Fungi and macroaggregation in deep-sea sediments

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Abstract

While fungi in terrestrial soils have been well studied, little is known of them in deep-sea sediments. Recent studies have demonstrated the presence of fungal hyphae in such sediments, but in low abundance. We present evidence in this study that one of the apparent reasons for the poor detection of fungi in deep-sea sediments is their cryptic presence in macroaggregates. Fungal biomass carbon from different core sections of deep-sea sediments from ~ 5000 m depth in the Central Indian Ocean was estimated based on direct microscopic detection of fungal mycelia. Treatment of sediment samples with ethylenediamine tetra-acetic acid (EDTA) enabled more frequent detection and significantly higher biomass than in samples without such treatment. Treatment with EDTA resulted in detecting various stages of breakdown of aggregates in the sediments, gradually revealing the presence of fungal hyphae within them. Experimental studies of a deep-sea, as well as 3 terrestrial isolates of fungi showed that all could grow at 200 bar and 5^{o}C in a nutrient medium as well as in deep-sea sediment extract. Hyphae of fungi grown in sediment extract under the above conditions showed various stages of accretion of particles around them, leading to the formation of aggregates. Such aggregates showed the presence of humic material, carbohydrate and proteins. We suggest that fungi in deep-sea sediments may be involved in humic aggregate formation by processes very similar to those in terrestrial sediments. The importance of such a process in carbon sequestration and food web in the deep-sea needs to be examined.

Introduction

The few studies that have been carried out on deep-sea fungi in recent years have provided mostly indirect evidence about their presence and activity, either by culturing or molecular signatures [32, 40, 14, 28]**.** Raghukumar *et al.* [33] and Damare *et al* [14] demonstrated the presence of fungal hyphae in deep-sea sediments, but admitted that they were hard to detect. It might be expected that the numerous studies on benthic meio- and microfauna and deep-sea bacteria [31, 7, 43, 26] would not have missed the presence of fungi if they were abundant. There are two possible reasons for the apparent paucity of fungal hyphae in deep-sea sediments. 1) Fungi might be present mostly as viable, but inactive spores; 2) Active fungal hyphae may be present, but in low abundance. We present evidence in this paper for yet another reason, namely that the apparent scantiness of fungi in deep-sea sediments might be due to their concealed nature in sediment aggregates, possibly of humic nature. Humic material in terrestrial soil is known to combine with soil particles to form microaggregates [8]. Fungal hyphae further act on these as binding agents to form macroaggregates by trapping fine particles into the microaggregates [22]. We show here as to how fungi might produce macroaggregation (similar to particulate organic matter) *de novo* from dissolved organic matter (DOM) under simulated deep-sea conditions.

Methods

Sample collection. A total of 36 sediment cores of ~30 cm length, on board the Russian research vessel AA Sidorenko (cruise #AAS 61) and 5 sediment cores on board the research vessel A. Boris Petrov (cruise #ABP 4) were collected from the Central Indian Basin (73-76^{o}E and 9-16^{o}S) from an average depth of 5000 m (Table 1).

Sediments were collected with an USNEL-type box corer of 50 cm^3 size. Subcores of sediments were collected from the center of the box corer with alcohol-sterilized PVC cylinders of 5 cm diameter. Subsections corresponding to 0-2, 2-4, 4-6, 6-8, 8-10, 10-15, 15-20 and 20-25 cm length were extruded from the sediment corer directly into sterile plastic bags to avoid any aerial contaminants. Sediment at the sampling sites was light to dark brown colored, consisted mainly of radiolarian oozes, and were intensely mottled indicating high bioturbation [35]. They were predominantly of a clayey-silt type with high water content and low shear strength and were loosely packed [23]. The mean grain size ($M_Z\phi$) ranged from 7.0-8.6 for the surface and from 6.6 - 8.6 for the subsurface sediments [42]. Total organic carbon (TOC) content of surface sediments was in the range of 1-5 mg g^{-1} dry sediment and 0.1–3 mg g^{-1} dry sediment in the subsurface sediments [36]. The average water content of the sediment was 595 % at 0-6 cm depth of the cores and 492 % below 6 cm (personal communication, Khadge).

Direct detection of fungal hyphae in the sediments and biomass calculations. Intact sediment samples from the centre of the subsections, each weighing 0.5 g, were suspended in 3 mL sterile seawater in sterile vials and fixed with 1 mL formalin solution (final concentration of ~ 10 %). To 0.6 mL of each fixed sediment sample, 100 μL of 0.1 % solution of filter sterilized calcofluor (Sigma Chemicals, USA), an optical brightener, was added and mixed thoroughly. Excess stain was washed off by centrifuging twice with sterile seawater. An aliquot of 50 μL corresponding approximately to 0.00625 g of sediment was placed on a microscope slide and covered with a cover glass of 22x50 mm size. The entire area of the slide preparation was examined under UV (excitation wave length 330-385 nm and barrier filter BA 420) of an epifluorescence microscope

(Olympus BX 60, Japan) to detect fluorescing fungal hyphae. Five such replicate slides were examined for each sediment subsections from all the cores. The total number of slides showing presence of fungi for any given subsection of all the stations in a cruise were pooled together and expressed as % frequency of fungal detection. Aliquots of the sediments mixed with 0.3 mL of 10 % ethylenediamine tetra-acetic acid (EDTA) solution were stained as above and estimated for % frequency of fungal detection as above [13]. The approximate length and breadth (width) of the total fungal hyphae detected under the microscope were measured directly with a calibrated ocular micrometer. Using these values, the volume of the filament was calculated assuming it to be a cylinder. The volume of the hyphae was then converted to the equivalent biomass in terms of C using the formula 1 μm^3 of fungal biomass is equivalent to 1 pg C [44]. This was then converted to µg of C g^{-1} of dry sediment [44] to obtain the contribution of fungal biomass in terms of C in those samples where fungi were detected. The differences between treatments with and without EDTA on percent frequency occurrence and fungal biomass were statistically analysed using t-test (Statistica, 5.0). Since the variability between samples was extremely high, the values for EDTA treatment were normalized to 100 % prior to the analysis.

Comparison of deep-sea and terrestrial isolates to form macroaggregates under simulated deep-sea conditions. The fungi were grown in sediment extract as the sole nutrient source. For preparation of sediment extract, deep-sea sediment sample from CIB was suspended in sterile seawater in a 1:2 ratio (w/v). To the sediment suspension, EDTA was added at a final concentration of 1 % [38] to facilitate the extraction of water-soluble nutrients into the seawater. The whole mixture was kept on a rotary shaker

overnight. In order to avoid bacterial growth, antibiotics (penicillin or ampicillin 40,000 U 100 mL^{-1} and streptomycin 0.1 g 100 mL^{-1}) were added to the sediment suspension during the extraction process. After 24 h, the suspension was filtered sequentially through sterile GF/F and 0.22 µM duraphilic filter papers (Millipore, USA). This filtrate was used as sediment extract for the following experiment.

Aspergillus terreus (# A 4634), although obtained from deep-sea sediments is a terrestrial fungus [15, 14] .Terrestrial isolates of the same species, MTCC# 279, 479 (*A. terreus*) and another species MTCC #635 (*A*. *sydowii*) obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India were also included in this study for comparison. These cultures were grown in a standard mycological culture medium, malt extract broth (MEB) containing 1.7 % malt extract and 0.3 % peptone (w/v) for 2 to 3 days and harvested just before sporulation. Malt extract agar (MEA) and malt extract broth were found to be generally suitable for isolation and growth of deep-sea fungi [14]. The fungal mycelia were homogenized using glass beads and used as inoculum. One mL of the inoculum was added to 4 mL of the sediment extract in a sterile plastic pouch. Two sets of experimental pouches were made. In the first, finely ground quartz (< 100 µm) was used as an inducer for aggregation [18] while the second set was without quartz. Both sets were incubated under hydrostatic pressure and temperature combinations of 200 bar / 30°C, 200 bar / 5°C, 1 bar / 30°C and 1 bar / 5°C to compare the effect of elevated hydrostatic pressure and low temperature on aggregate formation. The pressure and temperature in the sampling area were 490-590 bar and 3-4°C respectively. The fungi isolated from these conditions showed better growth at 200 bar pressure and therefore this pressure was chosen for further experiments. Atmospheric

pressure (1 bar) and room temperature (30^{o}C) were used as controls. A deep-sea culture vessel (Tsurumi & Seiki Co., Japan) filled with sterile distilled water and pressurized to the desirable hydrostatic pressure was used for culturing fungi under elevated hydrostatic pressure. Three replicates of each culture growing in sediment extract and MEB (1/5 strength) incubated under the above-mentioned culture conditions were used for estimating the fungal biomass as dry weight.

After incubation for 20 days under the above conditions, the contents of the pouches were fixed with formalin. The biomass was observed under the microscope for the presence of macroaggregates. The biomass obtained was examined for the presence of humic material by staining with benzidine [37], for polysaccharides with alcian blue and for proteinaceous material with coomassie blue [25].

Scanning electron microscopic observations of the deep-sea fungus #A 4634, grown in sediment extract at 200 bar pressure in the presence and absence of quartz powder was carried out. The fungal biomass was dehydrated in a graded acetone series (40-100%) and dried critically using liquid CO_2 in a critical point freeze drying apparatus (Structure Probe Inc., West Chester, PA, USA). It was sputter-coated with gold-palladium and examined under a JEOL scanning electron microscope (model 5800 LV, Japan).

Results

Direct detection of fungi in the deep-sea sediments. A range of 6-11 % of sediment subsections without EDTA treatment from the cruise #AAS 61 revealed presence of fungal hyphae after staining with calcofluor (Table 2). Sediment samples treated with EDTA prior to staining with calcofluor, revealed fungi in 6-61 % of subsections (Table 2).

In the cruise # ABP4, fungi were detected in 20-40 % of sediment subsections without EDTA treatment, whereas after treatment with EDTA a range of 20-80 % of sediment subsections revealed presence of fungi (Table 2). The difference between the treatments was highly significant ($p = 0.01$) for 0 – 8 cm subsections, but not for the entire core length for the cruise # AAS 61.However, this was not so for the sediment samples of 0-8 cm subsections from the cruise # ABP 4.

Treatment with EDTA resulted in disruption of the sediments to reveal numerous microaggregates, all of which contained fungal hyphae (Fig. 1A, 1B). Fungal carbon estimated from the total biovolume of fungal hyphae in untreated samples detected by epifluorescence microscopy ranged from 20 to 189 μg C, whereas in EDTA-treated sediment samples it ranged from 31 to 1931 μg (Table 2). In ABP4 cruise, 12-60 μg fungal carbon was present in untreated sediment samples, whereas in EDTA–treated samples this varied from 15-215 μg. The differences between the treatments were highly significant for the subsections of the entire core length in both the cruises (Table 2).

Growth of deep-sea and terrestrial fungi in sediment extract and their ability to form macroaggregates. The biomass produced by the deep-sea isolate # A 4634 in the sediment extract was almost the same as in MEB (1/5 strength) when incubated at 200 bar and 5^{o}C. The three terrestrial cultures showed better growth in MEB than in the sediment extract under similar incubation conditions (Table 3). The terrestrial cultures, except #MTCC 635, showed better growth at 1 bar and 30^{o}C than at 200 bar and 5^{o}C in sediment extract and MEB.

The fungi showed morphological differences when grown in MEB and sediment extract. The deep-sea and the terrestrial isolates when grown in MEB at 200 bar and 5^{o}C, showed abnormal swellings of hyphae (Fig. 2A), which were not seen when the cultures were grown in the sediment extract under similar conditions (Fig. 2B).

The deep-sea as well as the terrestrial fungi when grown in sediment extract showed dense aggregate formation with a network of hyphae hidden within (Fig. 3A). On treatment with EDTA fungal hyphae were clearly visible (Fig. 3B). The hyphae showed encrustations around them (Fig. 4A, B). Older hyphae were clumped together as macroaggregates when thus grown (Fig. 4C). Numerous microaggregates were connected by fungal hyphae to form complex macroaggregates (Fig. 4D). Surfaces of such hyphae were encrusted with particulate matter which stained brown indicating presence of humic substances (Fig. 5A), blue for proteinaceous matter (Fig. 5B) and exopolymeric substances (Fig. 5C). The aggregate formation was seen in the presence or absence of quartz powder when the fungi were cultured at 200 bar and 5^{o}C (Fig. 6 A, B). Aggregate formation was seen in cultures grown in sediment extract and incubated under 200 bar / 30^{o}C, 200 bar / 5^{o}C, 1 bar / 30^{o}C and 1 bar / 5^{o}C. The total organic carbon (TOC) content of the sediment extract used in the present study was 218 mg L^{-1} whereas, that of MEB (1/5 strength) was 58 mg L^{-1}. Both the media were clear and free of any particulate material at the time of inoculation. Aggregates were seen only after the growth of the fungi. Such aggregate formations were not evident in cultures grown in MEB under similar conditions (Fig. 7).

Discussion

Biomass is an important indicator of the ecological role of fungi and bacteria. Fungi may be present in sediments either in the form of dormant spores or physiologically active mycelia of indeterminate length, the latter being of relevance in biomass studies. We have attempted to detect mycelia directly under the microscope in order to determine fungal carbon biomass in the deep-sea sediments of the Central Indian Ocean. Our observations demonstrate that much of the fungal mycelia may be cryptic in deep-sea sediments, being present in aggregates that do not easily allow their detection. (Fig. 1A, Table 2). Such aggregates in terrestrial soils are formed by fungal and bacterial mediation [22]. Initially, reducing sugars and amino acids, formed as by-products of microbial metabolism undergo non-enzymatic polymerization to form a series of compounds like 3-carbon aldehydes, ketones and reductones and hydroxymethyl furfurals. These highly reactive compounds readily polymerize in the presence of amino compounds to form brown-coloured products, constituting humus [41, 38]. The humic material combines with soil particles to form microaggregates. Fungal hyphae further act as binding agents themselves to form macroaggregates by trapping fine particles of microaggregates [22]. While polysaccharide-mediated binding by fungi is an effective process for aggregate formation [2, 11], several studies have concluded that the primary contribution of fungi to aggregation is through hyphal entanglement of soil particles [27].

Experiments using deep-sea sediment extracts suggest that fungi were involved in *de novo* synthesis of aggregates from dissolved organic matter. Macroaggregates were formed from dissolved organic carbon by fungi growing under simulated deep-sea

conditions in sediment extract medium with TOC content of 218 mg L^{-1} leading to the build up of humic material. They were formed irrespective of the presence or absence of quartz powder in the medium indicating that nucleation was not required for their formation. Thus, fungi are not only hidden in such deep-sea sediment aggregates, but may also be actively involved in their formation. Aggregate formation was absent in malt extract broth which had a TOC content of 58 mg L^{-1}. The higher TOC content, as well as the chemistry of the deep-sea sediment extract might have led to aggregate formation, compared to MEB.

The increased sighting of fungi after treating the sediments with EDTA as reported here might be due to the instantaneous break up of ionic bridges formed by bivalent (Ca^{2+} and Mg^{2+}) and polyvalent (Al^{3+} and Fe^{3+}) cations in deep-sea sediment aggregates. Bivalent and polyvalent cations improve terrestrial soil structure through cationic bridging and formation of organo-metallic compounds and gels [1]. Deep-sea sediments are rich in metals [34] which form ionic bridges between sediment aggregates. The presence of aggregates concealing mycelia is well known in terrestrial soils [3]. A higher percentage frequency of detection, as well as significantly higher values of fungal biomass were obtained after EDTA treatment of the sediment samples than without (Table 2). The percent frequency of fungi and biomass in EDTA-treated samples were higher in the 0-6 cm depths which also had higher water content (Khadge, personal communication). Fungal biomass values in different subsections of the core were highly variable and generally lower in comparison to forest and grassland soils (Table 4). Fungal biovolume to biomass conversion factors used by van Veen and

Paul [44] have been used in this study. More precise conversion factors relevant to fungi growing under deep-sea conditions need to be developed.

The present observations and experiments suggest that fungi may form aggregates in deep-sea sediments. This process has several ecological implications. Biologically mediated cycling between dissolved and particulate organic matter (DOM and POM) is critical to the transfer and fate of organic material [45]. Processes transforming DOM to POM are crucial to the understanding of the cycling of organic material in the oceans. Bhaskar et al [6] have shown participation of bacteria and diatoms in formation of POM from DOM in the laboratory conditions.

Aggregate formation in sediments helps organisms residing within them in several ways. Fungi or bacteria can thus remain protected in certain particle size classes [39]. Aggregation prevents extracellular enzymes from diffusing away from the cells secreting them. Humic-enzyme-exopolysaccharide complexes have an important role to play in overall nutrient dynamics of the sediments
[9,10]. Aggregation protects the soil organic matter within water-stable aggregates, which is an important, biotically-regulated mechanism for accumulation and maintenance of organic matter in soil [17, 20, 4]. As in terrestrial soils [5], presence of large macroaggregates in deep-sea sediments with a network of fungal filaments may contribute significantly to the stabilization of soil aggregates. The polymers of fungal cell wall melanin and chitin are not easily degradable, while, phospholipids that form the main component of bacterial cell wall are readily degraded. Thus, fungal mediated C storage is expected to be more persistent in contrast to the bacterially sequestered one [3]. Understanding the relative contribution of C in the deep-sea sediments by fungi and

bacteria may allow prediction of longevity of stored C in deep-sea sediments. Such a sequestration might be enhanced by the formation of aggregates composed of humic material, as demonstrated in our experiments (Figs. 5A and C).

In addition, fungi in deep-sea sediments might also be important in the food web involving macro and meiofauna. Nematodes in terrestrial soil have been reported to be avid feeders of fungi [29, 30]. Nematodes form one of the major components of macro-benthos in deep-sea sediments, constituting more than 25 % of the benthic macro-benthos recorded in the sediments of the Central Indian Basin [21]. Fungal feeding habit of nematode is also a classification tool [29].

The significance of aggregate formation by deep-sea fungi in the above processes needs to be ascertained by further studies. More refined methods for studying deep-sea sediment aggregates, fungal biomass and their role may throw light on interesting processes in this extreme environment.

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Table 1. Details of the sediment samples collected from the Central Indian Basin during the cruises # AAS 61 and # ABP 4

Core #	Latitude	Longitude	Depth m	Date of sampling
Cruise # AAS 61				
BC 1R	09°59'661	76°29'220	5355	18-03-2003
BC 2RR	11°00'033	76°30'038	5320	30-03-2003
BC 3R	11°59'921	76°30'559	5280	20-03-2003
BC 4	13o00'032	76°29'915	5360	20-03-2003
BC 5	13°59'992	76°29'947	5180	21-03-2003
BC 6	14°59'904	76°29'895	4980	21-03-2003
BC 7	16°00'047	76°30'043	5070	22-03-2003
BC 8	15°59'981	75°30'000	5010	22-03-2003
BC 9	15°59'981	74°29'907	5350	23-03-2003
BC 10	15°59'775	73°29'999	4900	23-03-2003
BC 11	14°59'694	73°29'961	5480	23-03-2003
BC 12	15°00'011	74°30'026	5390	24-03-2003
BC 14	14°00'231	75°30'064	5145	25-03-2003
BC 15	14°00'180	74°30'457	5160	25-03-2003
BC 16	14°00'315	73°30'038	5120	26-03-2003
BC 17	13°00'317	73°30'038	4810	26-03-2003
BC 18	12°59'963	74°29'647	5050	27-03-2003
BC 19	12°59'769	75°29'688	5070	27-03-2003
BC 20	12°00'066	75°29'893	5200	28-03-2003
BC 21	11°59'986	74°29'428	5025	28-03-2003
BC 22	12°00'011	73°29'819	4900	28-03-2003
BC 23	10°59'955	73°29'937	5100	29-03-2003
BC 24	10°59'955	74°29'927	5050	29-03-2003
BC 25	10°59'964	75°29'842	5300	30-03-2003
BC 26	09°59'988	75°29'959	5290	04-03-2003
M3 BC 2	10°02'293	76°01'076	5270	31-03-2003
M3 BC16	10°02'596	76°00'953	5260	31-03-2003
M3 BC 11R	10°02'071	76°01'260	5320	01-04-2003
M3 BC 3	10°02'072	76°00'575	5270	01-04-2003
M3 BC 7	10°02'310	76°00'464	5310	01-04-2003
M3 BC 8	10°01'776	76°00'778	5280	02-04-2003

M3 BC 12	10°01'335	76°00'569	5280	02-04-2003
M3 BC 5	10°01'787	76°00'130	5280	02-04-2003
M3 BC 14	10°02'197	76°00'152	5270	02-04-2003
M3 BC 13	10°01'501	76°00'357	5283	03-04-2003
M3 BC A1/AR	10°00'322	76°06'646	5280	04-04-2003
Cruise # ABP 4				
BC 3	12°00'012	76°29'992	5359	12-04-2005
BC 8	16°00'006	75°29'999	5210	20-04-2005
BC 19	13°00'022	75°30'016	5096	16-04-2005
BC 25	10°59'997	75°29'999	5292	06-04-2005
M4 BC A1/B	10°10'536	76°03'864	5340	08-04-2005

Table 2. Direct detection and total fungal C in various depths in all the cores.

Sub-section (depth cm)	Cruise # AAS 61				Cruise # ABP 4			
	% frequency of detection of fungi (without EDTA treatment)	% frequency of detection of fungi (with EDTA treatment)	Fungal C (µg) in sediments showing presence of fungi (without EDTA treatment)	Fungal C (µg) in sediments showing presence of fungi (with EDTA treatment)	% frequency of detection of fungi (without EDTA treatment)	% frequency of detection of fungi (with EDTA treatment)	Fungal C (µg) in sediments showing presence of fungi (without EDTA treatment)	Fungal C (µg) in sediment showing presence of fungi (with EDTA treatment)
0 – 2	3	21	146	1931	8	24	60	215
2 – 4	2	7	98	355	0	0	ND	ND
4 – 6	3	9	189	455	4	0	26	ND
6 – 8	2	3	107	230	0	4	ND	15
8 – 10	2	1	34	31	4	8	12	51
10 – 15	1	0	23	ND	0	16	ND	102
15 – 20	2	2	20	107	4	4	22	15
20 – 25	2	2	26	67	0	0	ND	ND

ND - not detected;
T-tests were performed after normalizing the EDTA-treated values to 100 %.
T-test results for % frequency for AAS 61, 0 – 8 cm depth: d.f. 7; p value 0.01; significant at 1 % level.
T- test results for fungal C for AAS 61: d.f. 6; p value 0.003; significant at 1% level.
T- test results for fungal C for ABP 4 : d.f. 6 ; p value 0.012; significant at 1% level.

Table 3. Comparing growth of the deep-sea and terrestrial fungi in malt extract broth and sediment extract medium under various pressure and temperature combinations.

Culture	Sediment extract medium		Malt extract broth	
	1 bar / 30°C	200 bar / 5°C	1 bar / 30°C	200 bar / 5°C
	Biomass produced (mg)			
A 4634 (deep-sea mycelial fungus)	5.2 ± 2.6	24.9 ± 6.3	31.4 ± 3.9	29.1± 4.4
MTCC 279	28.4 ± 5.1	8.2 ± 2.8	28.0 ± 3.7	17.8 ± 3.8
MTCC 479	20.6 ± 3.3	17.4 ± 4.4	40.6 ± 2.9	29.2 ± 3.1
MTCC 635	6.2 ± 3.2	7.4 ± 3.6	8.3 ± 1.9	7.8 ± 3.1

Table 4. Comparison of CFU, fungal biovolume and carbon contribution to sediments of different habitats

Source	Location	CFU	Biomass (fungal carbon in μg g^{-1} dry sediment)	Reference
Sea water	Arctic	1000-3000 L^{-1}	---	[19]
Forest Soil	Brazil	50-75x10^4 g^{-1}	---	[46]
Grassland soil	Denmark	---	36	[24]
Grassland soil	Not mentioned	---	453	[12]
Forest soil	Not mentioned	---	3375	[12]
Forest soil	Denmark		250-3500	[16]
Deep-sea sediments (~5000 m depth)	Central Indian Basin	*18-2130 g^{-1}	7-54 μg g^{-1} dry sediment	Present work

* unpublished results

Legends to figures

Fig. 1. Direct detection of fungal hyphae from the deep-sea sediments (A) without ethylenediamine tetra-acetic acid (EDTA) treatment and (B) after treatment with EDTA. This was followed by staining with calcofluor and viewing under the UV light of an epifluorescence microscope. Bar = 10 µm.

Fig. 2. Growth of the deep-sea isolate *Aspergillus terreus* in (A) malt extract broth and (B) sediment extract grown under hydrostatic pressure of 200 bar and 30°C. Bar = (A) 5 µm, (B) 5 µm.

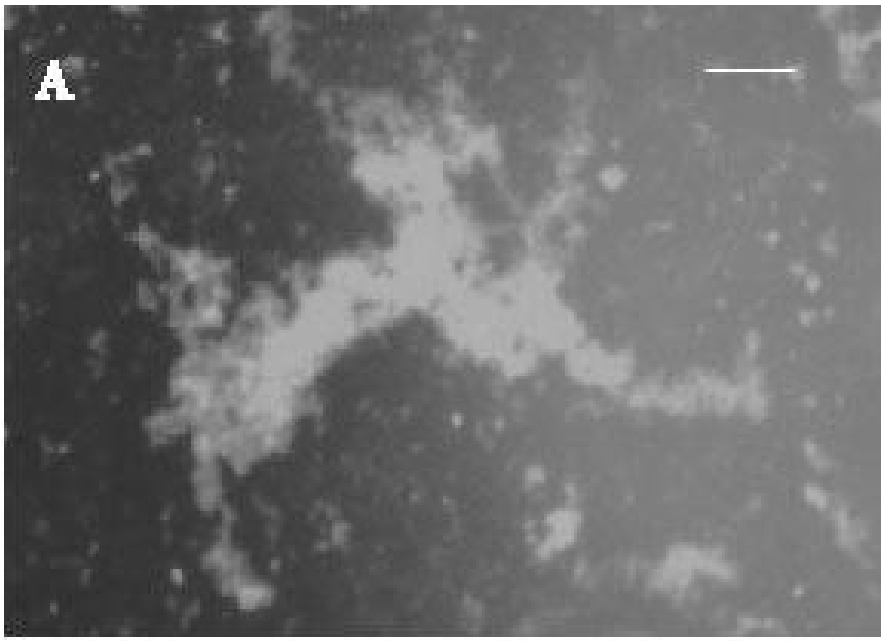
Fig. 3. The deep-sea fungal isolate # A 4634 grown in sediment extract at 200 bar 30°C, and viewed under the ultraviolet light of an epifluorescence microscope (A) without EDTA treatment and (B) after treatment with EDTA. Bar = 10 µm.

Fig. 4. The sequence of events leading to aggregation of fungal hyphae grown in sediment extract at 200 bar and 30°C is shown in (A) to (D). (A) and (B) show hyphae with numerous particles on the surface of hyphae. (C) a number of hyphae combined together to form a thick bundle along with the precipitate. (D) a complex network formed by the fungal hyphae along with the precipitate. A, B & D Bar = 5 µm; C = 2 µm.

Fig. 5. The aggregate produced by the deep-sea fungus # A4634 at 200 bar and 30°C (A) stained brown for humic substances, (B) blue for proteins and (C) blue for exopolymeric substances. Bar = (A) 10 µm, (B) 5 µm, (C) 10 µm.

Fig. 6. The deep-sea fungus # A4634 observed under scanning electron microscope after growing in sediment extract at 200 bar and 30°C and examined (A) without EDTA and (B) after EDTA treatment. Magnification in (A) = x1,300; (B) = x4,500.

Fig. 7. Growth of the deep-sea fungus # A4634 in malt extract broth showing absence of aggregate formation. Bar= 10 µm



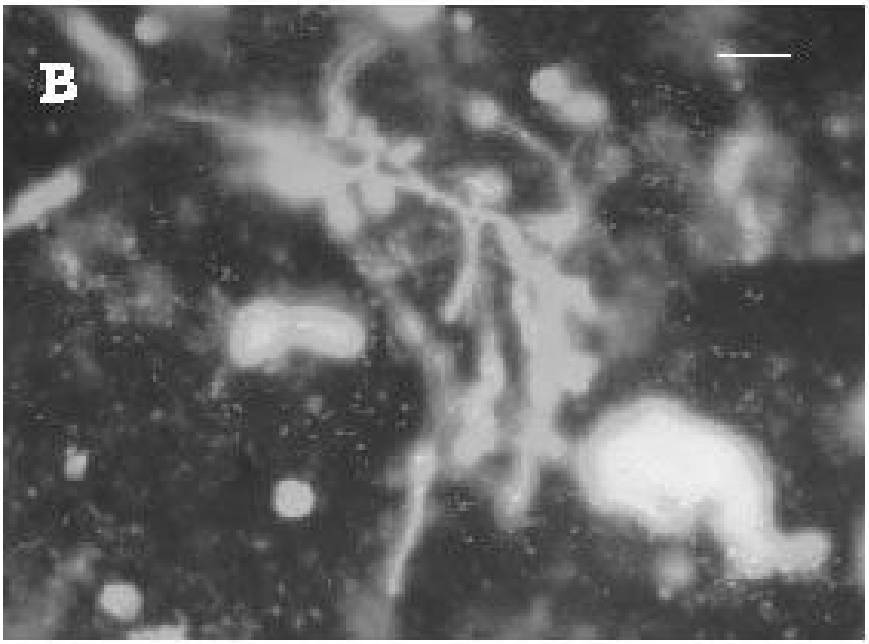


Fig. 1

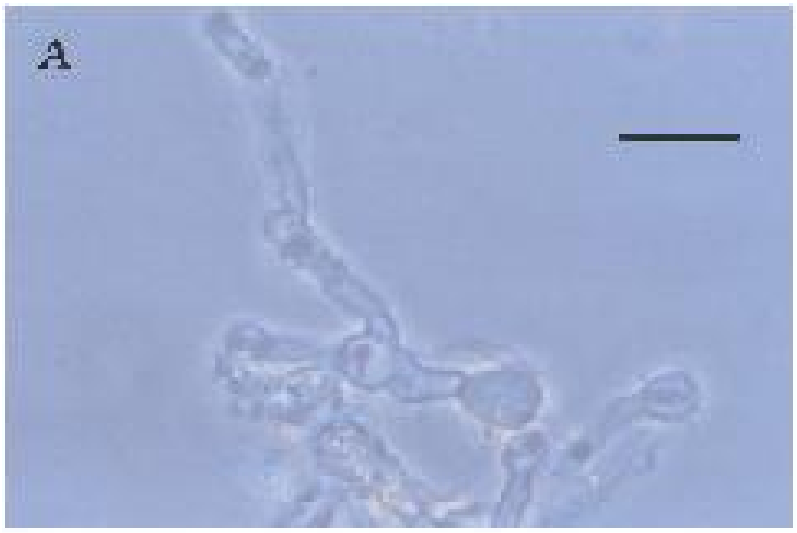


Fig. 2

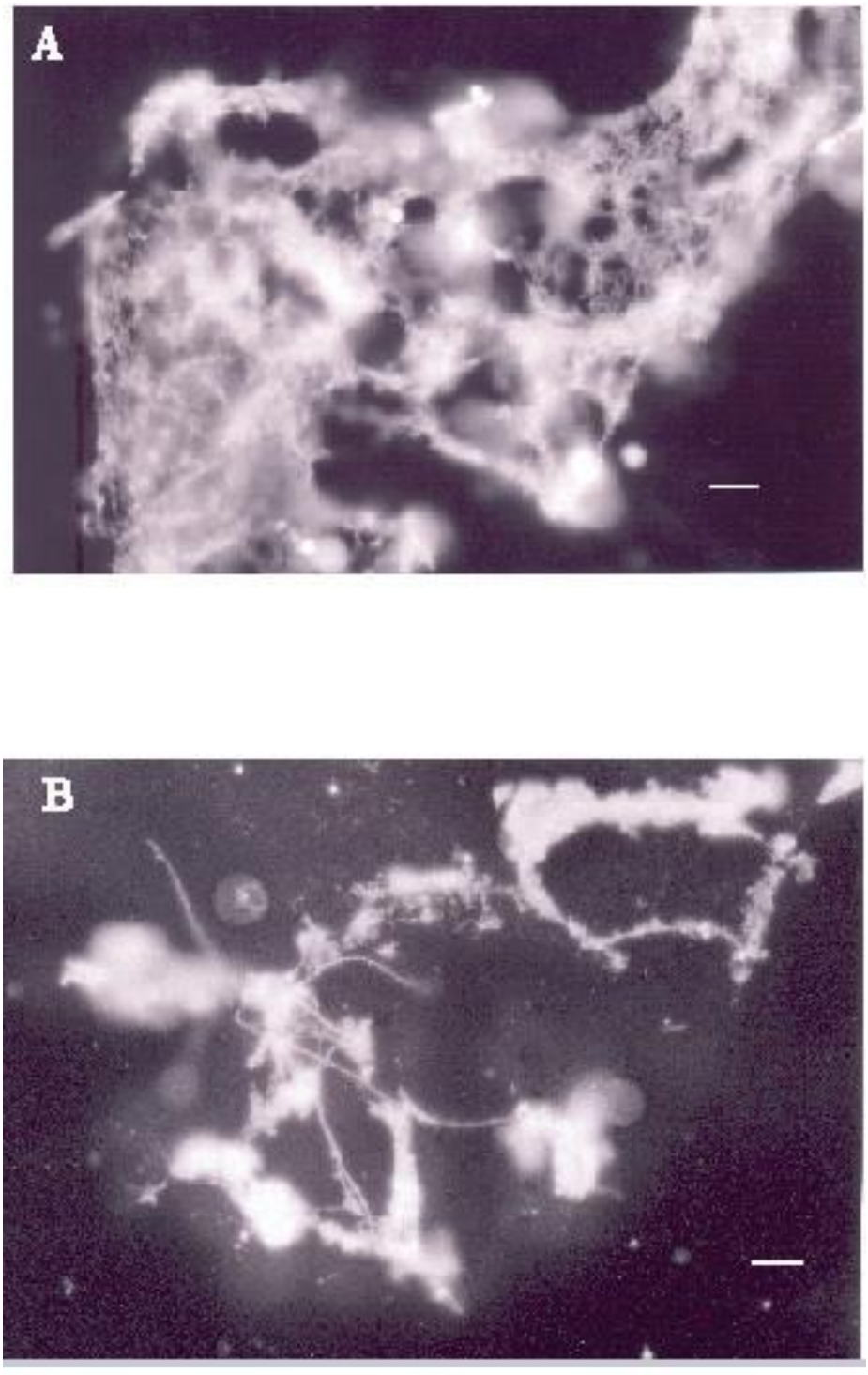


Fig. 3

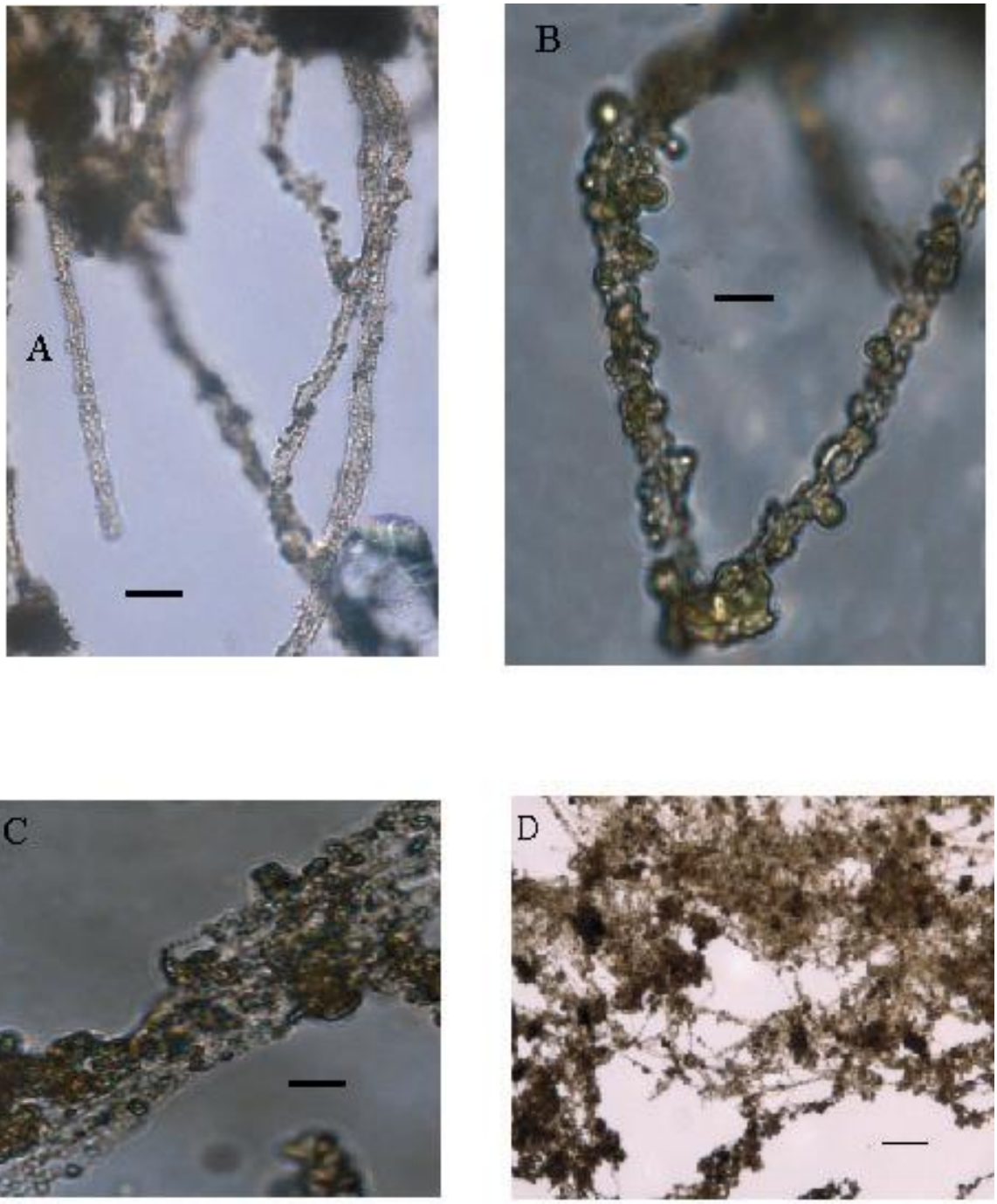
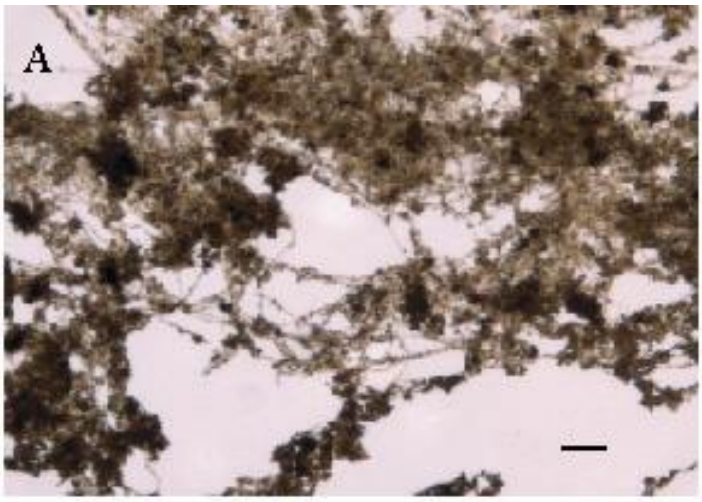
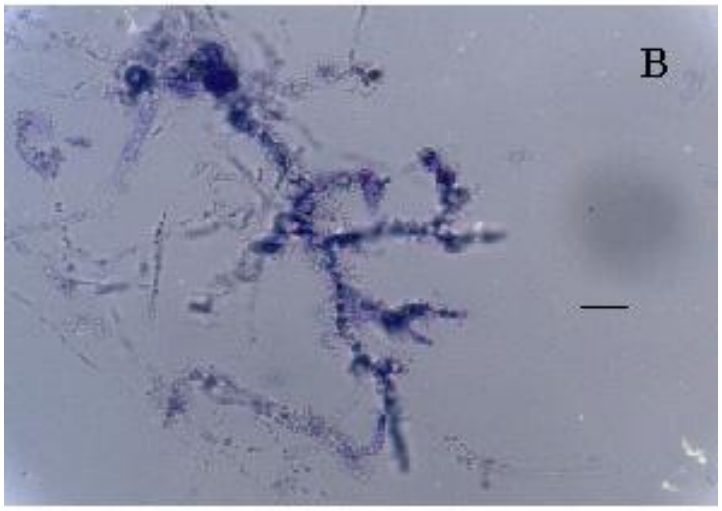


Fig. 4





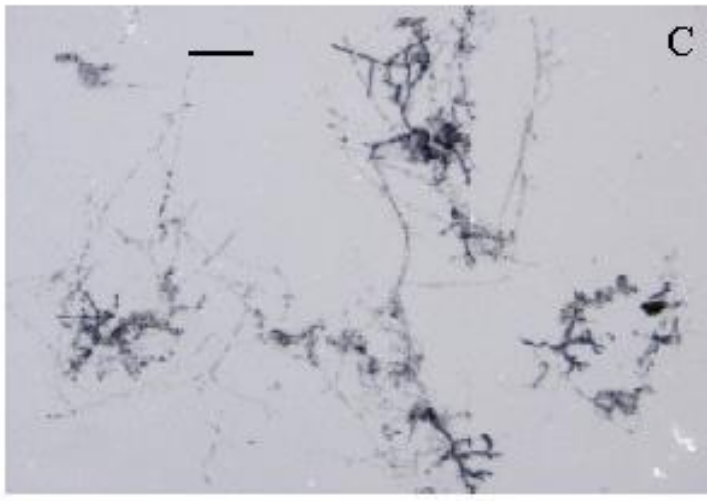


Fig. 5

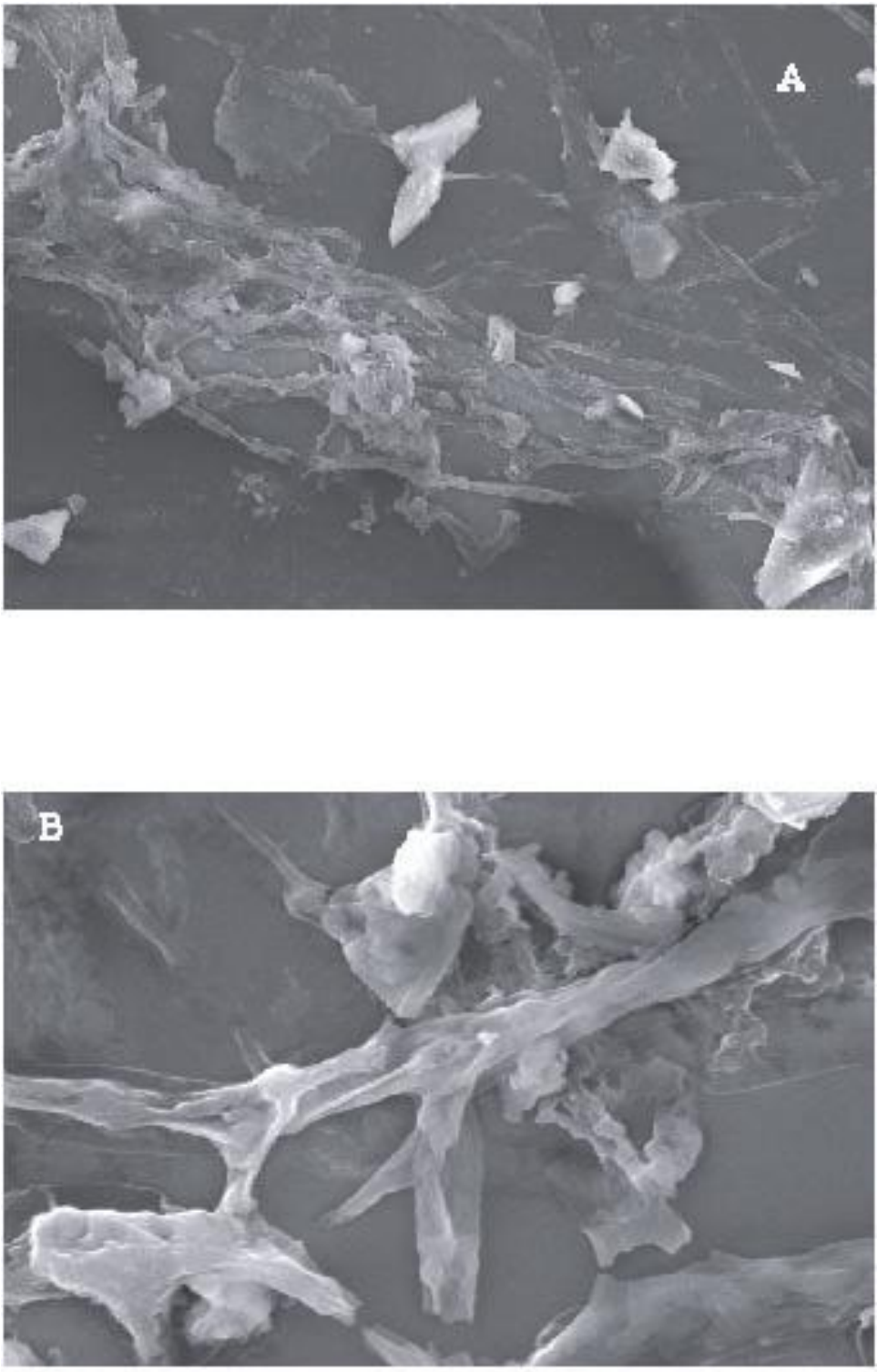


Fig. 6

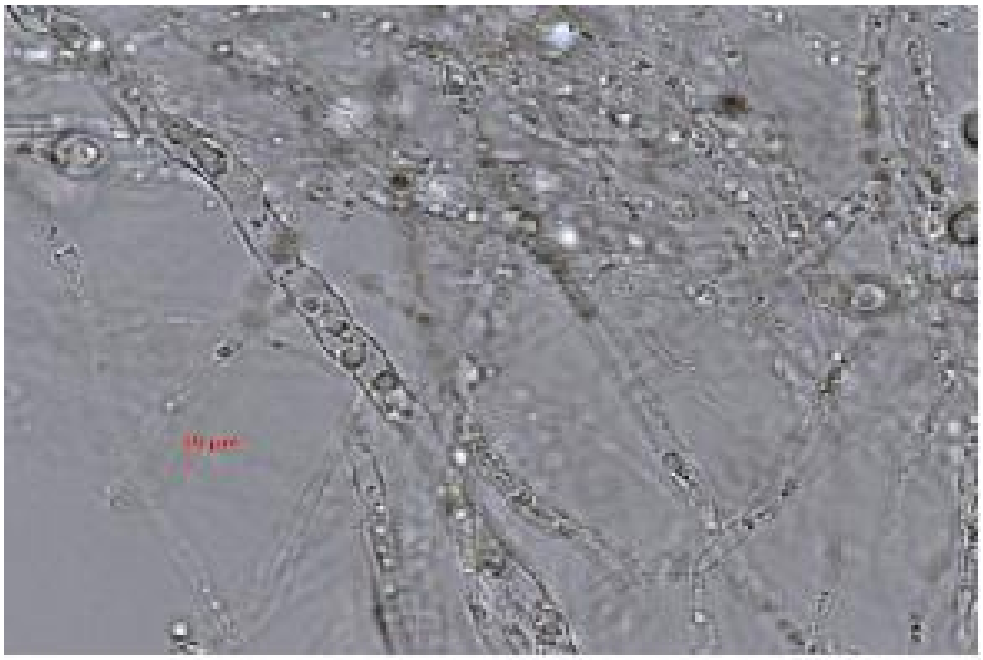


Fig. 7