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The biodiversity of *Actinomycetes* in the River Nile exhibiting antifungal activity

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Abstract

The taxonomic analysis of 114 actinomycete strains isolated from water of the River Nile and its bottom sediments showed that most of the water isolates belonging the genus *Streptomyces*. As well as, the majoring of the sediment isolates belongs to the genus *Micromonospora*. The overwhelming majority of *Streptomyces* species are *Str. antibioticus*, *Str. aunlatus*, *Str. violaceus* and *Str. antimycoticus*. *M. carbonaceae* and *M. purpureochromogenes* were also identified. Of 68 *Streptomyces* strains obtained, 11-exhibited significant antimycotic activity. Among the test fungi examined, *Aspergillus niger* and *Trichoderma viride* proved to be the most susceptible to the active substance present in the fermentation broths of *Streptmyces* strains. These active substances seem to be polyenes.

Introduction

Actinomycetes are widely spread in various bodies of water, where they play a great part in the carbon cycle due to their ability to grow at low concentrations of carbonaceous substances and to degrade recalcitrant organic matter (Kuznetsov, 1970). In recent years there has been a growing an awareness of the potential value of fresh water habitat as source of actinomycets that produce useful metabolic products. In fact some investigators emphasized that freshwater habitat are fruitful as those isolated organisms from terrestrial locales (Cross, 1981). A review of literature reveals that little is known concerning the actinomycetes exhibiting antifungal from this habitat. New sources of antimycotic agent would be welcome, particularly in view of the opportunistic capabilities of yeast and moulds in patients afflicted with terminal disease. It is obvious that agents currently available for the treatment of systematic fungal infections leave much to be desired (Medoff et al. 1983).

Actinomycetes in the River Nile are as yet poorly studied. The aim of the present work is to study the biodiversity of actinomycetes in water of the River Nile and its bottom sediments at Giza and Cairo and examinating the antimycotic activities of streptomycete isolates.

Material and methods

Sample collections

Water and bottom sediments were obtained over a period from January 2002 to June 2002. The samples were taken from Giza and Cairo sites. Nile water samples were collected from the surface layer and taken into 1 L sterilised flasks plugged with covered cotton plugs and aluminium foil. Bottom sediment samples were obtained using Eckman- Birge grab sampler.

Plating, isolation, characterization of the isolates

The samples were stored at 4 °C and analysed by plating their serial dilutions onto the selective agar media starch casein agar (Kuster and Williams, 1964), Malt – yeast extract agar (Pridham et al. 1956- 1957) and chitin agar (Hsu and Lockwood, 1975). All plates were incubated at 28 °C

for 2-3 weeks. The actinomycetes colonies were isolated and subjected to purification. The isolates were identified on the basis of their morphological and chemotaxonomical characteristics according to the method given by Bergey's Manuals (1994). The data obtained were introduced to the computer using SPSS program for similarity calculations. Selected strains were subjected to partial or full 16S rDNA analysis in order to confirm the determinative work (Ludwing and Strunk, 1997 and de Soete, 1983).

Fermentation

Streptomycetes, which isolated from water of the River Nile and its bottom sediments, were grown in submerged culture of seed medium and the supernatant broths and mycelial pellet were retained for testing purposes using the method of Tunac et al. (1985).

Determination of antifungal activity

Two yeasts *Candida albicans* and *Candida tropicalls* and three filamentous fungi *Aspergillus niger*, *Fusarium oxysporium* and *Trichodrma viride* were used as a test species for the detection of antifungal activity of the streptomycete isolates. The antifungal activity was determined by the plate diffusion method (Bauer et al. 1966).

Nature of antifungal activity

Approximately 5 ml of the centrifuged fermentation broths were extracted with an equal volume of n-butanol after which absorption spectra, in the UV and visible region (190-450), were determined using Beckman DU-8 spectrophotometer. Measurement of the absorption spectra were utilised to determine any of the antimycotic compounds detected were polyenes.

Results and discussion

Using the selective media and cultivation conditions described previously 114 streptomycete strains were isolated (Table 1). Most of the actinomycete strains belonging to the genera *Streptomyces* and *Micromonospora* were obtained using starch containing agar medium. The data of taxonomic analysis based on morphological characteristics of the actinomycets present in the water and sediments are shown in Table (2). The River water was dominated by representatives of the genus *Streptomyces* (83% of all water isolates) while the River sediments were common by genus *Micromonospora* (77 % of all sediment isolates). Using the polyphasic identification method, three streptomycete clusters were identified as *Str. antibioticus*, *Str. anulatus*, and *Str. antimycoticus* while five strains were identified as *Streptomyces* sp. The domination of streptomycetes in the River water can be explained by the access of the river water and run off from the surrounding soils, or may be due to the selective media used in the isolation procedure. It should, however, be noted that some streptomycetes isolated from the bottom sediments imply that the isolated streptomycetes may be only of allochthonous but also of autochthonous origin (Cross, 1982).

In case of *Micromonospora* isolates *M. carbonacae*, *M. purpureochromogenes* and *Micromonospora* sp. were detected. The dominance of *Micromonspora* isolates in the River sediments (Table 1) may be due to that this genus can find the particular microhabitats which satisfying their ecological requirements. Species of both genera may play an important part not only in their transformation of inorganic and organic compounds of the bottom sediment but also in secreting bioactive compounds into the sediment and water respectively.

A total of 11 strains of streptomycete, out of the 61 streptomycete isolate exhibited significant antifungal activity (Table 3). All strains except NR 41 inhibited *C. tropicalls*. Three isolates NR 24, NR 42 and NR 61 failed to inhibit *C. albicans*. Variable inhibitory patterns were evident when filamentous fungi were used as the test species. Strains NR5 and NR 58 proved to be inhibitory to *F. oxysporium* but not to *A. niger* or *T. viride*. On the other hand, isolates NR 42, NR 43 and NR 59 displayed activity against both *A. niger* and *T. viride* but not on *F. oxysporium*. The filamentous test organisms (*A. niger*) not inhibited by strains NR5 and NR 58.

The importance of polyene antibiotic in antifungal therapy (Medoff et al., 1983) prompted use to determine if all or some of the active substances reported in the present investigation was polyenic in nature. Table (4) illustrates that polyene chromophore groups were detectable in most of the broths and / or mycelia of the active streptomycetes. Specifically, 7 of 11 isolates were associated with polyene moieties. By far, the heptanes proved to be the most common group found; 7 of the strains elabo-

Table (1). Number of different actinomycete colonies grown on various selective media.

Medium	Strain number
Starch casein agar	58
Chitin agar	16
Melt yeast extract agar	40

Table (2). The taxonomies composition of the actinomycets present in water and its bottom sediments of River Nile.

Morphological characteristics	CFU number		Number of isolates		Genus
	Water	Sediment	Water	Sediment	
Polysporic actinomycetes, forming characteristic aerial and substrate hyphae	49	32	41	27	*Streptomyces*
Mostly monsporic actinomycetes, forming colour spore mass over round deep sitting colonies	22	31	20	24	*Micromonospora*

Table (3). Antifungal activity of strains of streptomycetes isolated from River Nile.

	Strain no.	*C. albicans*	*C. tropicalls*	*As. niger*	*F. oxysporium*	*T. viride*
Water	NR 1	2+	+	3+	2+	3+
	NR 5	+	+	-	+	-
	NR 11	+	2+	3+	2+	2+
	NR 23	2+	3+	+	2+	2+
	NR 24	2+	2+	2+	+	+
	NR 25	-	2+	3+	3+	3+
Sediment	NR 42	+	+	+	+	+
	NR 43	-	2+	2+	2+	3+
	NR58	2+	+	-	-	-
	NR 59	+	2+	2+	2+	2+
	NR 61	-	-	2+	2+	+

+ = Zones of 15- 20 mm2+= Zones of 21 – 26 mm
3+ = Zones of 27 – 32 mm - = no inhibition

rating this class of polyene. The pentanes, found in conjunction with two of the isolates. Of particular interest two streptomycete isolates NR58 and NR59, were apparently did not synthesize polyene-like substances. These strains have been selected for additional studies to analyze the nature of the active substances synthesized by each.

Table (4). Nature of antifungal activity of strains of streptomycetes isolates.

Chromophore class	Strain
Heptanes	NR1, NR5, NR23, NR24, NR25, NR42, NR43
Pentanes	NR11, NR61

Conclusion

In conclusion, water of River Nile dominated by *Streptomyces* species while *Micromonospora* are the major genus of its bottom sediments.The results reported in the present investigation reveal that the importance of isolation of streptomycetes exhibiting antimycotic activity. This fact is importance for several reasons. On one hand, it is commonly recognized that actinomycetes residing in the freshwater ecosystem have been relatively neglected (Goodfellow and Haynes, 1984). Several recent studies have demonstrated that actinomycetes of freshwater origin do, in fact, produce novel bioactive substances (Okami, 1986). Accordingly, additional investigations of such microorganisms appear warranted. Also of importance is the recognition that a need exists for the discovery of new antimycotic agents. This need is accentuated by the fact that many laboratories are presently screening for such agents.

In view of the results obtained in the present investigation, it seems reasonable to expect that active substances synthesized by actinomycetes recovered from freshwater habitats will be added to the growing list of antimycotic agents.

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