EGYPTIAN JOURNAL OF AQUATIC RESEARCH 1687-4285
VOL. 31 NO. 2, 2005: 125-144.

PHENOTYPIC CHARACTERIZATION AND NUMERICAL TAXONOMY OF SOME ACTINOMYCETES STRAINS ISOLATED FROM BURULLOS LAKE

ABOU-ELELA, G. M.* and GHANEM, N. B.•

National institute of Oceanography and Fisheries Alexandria. Egypt
•Botany Department, Faculty of Science .Alexandria University.

Keywords: Burullos Lake, actinomycetes, numerical taxonomy.

ABSTRACT

Twenty nine actinomycetes isolates randomly selected of 130 from Burullos Lake were investigated. These isolates were characterized taxonomically for 63 phenotypic characters including morphological; biochemical, nutritional, substrate utilization and anti-microbial analyses. The results were analyzed by numerical techniques using the simple matching coefficient (S_SM) and unweighted pair group average linkage (UPGMA) clustering. At 54% similarity level, the majority of actinomycetes strains were grouped into six phena (A, B, C, D, E and F) only two strains were grouped separately and formed two single clusters at this level. A representative strain from each phenon was chosen, they were identified as *Streptoverticillum morookaense*, *Nocardia brasiliensis, Streptomyces alanosinicus*, *Streptomyces globosus* and *Streptomyces gancidicus*

INTRODUCTION

Reliable identification of bacteria remains an important task in environmental microbiology. Phenotypic approaches using either conventional or commercial systems are still used in the majority of laboratories. Phenotypic identification of isolates from natural sources has limitations related, among others, to the influence of cultural conditions on morphological and biochemical characteristics and to the effect of environmental pressure on gene expression (Maruyama *et al*., 2000; Soutourina *et al*., 2001). The recently proposed class *Actinobacteria* (Stackebrandt *et al*., 1997) is comprised of high -G-C- content gram positive bacteria and includes the actinomycetes (order: actinomycetales), whose members have an unparalleled ability to produce diverse secondary metabolites. These bacteria are primarily saprophytic and are best known from soils where they contribute significantly to the turnover of complex biopolymers, such as lignocelluloses, hemicelluloses, pectin, keratin and chitin (Williams *et al*., 1984). Despite their importance in soil ecology, actinomycetes are best known as source of antibiotics. The cultivation of rare or novel actinomycetes taxa has become a major focus in the search for the detection of new pharmaceutical agents (Bull *et al*., 2000).

Actinomycetes can easily be isolated from fresh water and especially sediments of rivers and lakes. However, as stated by Al-Diwany and Cross (1978), who reviewed our present knowledge on actinomycetes in aquatic habitats, the occurrence of these organisms may simply mean that they survive at these sites because most of them are endowed with spores or cells which show higher resistance toward unfavorable conditions than most bacteria. Certain actinomycetes such as

• Corresponding author
*E-mail: Ghan_ka2006yahoo.com

Micromonospora spp. are inhabitants of mud, and *Nocardia amarae* belongs to the flora of activated sludge. The occurrence in water of other actinomycetes such as *Rhodococcus coprophilus*, has even been regarded as an indicator of farm effluent and animal pollution (Rowbotham and Cross, 1977). Most streptomycetes found in these habitats are probably derived from soil and are merely presented there as inactive but still viable arthrospores. However, some may find suitable conditions for growth.

This is the first report of numerical taxonomy of actinomycetes strains isolated from Burullous Lake. The present investigation was designed to present new insights into the microbial ecology to clarify the taxonomy of actinomycetes flora in fresh water lake environments using phenotypic, physiological and biochemical data and depending on numerical taxonomic procedures.

MATERIALS AND METHODS

Sites of sampling

Sediment and water samples were seasonally collected from 11 stations along the whole area of Burullos lake (Fig. 1) during 2003.

Strains and culture conditions

The studied population consisted of 29 strains from among 130 isolates which were randomly selected from Burullos Lake sediments (Abu–Elela *et al*, 2004). Actinomycetes generally randomly selected with colonial appearance and pigmentation after (2-6) weeks of incubation at 28ºC. Isolation medium consisted of: soluble starch,10.0g; KNO_3,2.0g; K_2HPO_4,2.0g; $MgSO_4$.$7H_2O$,0.05g; $CaCO_2$,0.02g ; $FeSO_4$, 0.01g and agar, 20.0 g and prepared with1 liter of lake water (Küster and Williams, 1964) . Culture stocks were maintained at -20 ºC in starch nitrate broth with 10% glycerol.

Cellular morphology, aerial hyphae, diffusible and substrate mycelial pigments, and the presence of spore mass, growth at different temperatures (20-50ºC), and the effect of different pH (5-9) were examined as described by (Shirling and Gottlieb, 1966). Isolates were screened for sodium chloride tolerance (0-10 %) according to (Tresner *et al.,* 1968).The utilization of different carbon and nitrogen sources were carried out according to (Pridham and Gottlieb, 1948). All tests were performed at 28ºC for up to 3 weeks.

For morphological characterization the following media were used Krasslinikov SRL agar (Mostafa, 1985), Glycerol- asparagines agar (Shirling and Gottlieb, 1966), Glycerol-nitrate agar (Mostafa, 1985), Czapek- Dox agar (Mostafa, 1985) and Inorganic salts-starch agar (Shirling and Gottlieb, 1966).

Electron microscopy was performed using the coverslip technique (Kawato and Shinobu, 1959). The coverslip was cut with a glass file and a suitable fragment with growth on it, it was mounted on a specimen stub, coated with gold palladium under vacuum, and examined with scanning electron microscope Jeol ISM-5300 operating at 10 Kv at the Central laboratory, Faculty of Science, Alexandria University.

Additional phenotypic characterization were performed using the standard procedures, catalase and urease production were examined by the method of (Weyland *et al*., 1970), melanin production according to (Shirling and Gottlieb, 1966), nitrate reduction (Williams *et al.,* 1983), sulphide precipitation (Cowan, 1974) and indole production (Molin and Ternstrom, 1986). Lecithinase was conducted on egg-yolk medium according to the method of Nitsh and Kutzner (1969), lipase (Elwan *et al.,* 1977), protease (Ammar *et al.,* 1991), pectinase (Ammar, *et al.,* 1995), amylase (Ammar *et al*, 1998). Degradation activities were detected according to (Nonomura and Ohara, 1969).

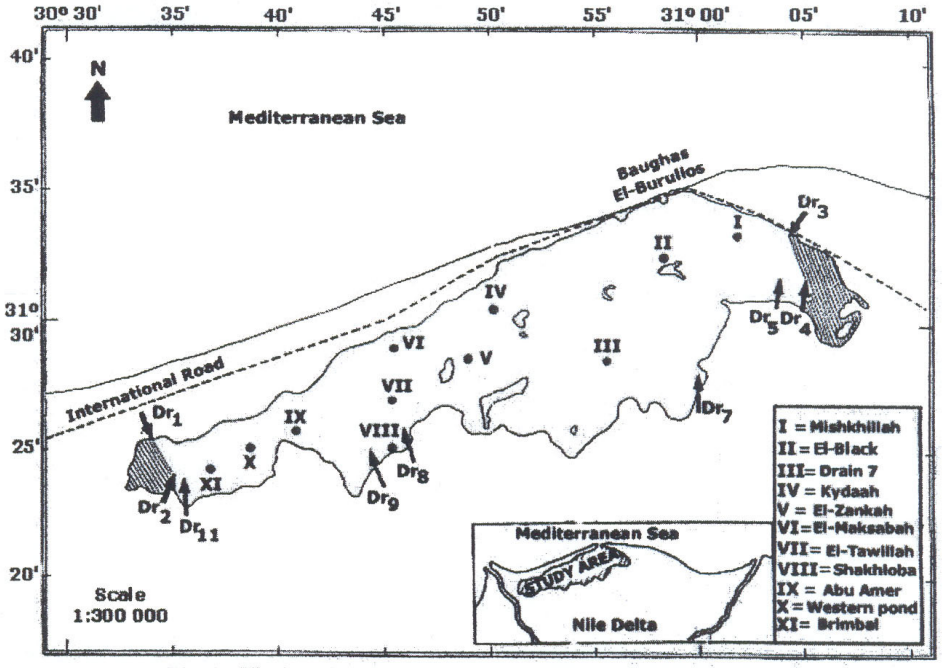

Fig. 1 : The location map of sampling stations in Lake Burullos

Antimicrobial activity :The ability of the tested strains to inhibit the growth of other micro-organisms as *Escherichia coli HP101*, *Staphylococcus aureus ATTC 29523* and *Bacillus subtilus* were determined as described by (Goodfellow *et al*., 1990). Zones of inhibition were scored as positive results after 24 h at 28C°. Resistance against phenol (0.002, 0.01 w/v), crystal violet (0.0001, 0.001 w/v), sodium azide (0.01w/v) and antibiotic resistance of the strains were examined for the ability to grow on medium supplemented with antibiotics one at a time using gentamycin (998 $\mu g.ml^{-1}$), erythromycin (804 $\mu g.ml^{-1}$), tetracycline (990 $\mu g.ml^{-1}$) and cefixine (997 $\mu g.ml^{-1}$) were detected according to (Williams *et al.*, 1983).

Detection of diaminopimelic acid (DAP) isomers and diagnostic sugars were carried out according to Becker *et al*. (1964) and Lechevalier and Lechevalier (1970 b) in AL-Azhar University Fermentation Biotechnology and Applied Microbiology and the identification was completed by comparison with standard strains.

Numerical analysis was not used for a direct taxonomical purpose but also to facilitate data handling and strains grouping. A total of 63 characters were coded as negative (0) or positive (1). The simple matching coefficient (SsM) (Sokal and Michenu, 1958) and the Jaccard coefficient (Sj) (Sneath, 1957) were used and clustering was achieved by unweighted pair group average linkage UPGMA (Sneath and Sokal ,1973). The computations were performed by using SYSTAT-PC program V 7 (Wilkinson *et al.,* 1992) on an IBM computer.

RESULTS

Clustering of actinomycetes strains using the S_SM coefficient with the UPGMA algorithm

Analysis of the selected strains of actinomycetes strains by numerical techniques using the simple matching coefficient (S_SM) and UPGMA clustering

yielded the dendogram in Fig.2 according to the distribution of positive characters of the major and minor clusters shown in (Table 1).The data show that at 54% similarity level, the majority of the strains were grouped into six phena (A, B, C, D, E and F) that contained the 29 isolates under investigation. Only two strains were grouped separately and formed two single clusters at this level.

Phenon A: This phenon comprised only four strains clustered at 54% similarity level, as shown in (Table 1). All strains grew well on the different used media except one strain that was unable to grow on inorganic salts starch agar. Substrate mycelia were yellow brown (2 strains), reddish brown (1 strain) and blue (1 strain). They were all possessing aerial mycelia, two strains were whitish gray, one strain was blue and the other one was yellow. Diffusible pigments were yellowish brown. Optimum growth was obtained at (30-40 ºC), one strain grew at 50 ºC and two strains grew at 20 ºC. The optimum pH for growth of these phenon members is 7.The most utilizable sugars were D-glucose, D-fructose and lactose; on the other hand L-arabinose was not utilized as a sole carbon source. The majority grew at 7% NaCl. Catalase is produced by one strain . Urease and nitrate reductase were observed in two strains where as sulfide production was detected only in one strain. There are no chitinase or gelatinase activities in all members. Only one strain exhibit antagonistic activity against *Staphylococcus aureus*. They were all susceptible to phenol (0.01w/v), two strains were resist crystal violet (0.0001 w/v) and only one strain resists it at (0.001w/v).

Phenon B: This Phenon contained three strains at 55% similarity level. They grew well on all utilized media. Two strains had yellow brown and one strain had violet substrate mycelium. The aerial mycelia shows different colours as recorded in (Table 1). All members were not pigmented. They were able to grow well at pH (5-8), the majority grew at pH 9 at temperature ranged from (20- 40 ºC). D-glucose was utilized as a sole carbon source by all strains. Where as D-Fructose was utilized by most strains. D-rhamnose, mannitol, galactose and xylose were utilized by only one strain. One strain tolerated up to 7% NaCl. Catalase and urease produced by most strains. Nitrate reductase was produced by all the phenon members. They all were able to degrade starch; most of them degraded cellulose and tyrosine. Only one strain showed antibiosis against *Escherichia coli* HP101. They were resistant to phenol (0.002 w/v) and crystal violet (0.0001w/v) and most of them resisted crystal violet (0.001w/v) and gentamycin at (998 $\mu g.ml^{-1}$).

Phenon C: The 6 strains included in this phenon were clustered at 65% similarity level. They were able to grow at all media. The substrate mycelia shows varying degrees of colors. Four of the phenon members possessed gray aerial mycelia where as one strain was violet and the second was pink gray. All were not pigmented.

They all have an optimum temperature of (30- 40 ºC) and a pH range from 6-9. They all utilized D-fructose and lactose. Three strains grew well at 7% NaCl. Two produced catalase and only one reduced nitrate to nitrite. Only two strains were able to degrade chitin and all of them had the ability to degrade starch, cellulose, and tyrosine. They did not show any activity toward gelatin. All had antagonistic effect against *Staphylococcus aureus*, and only one strain showed activity against *Escherichia coli* HP101, and also one strain against *Bacillus subtilis*. There were only two strains that exhibit resistance against (0.001w/v) crystal violet, Meanwhile All members resist phenol at (0.002w/v) and crystal violet at (0.0001w/v). They were susceptible to the used antibiotics.

Phenon D: Five strains are grouped in this phenon at similarity level 55%. As shown in (Table 1), one strain characterized by violet substrate mycelium. The rest have yellow brown. Four strains had gray aerial mycelium and only one had violet aerial mycelium. They could not grow in

Krasslinikov agar medium, but they could utilize the other media. Most of them were not pigmented but only one strain secreted a reddish brown pigments. They grew efficiently at temperature range (20- 40ºC); at pH (6-9) and only one succeeded in growing at pH 5. Lactose were utilized by all strains while, 80% of them utilized D-glucose, mannitol and xylose and two strains utilized D-fructose and rhamnose. Only one strain used raffinose, three strains used galactose while no one used L-arabinose. One strain tolerated up to 7% NaCl. Starch and tyrosine were degraded by all members. Melanin and catalase were produced but no sulfide precipitation by all members, two only reduced nitrate and produced urease. One strain gave antimicrobial activity against *S aureus* .All resisted phenol (0.002 w/v), two strains resisted crystal violet (0.001 w/v) but four strains resisted it at (0.0001 w/v), they were susceptible to all antibiotics used.

Phenon E: At 60% similarity level this phenon included three strains. As reported in (Table 1), two strains had reddish brown substrate mycelia and one had violet. One had yellow and two had gray yellowish pink aerial mycelia. Only one strain grew on Krasslinikov agar medium, all of them grew over all the utilized media. One strain produced reddish brown diffusible pigments. They strains grew at (20- 40 ºC). They are able to grow at different pH (6- 9). D-glucose and lactose utilized by all members of this phenon, two stains utilized rhamnose, mannitol, L-arabinose and D- xylose. D-Fructose and galactose were utilized by only one strain; raffinose was not utilized by any member of this phenon. With respect to NaCl tolerance, only one strain tolerated up to 7%. All produced catalase. Nitrate reduced to nitrite, sulfide precipitated and melanin produced by only one strain. They were all able to degrade starch and the majority degraded tyrosine. Two strains showed antibiosis against *Bacillus subtilis* where as one strain gave activity against *E. coli* and *S. aureus*. They were resistant to (0.002 w/v) phenol and (0.001w/v) crystal violet.

Phenon F: At 55% similarity level, six strains constituted this phenon, 3 strains characterized by reddish brown substrate mycelia, 2 strains had yellow brown and one strain had violet colored substrate mycelium. The majority had gray aerial mycelium and two members had yellow and only one had violet substrate mycelium (Table 1). All strains grew well on all used media except one strain that could not grow on Krasslinikov agar medium. Diffusible yellowish brown pigments were noticed only in three strains. All of them grew at (20-40ºC) with exception of one strain that grew up to 50º C, also all strains grew at pH (6- 9) , while three strains grew well at acidic range at pH 5.They used D-glucose, D- fructose, and D-xylose as a sole carbon source at a concentration of (1% w/v). The majority used rhamnose and mannitol. About 50% of the strains utilized raffinose lactose, L-arabinose and galactose. Most of them grew in presence of 4% NaCl , 50 % grew in 7% NaCl, and no one could grow at 10 %.

The biochemical tests showed that 50% produced catalase, the majority reduced nitrate to nitrite, three of them were able to produce urease, and precipitate sulfide. Melanin production was negative. They degraded starch, cellulose and tyrosine and four of them degraded gelatin but none of them degraded chitin. Only three strains showed antibiosis against gram negative bacterium *Escherichia coli* HP101 and the majority showed antibiosis against gram positive bacterium *Staphylococcus aureus*, all strains had antibiosis against *Bacillus subtilis*. they could resist (0.002 w/v) phenol and (0.001w/v) crystal violet. Only one strain resists gentamycin (998µg. ml^{-1}), and erythromycin (804 µg. ml^{-1}).

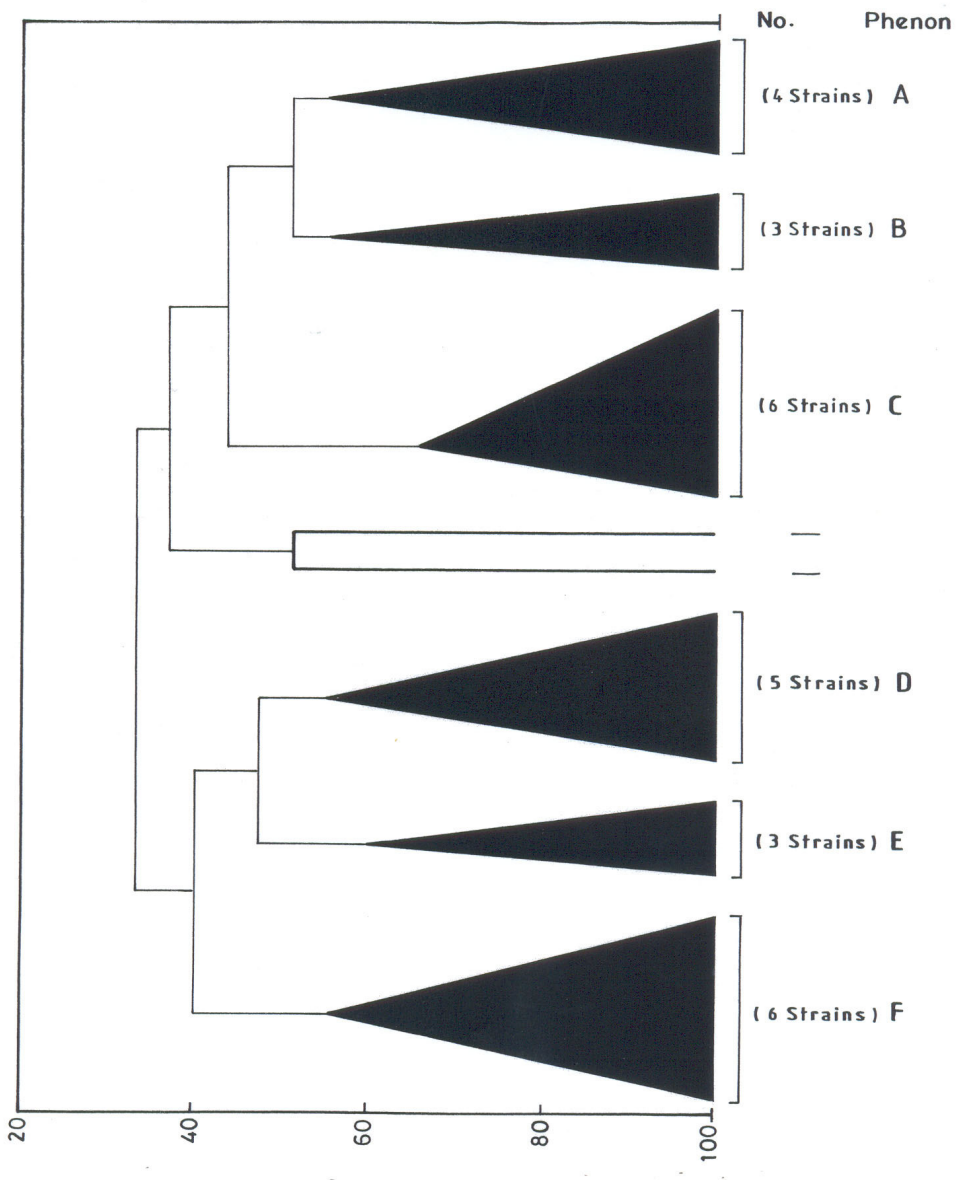

Fig. 2. Simplified dendogram showing the relationships among phena based on the S SM-UPGMA analysis

Table 1. Comparison of the frequencies of positive and negative characters for the six clusters of actinomycetes obtained by numerical taxonomy analysis.

Character :	Phenon No of strains	A 4	B 3	C 6	D 5	E 3	F 6
Growth on							
Krasslinikov agar		100	100	100	0	33	83
Glycerol asparagine agar		100	100	100	100	100	100
Glycerol nitrate agar		100	100	100	100	100	100
Czapek-Dox agar		100	100	100	100	100	100
Inorganic salts starch agar		75	100	100	100	100	100
Substrate mycelium							
Yellow brown		50	67	33	80	0	33
Reddish brown		25	0	50	0	67	50
Blue		25	0	0	0	0	0
Violet		0	33	17	20	33	17
Aerial mycelium							
Gray yellowish pink		0	33	0	0	67	0
Pink gray		0	67	17	0	0	0
Gray		0	0	67	80	0	50
Whitish gray		50	0	0	0	0	0
Blue		25	0	0	0	0	0
Violet		0	0	17	20	0	17
Yellow		25	0	0	0	33	33
Diffusible pigment							
Unpigmented		0	100	100	80	67	50
Reddish brown		0	0	0	20	33	0
Yellowish brown		100	0	0	0	0	50
Growth at							
20 ºC		50	100	100	100	100	100
30 ºC		100	100	100	100	100	100
40 ºC		100	100	100	100	100	100
50 ºC		25	0	0	0	0	17
Growth at PH							
5		50	100	50	33	0	50
6		75	100	100	100	100	100
7		100	100	100	100	100	100
8		75	100	100	100	100	100
9		75	67	100	100	100	100
Utilization of							
D-Glucose		75	100	83	80	100	100
D-Fructose		75	67	100	40	33	100
L-Arabinose		0	0	50	0	67	50
Lactose		75	0	100	100	100	50
Raffinose		50	0	67	20	0	50
Rhamnose		50	33	67	40	67	83
Mannitol		25	33	67	80	67	67
Galactose		25	33	50	60	33	50
Xylose		25	33	67	80	67	100

Cont.

Growth in NaCL%							
0		100	100	100	100	100	100
4		100	100	100	100	100	100
7		75	33	50	33	33	50
10		0	0	0	0	0	0
Degredation of							
Starch 1%		100	100	100	100	100	100
Cellulose 1%		50	67	100	0	0	100
Chitin 0.25%		0	0	33	0	0	0
Gelatin 0.4%		0	0	0	0	0	67
Tyrosine 0.1%		100	67	100	100	67	100
Enzyme activities							
Catalase		25	67	33	100	100	50
Urease		50	67	0	40	0	50
Nitrate reduction		50	100	17	40	33	67
Sulfide precipitation		25	0	0	0	33	50
Melanin production		0	0	0	100	33	0
Antibiosis against							
E.Coli		0	33	17	0	33	50
S.aureus		25	0	100	20	33	83
B.subtilis		0	0	17	0	67	100
Resistance to							
Phenol (0.01w/v)		0	0	0	0	0	0
Phenol (0.002 w/v)		100	100	100	100	100	100
Crystal violet (0.001 w/v)		25	67	33	40	100	100
Crystal violet (0.0001 w/v)		50	100	100	80	100	100
Gentamycin (998 µg.ml-1)		0	67	0	0	0	17
Erythromycin (804 µg.ml-1)		0	0	0	0	0	17
Tetracycline (990 µg.ml-1)		0	0	0	0	0	0
Cefixine (997 µg.ml-1)		0	0	0	0	0	0

Identification of the isolates

Strain (29) was carefully selected so that their characters were representative to the majority of the strains included in the phenon (A). It was identified as *Streptoverticillum morookaense* as shown by their phenotypic characterization in (Table 2), the strains grew on all media .Yellow brown substrate mycelium and gray aerial mycelium with long straight filaments of conidia, showing side branches at regular intervals (Fig. 3a), the diffusible pigment was gray yellowish brown. It is non motile. The cell wall hydrolysate contained LL-DAP and the sugars were not detected. It grew at (20 - 40C°) at pH (6-9) and NaCl (0-4%). All sugars were utilized except L-arabinose, lactose and ribose. All amino-acids were available. It degraded starch, tyrosin, xanthin, and esculin. Produced amylases, proteases and pectinases in addition to urease and nitrate reductase and precipitated sulfide. No melanin or indole production. No antimicrobial effect against all tested strain. It resisted phenol (0.002 w/v) and crystal violet (0.0001 w/v).

The representative of phenon (B) was strain (11) which is subjected to complete identification and identified as *Nocardia brasiliensis*. Its characters recorded in (Table2). It grew on all media with light yellowish brown substrate mycelium and pink gray aerial mycelium carrying chain of conidia (Fig. 3b). Cell wall analysis indicated that the cell wall contained meso-DAP and arabinose and galactose. No pigments. Growth occurred at (20- 40 C°) at pH (5-9) and NaCl (0-7%). D-glucose, D-fructose, rhamnose, galactose, xylose, trehalose, maltose, mannose and sodium citrate were utilized as carbon sources while L-valine, L-alanine and L-arginine were utilized as nitrogen sources. Catalase negative, no sulfide precipitation or melanoid or indole production. It degraded starch, tyrosin and esculin and hydrolysed protein, lipid and lecithin. Absence of antibiosis against the tested strains. It showed susceptibility to all antibiotics except gentamycin at (998 $\mu g.ml^{-1}$), and most chemicals.

Strain (38) is representative to phenon (C) had a great similarities to *Streptomyces alanosinicus*. As showed in the data in Table (2) .All media were suitable for growth. The substrate mycelium was reddish brown and the aerial mycelium was gray with spiral spore chain (Fig. 3c). The cell wall hydrolysate contained LL-DAP with no diagnostic sugars . No diffusible pigment was detected. Growth occurred at (20- 40C°); pH (6-9) and NaCl (0-4 %). Most sugars were utilized. Only L-alanine and L-arginine were utilized as nitrogen sources . No urease but nitrate reductase was detected. Melanin and indole were negative. No sulfide or catalase production. It degraded xanthin and esculin and produced amylases, cellulases, proteases, lipases, pectinases and lecithinases. It showed antibiosis against *E. coli* and *B. subtilis*. This strain resisted phenol (0.002 w/v) and crystal violet (0.0001w/v).

From phenon D, strain (31) was selected. It showed similarities to *Streptomyces globosus.* Its main features showed in (Table 2).This strain had yellowish brown substrate mycelium with gray aerial mycelium carrying spiral and rectiflexible spore chain which is non motile (Fig. 3d) . LL-DAP was detected in the cell wall and no diagnostic sugars. It grew on all media at (20 - 40°C) at pH (6-9) at salt concentration ranged from 0-7%. Sugars utilization as carbon sources was very limited where it only used D-glucose, lactose, xylose, maltose and sodium acetate but it utilized L-cycteine, L-alanine L-arginine and L-tryptophane as nitrogen sources. This strain produced catalase, urease, and nitrate reductase in addition to melanoid pigments but no production to indole. It degraded starch, tyrosine , esculin and xanthin and resisted phenol (0.002 w/v) and crystal violet (0.0001w/v). it showed no antibiosis against tested strains.

Strain (44) as a member of phenon (E) characterized by morphological,

physiological and biochemical features assigned it to *Streptomyces ruber*. Its characters recorded in (Table 2). It was able to grow on all media forming reddish brown substrate mycelium and gray yellowish pink aerial mycelium carrying chain of non motile spores (Fig. 3e). The cell wall hydrolysate contained LL-DAP with no sugars. The growth temperature ranged from (20-40° C) at pH (6-9) at saline range (0-4%).No utilization of L-arabinose, raffinose, sucrose, sodium citrate and ribose as carbon sources and no utilization to L-cyctein, L-phenylalanine and L-tryptophane as nitrogen sources. No catalase , urease or melanoid pigments. Indole negative. Starch, tyrosine and xanthine were degraded and protein, lipid and lecithin were hydrolyzed by this strain. Detection of antimicrobial agents against *E. coli* and *S. aureus*. It resisted phenol (0.002 w/v) and crystal violet (0.001 w/v) but showed a great susceptibility against all antibiotics tested.

Phenon F included strain (45) in which, the cell wall composition as in (Table 2) contained LL-DAP and no diagnostic sugars. It was identified as *Streptomyces gancidicus.* The substrate mycelium was yellow brown and the aerial mycelium was gray with chain of non motile spores (Fig. 3f). The diffusible pigment was gray yellowish brown. The strain could not grow at 50° C, pH range from (5-9) was available for growth at (0-7%) NaCl. The utilized sugars were D-glucose, D-fructose, rhamnose, mannitol, galactose, xylose, trehalose, sodium citrate and mannose while the utilized amino-acids were L-cycteine, L-valine and L-arginine. Urease and nitrate reductase positive but no sulfide production. No melanin or indole production. It degraded starch, tyrosine, gelatin, cellulose, esculin and xanthin in addition to production of lipases and proteases. Antibiosis was detected against all tested strains. Phenol (0.002 w/v), crystal violet (0.001 w/v) and sodium azide (0.01 w/v) had been resisted.

DISCUSSION

The use of numerical identification and the development of matrices for routine use have been successfully applied to various groups of bacteria (Vilhelmsson *et al.*, 1996; Garabito *et al.*, 1998).

Classification on the basis of numerical taxonomic criteria (Jones, 1978; Sneath, 1979) has pointed towards the order actinomycetales as a "natural" phenetic group (Jones, 1975). Taxonomic ideas on the actinomycetes were reviewed (Goodfellow and Minnikin, 1981). Large databases of physiological data are important sources of differentiating characters (Goodfellow *et al*, 1990; Kampfer and Kroppenstedt, 1991).

Taxa species circumscribed in numerical phenetic investigations have high information content and are either indispensable or sufficient to entitle an organism to group membership (Goodfellow, 1989).

Possible problems in numerical taxonomic studies can be minimized by the choice of suitable tests and statistics (O'Brien and Colwell, 1987). It is necessary that all the tests can be employed for all strains, that test reproducibility is high and that the majority of the phena show a low overlap with other phena (Sneath, 1979). Additionally, the results of numerical taxonomic studies should be compared to other numerical surveys and to classifications based on chemical, genetical and serological data (Goodfellow, 1986).

The isolated actinomycetes were subjected to numerical taxonomic study and were clustered at similarity level into 6 phena.

Phenon A included four strains, strain (29), shared in many characters with the reference strain *Streptoverticillium morookaense* in such that , both had gray yellowish brown substrate mycelia, the aerial mycelia consisted of long, straight filaments of conidia showing side branches at regular intervals and whitish to gray spore masses.

The cell wall hydrolysates of both strains contained LL-DAP with no diagnostic sugars. No motility. Absence of melanin. They utilized fructose, galactose and mannitol, degraded esculin, produced nitrate reductase and grew at 5% NaCl. Our isolate can not utilize raffinose and in this respect it differs from the reference strain. According to the recommended international Keys viz. (Buchanan and Gibsons, 1974; Williams; 1989; and Hensly, 1994), strain (29) identified as *S. morookaense*.

It is widely accepted that the genera *Streptomyces* and *Streptoverticillium* are closely related. Both have cell-wall I (Lechevalier and Lechevalier, 1970 a, b), a high content of GC in their DNA (Pridham and Tresner, 1974a, b) and high similarities in DNA homology (Kroppenstedt *et al*, 1981), and are lysed by the same phages (Wellington and Williams, 1981). Additionally, they contain similar menaquinones, fatty acids and polar lipids (Lechevalier *et al.*, 1977).The only support for distinguishing between *Streptomyces* and *Streptoverticillum* has come from DNA-RNA pairing studies (Gladek *et al.*, 1985).

Phenon B included only 3 strains with morphological and biochemical characteristics similar to members of nocardioform actinomycetes (Group 22 in Bergey's Manual of Systematic Bacteriology, Holt and Williams, 1994).

Strain (11) was similar to the reference strain *Nocardia brasiliansis* in that, both of them had pink gray aerial hyphae and the aerial mycelium consisted of chain of conidia. The cell wall hydrolysates contained meso-DAP with arabinose and galactose as diagnostic sugars. The two strains were non motile, lacking melanin pigments, positive for urease and nitrate reductase, degraded xanthine and esculin and utilized galactose and glucose where as mannitol, rhamnose and maltose not utilized. So it could be stated that actinomycete isolate (11) is suggestive of being related to *Nocardia brasiliensis*.

The presence of LL-diaminopimelic acid in strains number: 38 (phenon C), 31 (phenon D), 44 (phenon E) and 45 (phenon F) with no diagnostic sugars is consistent with the proposal that these strains belong to the genus *Streptomyces* and related genera (Williams *et al.*, 1989). As pointed out by (Williams *et al.,* 1983), there is no simple, rapid procedure for objective identification of streptomycetes. The problems of streptomycetes identification are largely a reflexion of the difficulties in streptomycetes taxonomy. Morphological or pigmentation characteristics were not included in streptomycetes taxonomy (Kampfer *et al*., 1991). Because it is generally accepted that more than one category of spore chains can be observed in the same species and the distinction between rectiflexibiles and spirals is not clear (Williams and Wellington, 1980; Williams *et al.,* 1989). The determination of the color of the spore mass is also not easy (Kutzner, 1981), and especially with respect to spore color and other morphological criteria. These traditional characters, which are often difficult to determine (Williams and Wellington, 1980, Kutzner, 1981), are inadequate for classification and identification.

Carbon source utilization tests have been found for in earlier studies to be characters with great differentiation potential. The utilization of sucrose, L-arabinose, inositol, mannitol, rhamnose and raffinose were recommended as an aid to species differentiation in the International *Streptomyces* Project (Shiriling and Gottlieb, 1972) and these tests have been used in several identification schemes (Nonomura, 1974; Szabo *et al.*, 1975).

As pointed out by Kampfer and Kroppenstedt (1991), streptomycetes taxonomy is still developing. The phenetic and diversity of streptomycetes is a major problem.

The four strains 38, 31, 44 and 45 showed great affinity to family streptomycetaceae (Buchanan and Gibbons, 1974; Williams, 1989; and Hensyl, 1994), especially genus *Streptomyces.*

Our isolate (38) had chemotaxonomic properties and cultural and morphological characteristics that are consistent with the reference strain *Streptomyces alanosinicus*. Both possess the same cell wall chemotype; produce gray aerial mycelia carrying gray spiral spore chains. No observed motility or produced melanin pigments. They utilized arabinose, fructose, galactose, glucose, mannitol, raffinose and xylose but no utility to sucrose. It was belong to *S. alanosinicus.*

Strain (31) and the reference strain *Streptomyces globosus* are related to each other. Their aerial mycelia are bearing spiral and rectiflexible spore chains forming gray spore masses. The cell wall contained LL-DAP with no sugars; absence of motility; glucose and xylose were utilized where as no utilization for fructose, galactose, mannitol, raffinose, sucrose. L-arabinose utilization was the only difference between our isolate (31) and the reference strain because our isolate can not utilize it. So that it was identified as *S. globosus*.

Strain (44) was closer to the reference strain *Streptomyces rubber*, they had grayish-yellowish pink spore masses, the spore chains were spiral, and the substrate mycelia were reddish brown. The same cell wall chemotype.

No detection to motility or melanin production. Both of them utilized arabinose, fructose, glucose, mannitol, rhamnose and xylose.

Strain (45) and the reference strain *Streptomyces gancidicus* shared in most characters. Their spore masses were in the gray series with gray yellowish brown substrate mycelia .Spore chains are spirals. Their cell hydrolysates contained LL-DAP with no diagnostic sugars. No motility. Absence of melanin. Both of them were able to consume arabinose, fructose, glucose, galactose, mannitol and xylose where as they were unable to utilize raffinose and sucrose.

Surprisingly, we were not able to isolate genera known to be present in marine environments such as *Micromonospora,* and *Rhodococcus* although *Streptomyces* was commonly observed. Streptomycetes were notably dominant in the sediment samples and in this respect we agreed with the results obtained by (Abou-Elela, 1999**;** Ghanem *et al*., 2000).

Table 2. Characteristic features of the identified strains

Characteristic	Phenon A (29)	Phenon B (11)	PhenonC(38)	Phenon D (31)	Phenon E (44)	Phenon F (45)
Growth on :						
Krasslinilov agar	+	+	+	-	+	+
Glycerol asparagine agar	+	+	+	+	+	+
Glycerol nitrate agar	+	+	+	+	+	+
Czapek dox agar	+	+	+	+	+	+
Inorganic salts starch agar	+	+	+	+	+	+
Morphological characters						
Substrate mycellium	yellow brown	light yellow brown	reddish brown	yellowish brown	reddish brown	yellow brown
Aerial mycelium	gray	pink gray	gray	gray	gray yellowish pink	gray
Spore chain /conidia	long straight filament of conidia showing at regular intervales side branches	aerial conidial chain	spiral	spiral and rectiflexible	spiral	spiral
Diffusible pigments	Gray yellowish brown	unpigmented	unpigmented	unpigmented	unpigmented	gray yellowish brown
Motility	non-motile	non-motile	non-motile	non- motile	non- motile	non-motile
Cell wall hydrolysate:						
Diaminopimelic acid	LL-DAP	meso-DAP	LL-DAP	LL-DAP	LL-DAP	LL-DAP
Sugar pattern	not-detected	arabinose &galactose	not-detected	not-detected	not-detected	not-detected
Growth at :						
20	+	+	+	+	+	+
30	+	+	+	+	+	+
40	+	+	+	+	+	+
50	-	-	-	-	-	-
Growth at PH :						
5	-	+	-	-	-	+
6	+	+	+	+	+	+
7	+	+	+	+	+	+
8	+	+	+	+	+	+
9	+	+	+	+	+	+
Utilization of carbohydrates						
D-glucose	+	+	+	+	+	+
D-fructose	+	+	+	-	+	+
L-arabinose	-	-	-	-	-	-
Lactose	-	-	+	+	+	-
Raffinose	+		-	-	-	-
Rhamnose	+	+	+	-	+	+
Mannitol	+	-	+	-	+	+
Galactose	+	+	+	-	+	+
Xylose	+	+	+	+	+	+
Sucrose	+	-	-	-	-	-
Trehalose	nd	+	+	nd	+	+
Maltose	+	+	+	+	+	-
Cellobiose	+	nd	nd	-	nd	nd
Sodium citrate	+	+	-	-	-	+
Sodium acetate	+	nd	nd	+	nd	nd
Ribose	-	-	-	nd	-	-

Mannose	nd	+	nd	nd	+	+	+	+
Utilization of amino-acids :								
L-cycteine	+	-	-	+	-	+	+	+
L-valine	+	+	-	-	+	+	+	+
L-alanine	+	+	+	+	+	+	-	-
L-phenyl-alanine	+	-	-	-	-	-	-	-
L-arginine	+	+	+	+	+	+	+	+
L-tryptophane	+	-	-	+	+	-	-	-
Growth in presence of NaCl %								
0	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
7	-	+	-	+	+	-	-	+
10	-	-	-	-	-	-	-	-
Degradation of								
Starch	+	+	+	+	+	+	+	+
Cellulose	-	-	-	-	-	-	+	-
Chitin	-	-	-	-	-	-	-	-
Gelatin	-	-	-	-	-	-	+	+
Tyrosine	+	+	+	-	+	+	+	+
Xanthin	+	-	-	+	+	+	+	+
Esculin	+	+	+	+	+	+	-	+
Enzyme activities								
Catalase	-	-	-	-	+	+	-	-
Urease	+	+	-	-	+	-	+	+
Nitrate reductase	+	+	+	+	+	+	+	+
Sulfide precipitation	+	-	-	-	-	+	-	-
Melanin production	-	-	-	-	+	-	-	-
Indole test	-	-	-	-	-	-	-	-
Hydrolysis of :								
Protein	+	+	+	+	-	+	+	+
Lipid	+	+	+	+	-	+	+	+
Pectin	+	nd	+	+	-	nd	nd	nd
Lecithin	nd	+	+	nd	nd	+	-	-
Antibiosis against								
E.coli	-	-	+	+	-	+	+	+
S.aureus	-	-	-	-	-	+	+	+
B.subtilis	-	-	+	+	-	-	-	+
Resistance to :								
Phenol (0.01 w/v)	-	-	-	-	-	-	-	-
Phenol (0.002 w/v)	+	+	+	+	+	+	+	+
Crystal violet (0.001 w/v)	-	+	-	-	+	+	+	+
Crystal violet (0.0001 w/v)	+	+	+	+	+	+	+	+
Sodium azide (0.01 w/v)	-	-	-	-	-	-	-	+
gentamycin (998 µg.ml-1)	-	+	-	-	-	-	-	-
Erythromycin (804 µg.ml-1)	-	-	-	-	-	-	-	-
Tetracycline (990 µg.ml-1)	-	-	-	-	-	-	-	-
Cefixine (997 µg.ml-1)	-	-	-	-	-	-	-	-

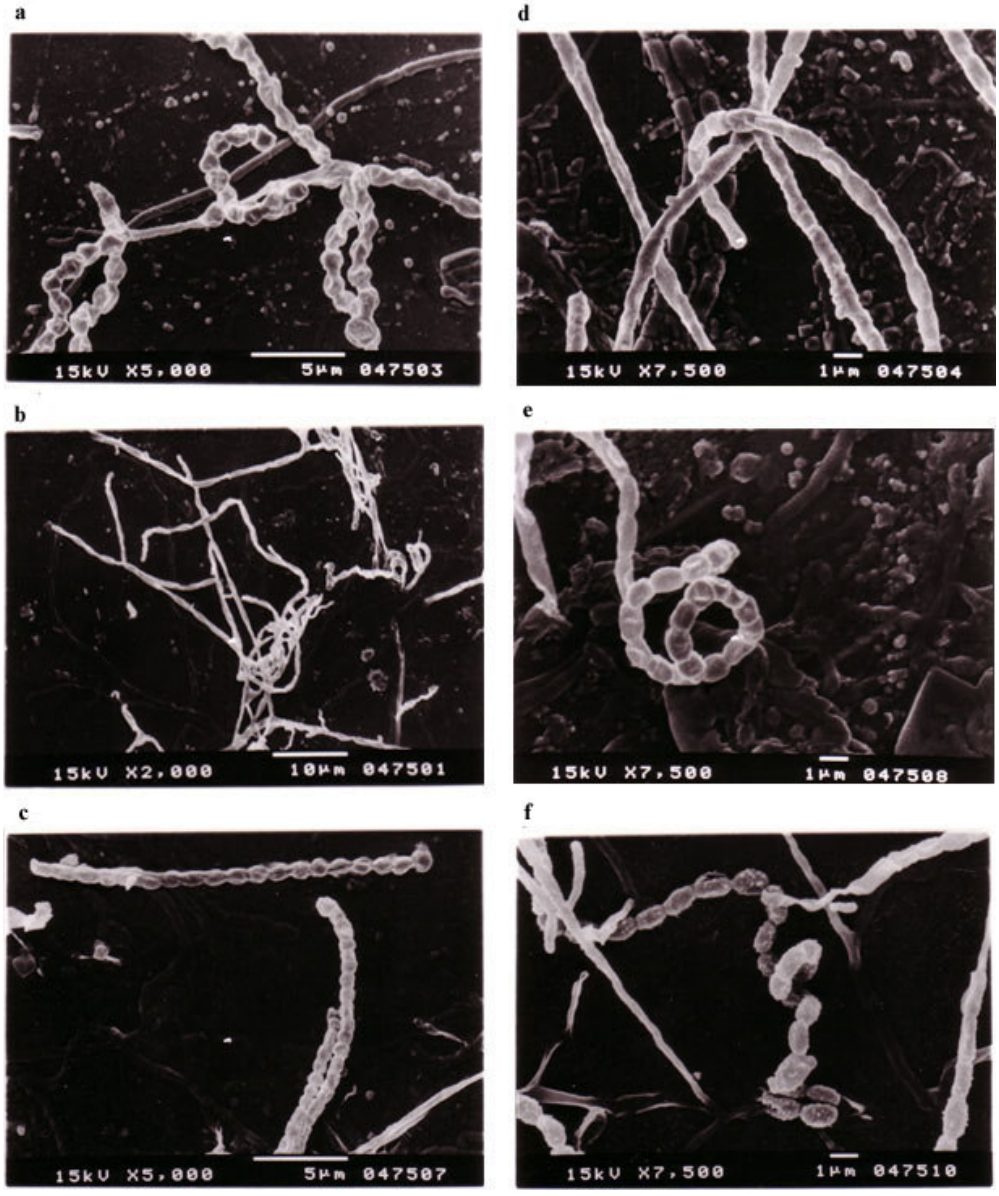

Fig. (3). Scaning electron micrographs showing the growth of different actinomycetes on starch nitrate medium after 14 days. (a) *Streptoverticillum morookaense* (b) *Nocardia brasiliensis* (c) *Streptomyces alanosinicus* (d) *Streptomyces globosus* (e) *Streptomyces rubber* (f) *Streptomyces gancidicus*.

REFERENCES

Abou-Elela, G. M. (1999). Studies on actinomycetes in marine water and sediments of Alexandria Beaches, (Egypt). Ph.D. thesis, Fac. Sci., Alex. Univ.

Abou-Elela, G. M.; N.B. , Ghanem and M. , Okbah (2004). Occurrence and distribution of some actinomycetes groups in Burullous Lake. Bull.Fac. Sci. Assuit Univ. 33 (2-d), 133-146.

Al-Diwany, L.J. and T., Cross (1978). Ecological studies on nocardioforms and other actinomycetes in aquatic habitats. PP. 153-160. In: Mordaski, M.; Kurylowriez, W. and Jeljaszewiez; J. (eds.) *Nocardia* and *Streptomyces.* Proceedings of the International Symposium on *Nocardia* and *Streptomyces.* Warsae, October 4-8 1976. Stuttgart, New York. Gustavfischer Verlag.

Ammar, M.S.; M.S., El-Gamal; S.S., El-louboudy; and A.M., Ibrahim (1995). Fermentation of mesophilic proteases by *B.subtilis*, S-19 in relation to certain nutritional factors under solid state fermentation in relation to vitamins, amino acids, heavy metals and certain plant growth regulators. 1st Internl.Sci. Confr. (Sci., and developm.) Cairo 20-23, March (1995).

Ammar, M.S.; S.S., El-louboudy and U.M., Abdul-Raouf (1991). Purification and properties of mesophilic protease(s) produced by *Bacillus anthracis,* S-44. Isolated from a temple in Aswan. Al Azhar Bull. Sci., Vol.2 (1): 325-338.

Ammar, M.S.; M., EL-Esawey; M., Yassin and Y.M., Sherif (1998). Hydrolytic enzymes of fungi isolated from certain Egyptian Antiquities objects while utilizing the industrial wastes of Sugar and Integrated Industries Company (SIIC). Egypt. J. Biotechnol., Vol.3. Jan. 1998:PP. 60-90.

Becker, B.;, M.P., Lechevalier ; R.E. Gordon and H.A., Lechevalier (1964). Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. Appl. Microbiol., 12:421-423.

Buchanan, R.E. and N.E., Gibbons (ed.) (1974). Bergey's Manual of Determinative Bacteriology 8th edition. The Williams and Wilkins company / Baltimore.

Bull, A.T.; A.C., Ward and M., Goodfellow (2000). Search and discovery strategies for biotechnology : the paradigm shift. Microbiol . Mol. Biol. Rev. 64: 573-606.

Cowan, S.T. (1974). Cowan and Steel's Manual For The Identification Of Medical Bacteria 2nd Edition Cambridge, Univ. Press.!!

Elwan, S.H.; M.R., El-Nagar and M.S., Ammar (1977). Characteristics of lipase(s) in the growth filtrate dialystate of *Bacillus stearothermophilus* grown at 55C using a tributryin-cup plate assay. Bull. Of the Sci.,Riyadh Univ., vol. 8: 105-119.

Garabito, M.J.; M.C., Marquuuez and A., Ventosa (1998). Halotolerant *Bacillus* diversity in hyper saline environments. Can. J. Microbiol. 44, 95-102.

Ghanem, N.B.; S.A., Sabry and, G.A., Abou-Elela (2000). Isolation and enumeration of marine actinomycetes from seawater and sediments in Alexandria. J. Gen. Appl. Microbiol. 46: 105-111.

Gladek, A.; M., Mordarski; M. Goodfellow and S.T., Williams (1985). Ribosomal ribonucleic acid similarities in the classification of *Streptomyces*. FEMS Microbiology Letters 26, 175-180.

Goodfellow, M. (1986). Actinomycetes systematics: present state and future prospects. In: Biological, biochemical and biomedical aspects of actinomycetes. Part B. PP. 487-496. Edited by. Szabo, G.; Tiro, S. and Goodfellow, M. Budapest: Academiai Kiado.

Goodfellow, M. (1989). Suprageneric classification of actinomycetes. In: Bergey's Manual of Systematic Bacteriology. Williams, S.T. (ed.).P. 2333.The Williams and Wilkins Co., Baltimore.

Goodfellow, M. and D.E., Minnikin (1981). In: The Prokaryots. Starr, M. P; Stalp, H.; Truper, H.G. (eds.).p. 1811. Springer: Berlin.

Goodfellow, M.; L.J., Stanton; K.E., Simpon and D.E., Minnikin (1990). Numerical and chemical classification of actinoplanes and some related actinomycetes. J. Gen. Microbiol. 136, 19-36.

Hensly, W.R. (1994). Bergey's Manual of Systematic Bacteriology 9th edition. John. G. Holt and Stanley T. Williams (eds.). Williams and Wilkins, Baltimore, Philadeiphia, Hong Kong, London, Munich, Sydney, Tokyo.

Holt, J.J. and H.S., Williams (1994). Bergey's Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore.

Jones, D. (1975). A numerical taxonomic study of coryneform and related bacteria. J. Gen. Microbiol. 87, 52-96.

Jones, D. (1978). Composition and differentiation of the genus *Streptococcus*. In: Streptococci. Skinner and Quesnel (eds.). p.1. Academic Press: London, New York.

Kampfer, P. and M., Kroppenstedt (1991). Probalistic identification of streptomycetes using miniaturized physiological tests. J. Gen. Microbiol. 137, 1893-1902.

Kampfer, P.; R. M., Kroppenstedt and W., Dott (1991). A numerical classification of the genera *Streptomyces* and *Streptoverticillium* using miniaturized physiological tests .J. Gen. Microbiol. 137, 1831-1891.

Kawato, N. and Shinobu, R. (1995). On *Streptomyces herbaricolor*, nov. sp. supplement : A simple technique for the microscopic observation. Nat. Sci. 8, 114-119.

Kroppenstedt; R.M.; F., Korn-Wendisch; V.J. Fowler and E. Stackebrandt (1981). Biochemical and molecular genetic evidence for the transfer of Actinoplanes armeniacus into the family streptomycetaceae . Zentralblatt fur Bakteriologie. Mikrobiologie und hygiene (I Abteilung, Originale C) 2, 254-262.

Kuster, E. and S.T., Williams (1964). Selective media for isolation of streptomycetes. Nature, London 202, 928-929.

Kutzner, H.J. (1981). The family streptomycetaceae. In: The Prokaryotes: a Hand book on habitats, isolation and identification of bacteria. Strarr, M.P.; Stolp, H. ; Truper, H.G.; Balous , A. and Schlegel, H. (eds.) P. 2028. Springer: Berlin.

Lechevalier, H. A. and M.P. , Lechevalier (1970 a). A critical evaluation of the genera of aerobic actinomycetes. In The Actinomycetales, pp. 393-405. Edited by H. Prauser. Jena: Gustav Fisher Verlag.

Lechevalier, M.P. and H. A., Lechevalier (1970 b). Chemical composition as a criterion in the classification of aerobic actinomycetes. Int. J. Syst. Bact. 20, 435-443.

Lechevalier, M.P.; C. Debievre and H.A., Lechevalier (1977). Chemotaxonomy of aerobic actinomycetes: phospho-lipid composition. Biochem. Syst. Ecol. 5,249-260.

Maruyama, A.; D., Honda; H., Yamamoto; K., Kitamura and T. Higashihara (2000). Phylogenetic analysis of psychrophilic bacteria isolated from the Japan Trench, including a description of the deep-sea species *Psychrobacter pacificensis sp.* nov. Int . J. Syst . Evol. Microbiol. 50 Pt 2, 835-846.

Molin, G. and A., Trenstrom (1986). Phenotypically based taxonomy of psychrotrophic *Pseudomonas* isolated from spoiled meat, water, and soil. Int. J. Syst. Bacteriol. 36; 257-274.

Mostafa, S. A. (1985). Studies on certain actinomycetes from Egyptian soil with

special reference to their metabolites. Ph.D. thesis, Fac. Sci., Alex Univ.

Nitsh, B. and H.J. , Kutzner (1969). Egg-yolk agar as diagnostic medium for *Streptomyces sp*., 25: 113.

Nonomura, H. (1974). Key for classification and identification of 458 species of the streptomycetes included in the ISP. J. Ferm. Technol. 52, 78-92.

Nonomura, H. and Y., Ohara (1969). Distribution of actinomycetes in soil. A culture method effective for both preferential isolation and enumeration of *Microbispora* and *Streptosporangium* stains in soil. J. Ferment. Technol. 47, 463-469.

O'Brien, M. and R.R.,Colwell (1987). Characterization tests for numerical taxonomy studies. Methods in Microbiol. 19, 69-104.

Pridham, T.G. and D., Gottlieb (1948). The utilization of carbon compounds by some actinomycetes as an aid for species determination. J. Bacteriol., 56 (1): 107-114.

Pridham, T.G. and H.D., Tresner (1974 a). Family VII. Streptomycetaceae Waksman and Henrici 1943, 339. In Bergey's Manual of Determinative Microbiology, 8th edn, pp. 747-748. Edited by R.E. Buchanan & N.E. Gibbons. Baltimore: Williams &Willkins.

Pridham, T.G. and H.D., Tresner (1974 b). *Streptomyces* Waksman and Henrici 1943, 339. In Bergey's Manual of Determinative Microbiology, 8th edn, pp. 748-829. . Edited by R.E. Buchanan & N.E. Gibbons. Baltimore: Williams &Willkins.

Rowbotham, T.J. and T., Cross (1977). Ecology of *Rhodococcus coprophilus* and associated actinomycetes in fresh water and agricultural habitats. J. Gen. Microbiol. 100, 231-240.

Shirilling, E.B. and D., Gottlieb (1966). Methods of characterization of *Streptomyces* species . Int. J. Syst. Bact., 16:313-340.

Shirilling, E.B. and D., Gottlieb (1972). Cooperative description of type strains of *Streptomyces*. V. Additional descriptions. Int. J. Syst. Bacteriol. 22,265-394.

Sneath, P. H. A. (1979). Basic program for character separation indices from an identification matrix of percent positive characters. Computers and Geosciences.5,349-357.

Sneath, P.H.A. (1957). The application of computers to taxonomy. J. Gen. Microbiol. 17, 201-226.

Sneath, P.H.A. and R.R. , Sokal (1973). Numerical Taxonomy. The principles and Practice of Numerical Classification. San Francisco: W.H. Freeman.

Sokal, R.R. and C.D., Michenu (1958). A statistical method for evaluating systematic relationships. University of Kansas Scientific Bulletin 38, 1409-1438.

Soutourina, O.A.; E.A., Semenova; V.V., Parfenova; A., Danchin and P., Bertin (2001). Control of bacterial motility by environmental factors in polarly flagellated and peritrichous bacteria isolated from Lake Baikal. Appl. Environ. Microbiol. 67, 3852-3859.

Stackebrandt, E.; F.A., Rainey and N.L., Ward-Rainey (1997). Proposal for a new hierarchic classification system. *Actinobacteria* classis nov. Int. J. Syst. Bacteriol. 47: 479-471.

Szabo, I.M.; M., Morton; I., Buti and C., Fermandez (1975). A diagnostic key for the identification of species of *Streptomyces* and *Streptoverticillium* included in the International *Streptomyces* Project. Acta. Bot. Acad. Sci. Hung. 21,387-418.

Tresner, H.D.; J.A., Hayes and E.J., Backus (1968). Differential tolerance of streptomycetes to sodium chloride as a taxonomic aid. Appl. Microbiol. 16, 1134-1136.

Vilhemsson, O.; H., Hafsteinsson and J. K., Kristjansson (1996). Isolation and characterization of moderately halophilic bacteria from fully crude salted cod (bachalao). J. Appl. Bacteriol.

Wellington, E.M.H. and S.T., Williams (1981). Host range of phage isolated to *Streptomyces* and other genera. Zentralblatt fur Bakteriologie. Mikrobiologie und Hygiene (IAlbteilung), Supplement 11, 93-98.

Weyland, H.; H., Ruger and H. , Schwarz (1970). Zur isolierung and identifizierung mariner. BaKterien. Veroeff. Inst. Meereforsch Bremer-haven 12,24-296.

Wilkinson, L.; M.A., Hill; S., Miceli; G., Birkenbeuel and E., Vang (1992). Systat for Windows, Version 5 Eddition. Evanston, 1L; Systay, Inc., Illinois.

Williams, S.T.; S., Lanning and E.M.H., Wellington (1984). Ecology of actinomycetes, P. 481-528. *In* M. Goodfellow, M. Mordarski and S.T. Williams (ed). The biology of the actinomycetes. Academic press. London, United Kingdom.

Williams, S.T. (1989). Bergey's Manual of Systematic Bacteriology Vol. 4, Stanely, T. Williams. Williams and Wilkins (eds.), Baltimore, Hong Kong, London, Sydney.

Williams, S.T. and E.M.M., Wellington (1980). Micromorphology and fine structure of actinomycetes. In: Microbiological Classification and Identification. Goodfellow, M. and Board, R.G. (eds.). p. 139. Academic Press: London.

Williams, S.T.; M., Goodfellow and M., Alderson (1989). Genus *Streptomyces* (Waksman & Henrici, 1943) 339 Al. In Bergey's Manual of Systematic Bacteriology, Vol. 4, pp. 2452-2492. Edited by S. T. Williams, M.E. Sharp and J.G. Holt. Baltimore: Williams and Wilkins.

Williams, S.T.; M., Goodfellow; G., Alderson ; E.M.M., Wellington; P.H.A., Sneath and M.J., Sackin (1983). Numerical classification of *Streptomycetes* and related genera. J. Gen. Microbiol. 129, 1743-1813.