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ISOLATION AND CHARACTERIZATION OF TEN MYCELIAL ORGANISMS FROM DIFFERENT AQUATIC HABITATS OF DHAKA, BANGLADESH

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Abstract

Aquatic mycelial organisms, belonging to Actinobacteria were isolated from 12 samples collected from eight sites of Dhaka Metropolitan City. From a total of 39 isolates, ten were studied in detail and their identification was attempted. Out of the ten isolates seven belonged to the genus *Streptomyces* and the remaining three were members of *Pseudonocardia*, *Microbispora* and *Saccharomonospora*. The pigment produced by strain DN-10 was pH dependent and it changed from red in acidic to blue in alkaline condition. The optimum temperature for growth of the isolates was in between 30 and 37°C. Strain B-3 and DN-13 were found to be pectin-degraders.

Introduction

Microorganisms grow wherever they find their nutrients and abode. Aquatic habitats including oceans, streams, rivers, springs, lakes, ponds and reservoirs contain and innumerable numbers of microbes of different kinds. Submerged plant parts, even aquatic animals also serve as habitat for microorganisms. The microorganisms, which are composed of a mass of thread like branched filaments are called mycelial organisms (Alexopoulos and Mims 1989). The mycelial habit is quite common in microorganisms. In filamentous bacteria the filamentous habit is due to the absence of cross wall formation or to the absence of cell separation (Brock 1966). Actinomycetes comprise a group of branching coenocytic, unicellular, achlorophyllous microorganisms. They form a mycelium which may be of a single kind, designated as substrate or vegetative; or of two kinds, substrate mycelium and aerial or reproductive mycelium (Waksman 1967). The recently proposed class *Actinobacteria* (Stackebrandt *et al.* 1997) is comprised of high G + C content Gram-positive bacteria and includes the actinomycetes (in the order Actinomycetales). None of Actinomycetales is sheathed, stalked or photosynthetic. Members of Actinomycetales are found almost everywhere, and the largest reservoir of these microorganisms is soil. Actinomycetes are found in the mud at the bottom of ponds, lakes, streams, and rivers. They are also found in fresh water, although the numbers are extremely low (Labeda and Shearer 1990). Work on aquatic mycelial organisms is very scanty in Bangladesh and thus this is an unexplored field of research. The organisms isolated from our aquatic habitats could possibly produce novel enzymes, metabolites and antibiotics.

Materials and Methods

Water, sediment and submerged twigs of plants were collected as samples from eight different sites of Dhaka Metropolitan city. Serial dilution plate technique and direct inoculation method were used for the isolation of mycelial organisms. For the isolation of these organisms different types of culture media *viz.* Oat meal agar (Shirling and Gottlieb 1966), glucose asparagine agar (Waksman 1967), starch-casein agar (Küster and Williams 1964), modified Bennett's agar (Iwasa *et al.* 1970) nutrient agar (NA) and nutrient broth (NB) media were used. The pH of the collected samples ranged from 6.4 -7.8 and the pH of the isolation media were adjusted between 7.2 - 7.4.

The media used for morphological studies were yeast extract malt extract agar (ISP-2), oat meal agar (ISP-3), inorganic salt-starch agar (ISP-4), glycerol asparagine agar (ISP-5), tyrosine agar (ISP-7), nutrient agar (NA) and modified Bennett's agar (mBA). The amount of growth,

sporulation, aerial mass colour, reverse colour and diffusible pigment of the selected isolates were recorded. The pattern of growth and the arrangement of spores were studied under microscope. Photomicrographs were taken with a Nikon Optiphot, microscope fitted with photomicrographic attachment (UFX-II A, Nikon, Japan).

According to the methods suggested in the Bergey's Manual (Sneath *et al.* 1986 and Williams *et al.* 1989) the physiological and biochemical tests of the isolated mycelial organisms were carried out. Manual of Microbiological Methods (SAB 1957) and Understanding Microbes (Claus 1995) were also consulted.

Results and Discussion

Thirty nine aquatic mycelial organisms hence forward designated as strains, were isolated from different aquatic habitats of Dhaka Metropolitan city. During this study samples from three sites *viz.* Dhanmondi lake, Bangla Academy pond and a water tank, yielded growth of mycelial organisms. Oat meal agar medium was found better for the isolation of aquatic actinomycetes.

Seven-day-old cultures of the isolated strains were studied for their morphological characters (Table 1). The strain B-1 showed better growth on NA and revealed an abundant aerial mycelium. Spiral spore chains were observed in strains DN-1, DN-6, DN-10 and S-3. Retinaculiaperti spore chain was observed in strain DN-5 and rectiflexibiles spore chains were present in strain DW-2 and B-3. The spores were spherical and non-motile, but those of B-1 were sub-spherical (Fig. 1). All the isolated organisms were Gram-positive.

Cultural characteristics of the ten strains are shown in Table 1. All the strains showed better growth in ISP-4 and mBA media. But strain B-1 grows only in NA and ISP-5 media, better growth was observed in NA medium. Strains DN-1, DN-6, DN-10, DN-13, DW-1 and S-3 produced diffusible pigment in different media, strains No. DN-5, B-1 and B-2 did not produce diffusible pigment.

Physiological and biochemical characteristics of ten strains are shown in Table 2. All the tested strains were found to produce amylase. Strain No. DN-13 and B-3 could hydrolyze pectin. Out of ten strains only DN-1 and DN-10 were able to produce melanoid pigment and hydrogen sulfide.

Most of the isolated strains showed sensitivity towards antibiotics except penicillin G 10 µg (P-10). DN-1, DN-5 and B-3 were totally resistant to penicillin.

Effect of four growth inhibitory compounds *viz.* crystal violet, phenol, sodium azide and sodium chloride were tested on the selected strains. Phenol (0.1%) could not inhibit the growth of DN-5 but this compound inhibited remaining selected strains. Out of ten strains six were inhibited by crystal violet at a concentration of 0.0001% (w/v). Sodium azide was used in two different concentrations *viz.* 0.01 and 0.02%. All the strains except DN-10 grew well at these two concentrations (Table 2).

Tresner *et al.* (1968) suggested differential tolerance to NaCl of streptomycetes as a taxonomic criterion. They also reported that those species which did not produce melanin, are more NaCl tolerant than melanin-producing species. In this study, tolerance of selected isolates to NaCl at different concentrations was tested. Only two isolates (B-1 and B-3) isolated from only one habitat could tolerate up to 13% NaCl (Fig. 2). Strain DN-1 is melanin producer and it could tolerate only 4% NaCl. On the other hand, strain DN-10 also produced melanin and could tolerate 7% NaCl and were growth was observed at 10 and 13% NaCl.

Temperature is one of the physical factors that governs the distribution and activities of actinomycetes in natural habitats. Goodfellow and Williams (1983) reported that most of the actinomycetes behave as mesophiles with an optimum growth at 30ºC. There were also thermo-tolerant and thermophilic actinomycetes (Li-Hua Xu *et al.* 1998). In the present study, ten strains

were grown at different temperatures. None of them were able to grow at 4 and at 50ºC except DW2. The optimum temperature for growth of most of the selected strain was between 30 and 37°C (Fig. 3). This result showed similarities with the work of Goodfellow and Williams (1983). All the isolates were able to grow at 45°C.

Table 2. Physiological and biochemical properties of the isolated ten mycelial organisms.

Tests	Strains									
	DN-1	DN-5	DN-6	DN-10	DN-13	DS-2	DW-2	S-3	B-1	B-3
Nitrate reduction	+	-	-	+	+	+	+	+	+	-
Lecithinase activity	+	-	+	-	+	-	+	+	-	-
Proteolytic activity	-	+	+	+	+	+	+	+	+	+
Citrate utilization	+	+	-	+	-	+	-	+	-	-
Hydrolysis of:										
Starch	+	+	+	+	+	+	+	+	+	+
Casein	-	+	+	-	+	+	+	+	-	-
Gelatin	-	-	-	+	+	+	-	+	+	+
Esculin	+	+	+	+	+	-	-	+	+	-
Pectin	-	-	-	-	+	-	-	-	-	+
Production of:										
Melanoid pigment	+	-	-	+	-	-	-	-	-	-
Hydrogen sulfide	+	-	-	+	-	-	-	-	-	-
Indole	+	+	+	-	+	+	+			
Decomposition of:										
Urea	-	-	-	-	-	-	-	+	+	+
Xanthine	-	+	+	+	-	-	-	+	+	-
Tyrosine	+	+	+	-	+	+	-	+	-	-
Resistance to:										
Neomycin (50 µg/ml)	-	-	-	-	-	-	-	-	-	-
Rifampicin (50 µg/ml)	-	-	-	-	-	-	-	-	-	-
Penicillin G (10 µg/ml)	+	+	-	-	-	-	-	-	-	+
Growth at 45°C	+	+	+	+	+	+	+	+	+	+
Growth at (% w/v)										
NaCl (7.0)	-	-	+	+	+	+	-	Trace	+	+
Sodium azide (0.01)	+	+	+	-	+	+	+	+	Trace	+
Phenol (0.1)	Trace	+	-	-	-	-	-	Trace	-	-
Crystal violet (0.0001)	+	+	-	+	+	-	-	-	-	-
Growth at pH										
4.5	+	-	-	+	-	-	-	+	-	-
6.5	+	+	+	+	+	+	+	+	-	+
8.5	+	+	+	+	+	+	+	+	+	+
Acid from:										
Fructose	-	+	-	+	-	-	-	-	+	-
Glucose	+	+	+	+	+	+	-	-	+	-
Galactose	+	+	+	+	+	+	-	+	+	-
Inositol	-	-	+	-	-	-	-	-	+	-
Inulin	+	+	+	+	+	+	-	+	+	-
Lactose	-	+	-	+	-	-	-	+	-	-
Maltose	-	-	-	+	-	-	-	+	+	-
Mannitol	-	+	+	+	-	+	-	+	+	-
Salicin	+	+	+	+	+	+	-	+	+	-
Sucrose	-	-	+	+	-	-	-	+	+	-
Trehalose	+	+	+	+	-	+	-	+	+	-

'+' indicates positive hydrolysis, production, decomposition, resistance, growth and acid production, nitrate reduction, lecithinase activity, proteolytic activity and citrate utilization and '-' indicates negative of those.

Goodfellow and Williams (1983) reported that most of the actinomycetes behave as neutrophiles in culture, growing between pH 5.0 and 9.0 with an optimum close to neutrality. Acidophilic and acidoduric strains had been isolated from acidic soils and other materials (Williams *et al.* 1971, Khan and Williams 1975). pH is clearly a major factor for determining their

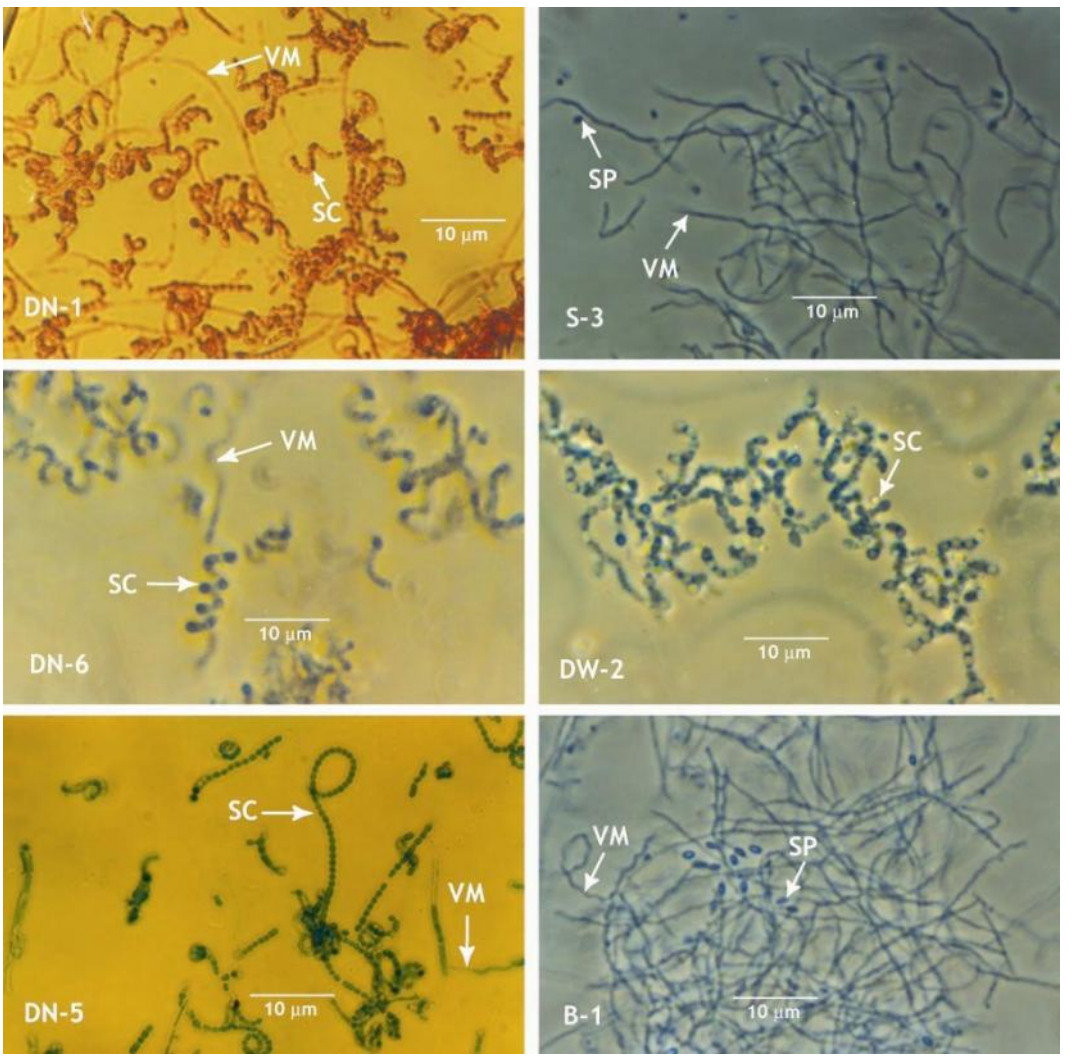

Fig.1. Photomicrographs of the six isolated mycelial organisms DN-1 and S-3 (*Streptomyces violaceusniger);* DN-5 *(S. fragilis);* DN-6 (*S. flaveolus);* DW-2 (*Streptomyces purpureus),* B-1 (*Pseudonocardia thermophila).* Bar = 10 µm. SC = spore chain; VM = vegetative mycelium; SP = spore .

distribution and activity. The growth responses of ten strains were tested at different pH levels, ranging from 4.5 to 8.5. The optimum growth of the selected isolates was close to neutrality. In both media at pH 4.5 and 6.5 DN-10 produced red pigment but at pH 8.5 the pigment is blue. It produced brown colonies at pH 4.5, blue colonies at pH 6.5 and white colonies at pH 8.5.

The morphological, physiological and biochemical characters of the ten selected strains were recorded and compared with those of the standard organisms as described in the Bergey's Manual of Systematic Bacteriology (Williams *et al.* 1989). Strain DN-1 and S-3 closely resembled *Streptomyces violaceusniger*, while DN-5 showed resemblance with *Streptomyces fragilis.* DN-6 was related to *Streptomyces flaveolus*, DS-2 to *Microbispora bispora*, DW-2 to *Streptomyces purpureus*, B-1 to *Pseudonocardia thermophila* and B-3 resembled *Streptomyces halstedii.*

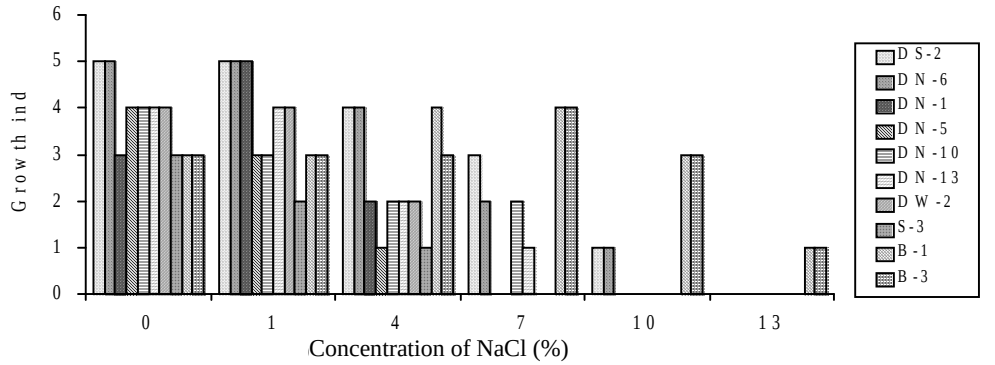

Fig. 2. Growth response of ten selected strains at different concentration of NaCl. In growth index 'O' as not growth and '5' as maximum growth.

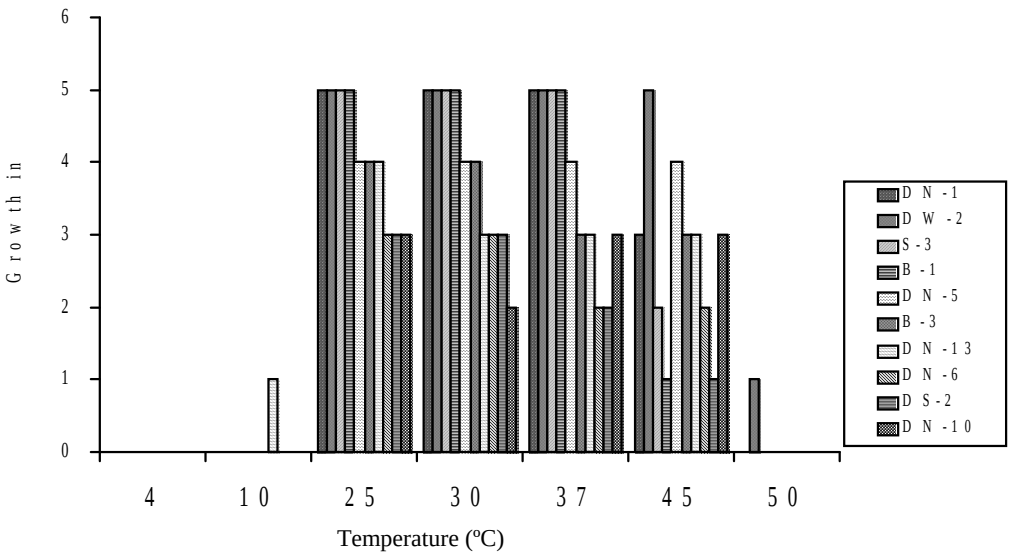

Fig. 3. Growth response of ten selected strains towards different temperature. In growth index 'O' as not growth and '5' as maximum growth.

Strain DN-10 was a member of genus *Streptomyces* but it could not be identified up to the species level. Similarly, DN-13 closely resembled the genus *Saccharomonospora*.

The present work was designed to study, isolate and characterize of aquatic mycelial organisms growing in Dhaka Metropolitan city. The potentiality of this group of organisms is yet to be worked out.

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