	<i>Labyrinthuloides yorkensis</i>
			100	4	— do —
			150	4	— do —
	77	8°01.6 N 56°59.9 E	5	2	— do —
			95	< 1	—
			190	2	— do —
	79	9°58.4 N 56°01.9 E	5	12	— do —
			175	8	<i>Ulkenia amoeboidea</i>
	82	9°59.9 N 59°00.2 E	5	2	<i>Labyrinthuloides yorkensis</i>
			105	8	— do —
			175	< 1	— do —
			200	2	— do —
	85	9°59.2 N 61°59.9 E	5	60	— do —
			105	19	<i>Ulkenia amoeboidea</i>
			170	17	<i>Labyrinthuloides yorkensis</i>
	88	10°00.4 N 64°58.5 E	5	28	<i>Ulkenia amoeboidea</i>
			80	36	<i>Labyrinthuloides yorkensis</i>
			125	38	<i>Ulkenia amoeboidea</i>
			125	38	<i>Labyrinthuloides yorkensis</i>
	91	9°59.0 N 68°00.8 E	5	< 1	<i>Ulkenia amoeboidea</i>
90			69	<i>Labyrinthuloides yorkensis</i>	
125			19	<i>Ulkenia amoeboidea</i>	
99	15°00.0 N 70°59.7 E	5	< 1	<i>Labyrinthuloides yorkensis</i>	
		75	25	<i>Ulkenia amoeboidea</i>	
		125	11	<i>Labyrinthuloides yorkensis</i>	
100	15°01.1 N 71°59.6 E	5	< 1	—	
		50	< 1	—	
		100	< 1	—	
RV <i>Gaveshani</i> 136 22.4 — 3.5.84	Kavaratti Island, Lakshadweep	Sample a	0	10675	Unidentified
		Sample b	0	6500	Unidentified
RV <i>Gaveshani</i> 147 7.1. — 25.1.85 29.4.85	Agatti Island, Lakshadweep Zuari Estuary Goa.	Sample a	0	5350	Unidentified
		Sample b	0	860	Unidentified
		Dona Paula,	0	66	

The medium was autoclaved without the antibiotics. To this was added 20 ml of an antibiotic stock solution in seawater of the same salinity as the medium to yield the required amount of antibiotics. Nystatin which is insoluble in water, was brought into a fine suspension in the stock solution by using a magnetic stirrer.

RESULTS

This method was used very satisfactorily to isolate thraustochytrids from the oceanic waters of the Arabian Sea and the coral reef lagoon waters of Lakshadweep islands. Medium 1 was used in both the cases. The number and species of the thraustochytrids isolated during the three different cruises from various stations (Fig. 1) are reproduced in Table I.

The oceanic water samples collected during the cruise with ORV *Sagar Kanya* yielded a low number of thraustochytrides, from 2 to 60/l. *Ulkenia amoeboidea* (Bahnweg and Sparrow) Gaertner and *Labyrinthuloides yorkensis* Perkins were the only species isolated from this area. The colonies of the former were orange and those of the latter creamish in colour, both being flat, monolayered and spreading after 10 days' incubation.

Difficulties are encountered when isolating thraustochytrids from coastal waters. The high load of mold fungi and yeasts in these waters results in their rapid growth suppressing the growth of thraustochytrids. Therefore, attempts to find out the tolerance of thraustochytrids to Cycloheximide, Griseofulvin, Malachite green and Nystatin were made, so that one of these antifungal compounds could be incorporated in the isolation medium to isolate the thraustochytrids. The results are shown in Table II.

None of the antifungal compounds tested appeared to be promising for the purpose. Nystatin allowed the growth of thraustochytrids at a concentration of 5 $\mu\text{g/ml}$ when incorporated into agar medium, but not at 25 and 50 $\mu\text{g/ml}$. However, growth was normal when these amounts of Nystatin were used in seawater/pollen cultures.

Nevertheless, during the cruises 136 and 147 of RV *Gaveshani* to the Lakshadweep islands in the Arabian Sea, a very high number of an unidentified species of thraustochytrid was isolated in enormous numbers from the lagoon waters (Fig. 1, Table I), in spite of incorporating 50 $\mu\text{g/ml}$ of Nystatin in the isolation medium. The solution for rinsing (see Materials and Methods) contained besides Streptomycin and Penicillin, Nystatin at a concentration of 50 $\mu\text{g/ml}$.

Some preliminary experiments indicated that thraustochytrids possess very good lipolytic ability. This test was carried out by growing different species

Table II. Effect of some antifungal compounds on growth and isolation of thraustochytrids.

Compound	Concentration	Effect on	Medium	Remarks
Cyclohexamide	100 μg /ml	<i>Ulkenia visurgense</i> Cult No. 41	Seawater/pollen	No growth
		<i>Thraustochytrium</i> <i>motivum</i> Cult. No. 40	— do —	No growth
		<i>Thraustochytrium</i> <i>striatum</i> Cult No. 1	— do —	No growth
Nystatin	50 μg /ml	<i>Ulkenia visurgense</i> Cult No. 41	— do —	Growth normal
		<i>Thraustochytrium</i> <i>striatum</i> Cult. No. 1	— do —	Growth normal
		<i>Thraustochytrium</i> <i>motivum</i> Cult. No. 40	— do —	Growth normal
		<i>Ulkenia visurgense</i> Cult No. 41	Thraustochytrid isolation medium 1	No growth
		<i>Thraustochytrium</i> <i>striatum</i> Cult KMPB 6008	— do —	No growth
		Cult. No. 34 from Kavaratti lagoon	— do —	Good growth
		Isolation from Zuari estuary	— do —	No. thraustochytrids could be isolated.
	25 μg /ml	<i>Thraustochytrium</i> <i>striatum</i> Cult. No. KMPB 6008	— do —	No growth
	5 μg /ml	<i>Thraustochytrium</i> <i>striatum</i> Cult. No. KMPB 6008	— do —	Growth normal
Malachite green	50 μg /ml	<i>Schizochytrium</i> sp. Cult No. 55	— do —	No growth
		<i>Thrustochytrium</i> <i>motivum</i> Cult	— do —	No growth

of thraustochytrids on Medium 2 presented in Materials and Methods and by observing for zones of precipitation around the colonies, indicating lipolytic activities (Table III). Hence, the same medium was used once to isolate these organisms from the Zuari estuary (Table II).

Table III. Lipolytic activity of some thraustochytrids, as judged visually from zone of precipitation.

Isolate	Degree of Activity
<i>Thraustochytrium striatum</i>	
KMPB 6008	++++
Unidentified from decaying <i>Sargassum</i> sp.	
Cult. No. 69	++++
Unidentified from decaying <i>Sargassum</i> sp.	
Cult. No. 70	++++
<i>Thraustochytrium motivum</i>	
Cult. No. 40	++
<i>Thraustochytrium</i> sp. from <i>Hypnea</i>	
Cult. No. 62	+++

DISCUSSION

In the experience of the author, the essential features of a medium to isolate thraustochytrids seem to be : a) addition of vitamins and b) a soft agar. Earlier experiments indicated that much less or no thraustochytrids are obtained when these conditions are varied. It is now known that many thraustochytrids require one or more vitamins for growth (Bahnweg, 1979).

Similar to the experience of Bahnweg and Sparrow (1974), it has been found in the present study that Millipore filters do not favour the isolation of thraustochytrids, whereas glassfibre filters do. This might be due to the capillary retention of water by the glassfibre filters, providing more congenial conditions for the growth of these organisms.

There are some advantages and disadvantages of a plating technique such as the one employed in the present study, when compared to the pine pollen technique. In the latter, the species of the thraustochytrids could be tentatively identified even while examining the isolation flasks for the presence or absence of the thraustochytrids, while in the former technique, only differences in colony colour can be noticed and the species cannot be even tentatively identified, since most of the thraustochytrids look alike on media in isolation plates. For further identification, representative colonies will have to be sub-cul-

tured on pine pollen/seawater and the development studied. However, the total number of thraustochytrids in a sample can be estimated far more rapidly with the plating technique than with the pine pollen technique. Besides, the range of thraustochytrids that can be estimated is far greater for the plating technique. Thus, if 50 ml of water is filtered and plated, 1 colony resulting in the Petri dish would indicate 20 fungi/l, while a crowded Petri plate with 50 colonies would indicate 1000 fungi/l. This range cannot be obtained with the pine pollen technique (Gaertner, 1968). Using replicates for the estimation allow for statistical analyses in the plating technique. The pine pollen technique is still extremely useful and the choice of the method, plating or baiting will depend on the kind of work, such as quantitative or qualitative.

Mold and yeast growth can become a terrible problem, both during work on board and during the subsequent incubation for 10 days. This problem is more acute when working with coastal waters. Although probably this problem cannot be completely overcome, mold and yeast growth could perhaps be slowed down by using full strength seawater and a carbon source other than glucose. Medium 2, fulfilling these conditions was used successfully to enumerate these organisms from estuarine waters, without much interference from molds and yeasts. However, it cannot be claimed at the moment that such a medium was not selective to certain forms. It is also possible that a plating technique could be used in future with selective media to isolate thraustochytrids with different physiological characteristics.

ACKNOWLEDGEMENT

The author is thankful to Dr. T. S. S. Rao, Head, Biological Oceanography Division, Dr. V. V. R. Varadachari, Director, National Institute of Oceanography for their encouragement and to the Council of Scientific and Industrial Research for awarding the post of a Pool Officer, during the tenure of which this work was carried out. Thanks are also due to Dr. D. Chandramohan, Biological Oceanography Division, for discussions and encouragement.

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