Zagazig J. Pharm. Sci., December 1995,
Vol. 4, No. 2, pp. 98-108

ISSN 1110-5085

BIOLOGICAL CHARACTERISTICS AND TAXONOMY OF BACTERIA BELONGING TO THE GENUS *STREPTOMYCES* WITH RED COLOURED AERIAL MYCELIUM

Ahmed A. Ismail and Fathy A. Mansour *

*Departments of Botany, Faculty of Science, Zagazig and *Mansoura Universities, Egypt.*

ABSTRACT

A number of 13 experimental isolates of *Streptomycetes* were characterized by the production of red coloured aerial mycelium and classified on the basis of the criteria recommended by the methods of International *Streptomyces* Project (ISP). The physiological and biochemical characteristics of the identified isolates are suggested to be useful in completing the description of a strain or species, even if they are not very significant or indicative on their own.

INTRODUCTION

The taxonomic position of the family *Streptomycetaceae* in the order *Actinomycetales* (based on the biochemical criterion) and the classification of the family to the genus level (based on the gross morphology) have been discussed before by some authors[1,2].

Members of the genus *Streptomyces* are characterized by the production of non motile aerial spores. While sporophores do not exhibit verticillate branching and sporangia - like vesicle are not formed.

It is well known that *Streptomycetes* have the characteristic procaryotic cell organization and the cellular dimensions of their hyphae placed them among bacteria[3].

Numerous systems of classification were devised to accommodate the increasing number of *Streptomyces* species. Most of the systems are based on few subjectively chosen morphological and pigmentation properties which were rarely studied and standardized the growth conditions. Several attempts have recently been made to overcome the diversity of criteria and methods applied for the description, classification and naming of *Streptomycetes* in the International *Streptomyces* Project[4-8] (ISP).Other diagnostic keys have been developed by rising the data of the ISP[9-11].

The aim of the present work is to study the classification and identification of some soil isolates of *Streptomycetes* of the northern part of the Nile Delta region of Egypt that produce red coloured aerial mycelium. Evidences were laid upon the use of additional physiological and biochemical test to complete the description of species or strain. The same characteristics were previously performed for the white isolates[12].

MATERIAL AND METHODS

A number of 13 isolates of *Streptomycetes* belonging to the red colour series were isolated from the northern part of the Nile Delta region, Egypt. The soil samples were collected according to the procedure adopted and reported before[13].

The ability of isolates to form melanoid pigments and soluble colours other than melanoids was performed according to the ISP methods[4].

The ability of isolates to utilize different carbon sources was previously studied according to the ISP carbon utilization medium modified form[14].

In addition, the antimicrobial activities of the studied isolates were carried out against the following test organisms.

1- Bacteria:

i- Gram-positive bacteria: *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus , Micrococcus lutea.*

ii-Gram-negative bacteria: *Escherichia coli, Pseudomonas aeruginosa.*

2-Yeast: *Sacharomyces cerevisiae.*

3-Fungi: *Penicillium chrysogenum.*

Hydrolysis of starch was tested according to the methods of **Harrigan**[15].

Gelatin liquifaction and coagulation, and peptonization were adopted according to previous methods[16]. The method described by **Williams *et al*** [17] was used in detecting sodium chloride tolerance. Cellulose decomposition was tested according to **Crawford et al** method [18].

Reduction of nitrate to nitrite was performed according to Trommosdorf's method[19].

The formation of hydrogen sulphide was detected by the methods adopted by **Kuster** and **Williams**[20].

The ability of the isolates to utilize oxalate as well as other salts of organic acids was examined according to the procedure adopted by **Nitsch *et al*** [21].

Hydrolysis of urea was determined following the method of **Nitsch** and **Kutzner**[22].

Hippurate hydrolysis of urea was determined following the procedure adopted by **Ziegler** and **Kutzner**[23].

The ability of the experimental isolates to utilize different nitrogen sources were examined on gauze no. 1 medium as recommended by **Shirling *et al***[4].

Zagazig J. Pharm. Sci., December 1995,
Vol. 4, No. 2, pp. 98-108

The ability of the experimental isolates to utilize different vegetative oils was studied on the ISP medium as recommended by **Shirling *et al*** [6].

RESULTS AND DISCUSSION

Biological characteristics and taxonomic identification of isolates of the red series:

The red coloured series comprises 13 isolates which were characterized by straight spore chains and smooth spore surface. Consequently they could be collected in one section (RDS). This section, according to the chromogenicity test, is subdivided into two subsections, subsection RDS.A (7 isolates) with positive melanin reaction and subsection RDS.B (6 isolates) with negative melanin reaction.

Subsection RDS.A:

The 7 isolates of this subsection showed distinct variations with regard to pigmentation of the substrate mycelium, so that they had been classified into two groups; group RDS. A-i(2 isolates) which produced reddish-brown or brown substrate mycelium and group RDS. A-ii (5 isolated), which showed non-distinctive substrate mycelium.

Diagnostic characteristics of the isolates of group RDS.A-I of the red series:

-Spore chain morphology:Section Rectiflexibiles (Fig.1).

-Spore surface: Smooth (Fig. 2).

-Colour of colony :Rose gray or pink to pinkish-gray coloured aerial mycelium is produced on the media employed(Table 1).

-Reverse side of colony: the colour of substrate mycelium is reddish-brown on all media used (Table1).

-Colour in medium: Melanoid pigments are produced in tyrosine agar, peptone-yeast iron agar or tryptone yeast broth. However, reddish-brown pigment (PH-sensitive) is also formed in these media (Table1).

-Utilization of different carbon sources: Glucose, arabinose, xylose, rhaminose, fructose, inositol, mannitol, raffinose and sucrose are all utilized for growth of the two isolates of this group. .

-Growth on czapek's medium: scanty growth is shown on the medium (Table 1).

-Sodium chloride tolerance: > 6 % - ≤ 8 %.

-Antagonistic properties: the two isolates possess no antimicrobial activities against the test organisms.

-**Taxonomical** identification: According to the **Bergey's** Manual[24] and surveying the literature on description of *Streptomyces* spp.[4,9] isolates NO. R. 14 and R.118 are identified to be strains belonging to *Streptomyces sphaeopurpureus*[25].

-Additional characteristics for the isolates of group RDS.A-I:

The results presents in Table (2) indicate that the two isolates of this group are identical in their physiological and biochemical characteristics. They were, therefore, considered to be two identical strains belonging to *Streptomyces phaeopurpureus*[25].

Group RDS.A-II :

This group included 5 isolates was further differentiated into two subgroups according to their ability to utilize different carbon-sources. The two subgroup were: subgroup RDS.A-iia which included isolates NO.R.96 and R.104, and subgroup RDS.A-iib which comprised isolates No. R.16, R.102 and .R.114.

Table (1) : Cultural characteristics of 7-14 days old cultures of isolate of group RDSA-i.

Medium	Colour of colony	Colour of reverse side of colony*	Colour in medium**	Growth
Inorganic salts-starch agar	Rose-grey	Reddish-brown	Reddish-brown	++
Glycerol-asparagine agar	Rose-grey	Reddish-brown	Reddish-brown	++
Malt-yeast-extract agar	Rose-grey	Reddish-brown	Reddish-brown	+++
Oat meal agar	Rose-grey	Reddish-brown	Reddish-brown	++
Czapek's solution agar	Red-grey	Reddish-brown	Reddish-brown	+
Starch-nitrate agar	Rose-grey	Reddish-brown	Reddish-brown	+++
Glucose-nitrate agar	Pink to pinkish grey	Reddish-brown	Reddish-brown	+
Glycerol-nitrate agar	Pink to pinkish grey	Reddish-brown	Reddish-brown	+
Potato agar	Pink to pinkish grey	Reddish-brown	Reddish-brown	+
Fish meal agar	Pink to pinkish grey	Reddish-brown	Reddish-brown	+++

+++ = Good growth ++ = Moderate growth + = Scanty growth

* The substrate mycelium pH-indicator property changed : from reddish brown to yellow upon adding 0.05N HCl and to pale brown upon adding 0.05N NaOH.

** Such pigment was pH-sensitive : exhibiting the same pattern of changes noted in the substrate mycelium pigment.

Table (2): Physiological and Biochemical Characteristics of Isolates of the Red-Coloured Series.

Isolate No.	Utilization of: Peptonization of milk	Coagulation of milk	Hydrolysis of starch	Liquefaction of gelatin	Reduction of nitrate	Decomposition of cellulose	Production of H_2S	Hippurate conc. (% 100 ml): 0.5	1.0	1.5	2.0	Oxalate conc. (% 100 ml): 0.5	1.0	1.5	2.0	Organic acid salts: Benzoate	Tartarate	Citrate	Types of oil: Linseed oil	Castor oil	Coconut oil	Sesame oil	Olive oil	Clove oil	Clotrimazole oil
R.11	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-
R.11A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-
R.9b	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	+	+	+	+	+	+	+	-
R.10A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R.16	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R.10B	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R.11d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
R.5	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
R.9b	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
R.28	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
R.35	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
R.37	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+
R.59	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+

+ = Good growth. + = Weak growth. - = No growth

Zagazig J. Pharm. Sci., December 1995,
Vol. 4, No. 2, pp. 98-108

Table (2): Continued

Isolate No. / Utilization of	Amm. nitrate	Amm. nitrite	Amm. Chloride	pot. nitrate	pot. thiocyanate	Sod. nitrate	Sod. nitrite	Urea	peptone	Casein	Asparagine	Glycine	Alanine	Lysine	Serine	Methionine	Leucine	Tyrosine	Glutamic acid	Isoleucine	Valine	Tryptophan
R. 14	+	-	+	+	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	+	+
R. 118	+	-	+	+	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	+	+
R. 96	+	-	+	+	-	+	-	-	+	+	+	+	+	+	-	+	±	+	-	+	+	-
R. 104	+	-	+	+	-	+	-	-	+	+	+	+	+	+	-	+	+	+	-	±	+	-
R. 16	+	+	-	+	-	+	+	-	+	+	+	+	-	-	-	-	+	+	-	-	+	-
R. 102	+	+	-	+	-	+	+	-	+	+	+	+	-	-	-	-	+	+	-	-	+	-
R. 114	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	-
R. 8	+	-	-	+	-	-	-	-	+	+	+	+	+	-	-	+	+	-	-	+	+	+
R. 86	+	-	-	+	-	-	-	-	+	+	+	+	-	-	-	+	+	-	-	+	+	+
R. 28	+	+	+	+	-	+	+	-	+	+	+	+	-	-	+	+	-	-	-	-	+	+
R. 85	+	+	+	+	-	+	+	-	+	+	+	+	-	-	+	+	-	-	-	-	+	+
R. 27	+	-	-	+	-	-	-	-	+	+	+	+	+	-	+	-	±	-	-	+	+	-
R. 59	+	-	-	+	-	+	-	-	+	+	+	+	+	-	+	+	+	-	±	+	+	-

+ = Good growth ± = Weak growth, - = No growth

Diagnostic characteristics of the isolates of sub-group RDS A-II.A of the red color series:

-Spore chain morphology: Section Rectiflexibiles (Fig. 3).

-Spore surface: Smooth (Fig. 4).

-Colour of colony: Yellowish-red to grayish-red coloured aerial mycelium is produced on the media used (Table 5).

-Reverse side of colony: the substrate mycelium is not distinctive (Table 15). (greenish-yellow or greenish-pale yellow; olive brown and greenish-brown on the media used) the substrate mycelium pigment is not pH-sensitive.

-Colour in medium: Melanoid pigments were formed in tyrosine agar, peptone yeast iron agar or tryptone - yeast broth. On the other hand , no pigment other than melanoids was detected in the media used (Table 5).

-Utilization of different carbon sources: the two isolates of this subgroup appeared to be able to utilize glucose, arabinose, xylose and (to a slight extent) fructose, but they failed to utilize rhaminose, inositol, mannitol, raffinose and sucrose for growth.

-Growth on Czapek's medium: scant growth is observed on this medium (Table 3).

-Sodium chloride tolerance: The two isolates show NaCl tolerance up to 6%.

-Antagonistic properties: The two isolates (No.R. & 96 R.104) exhibit slight effect against all the tested organisms except *Pseudomonas aeruginosa*.

-**Taxonomical identification**: Followigh the diagnostic key of **Bergey's Manual**[2,4] and surveying the literatures[5-9] isolates No. R.96 and R.104 of sub-group RDS.A-ii.a were proved to belong to *S. roseoviridis* **Preobrazhenskaya** [29].

Additional characteristics for isolates of sub-group RDS A-II.A:

As could be deduced from the results given in Table (2), the two isolates of this subgroup had differences in their physiological and biochemical behaviors and they were, therefore, considered to be two different strains belonging to *S. roseoviridis*[29].

Diagnostic characteristics of the isolates of subgroup RDS.A-II.b of the red colour series :

This subgroup which includes 3 isolates was characterized by:

-Spore chain morphology: section rectiflexibiles (Fig.5)

-Spore surface: smooth (Fig.6)

-Colour of colony: Grayish-red coloured aerial mycelium is produced on all media used (Table 4).

Reverse side of colony: The substrate mycelium shows non distinctive pigment (pale yellow to grayish-yellow on the media employed).The pigment of the substrate mycelium's not pH-sensitive (Table 4).

-Colour in medium: Melanoid pigments were formed in tyrosine agar, peptone-yeast iron agar or tryptone-yeast broth. No pigment other than melanoids is noted in the media used (Table 4).

-Utilization of different carbon sources: Glucose and fructose are utilized for the growth of three isolates, but no growth is detected on arabinose, xylose, rhaminose, inositol, mannitol, raffinose and sucrose.

-Growth on Czapek's medium: Scanty growth is shown on this medium (Table 4).

-Sodium chloride tolerance: > 4% - ≤ 6%.

-Antagonistic properties: The three isolates of this subgroup only inhibit the growth of *B. subtilis*, *B. megaterium*, *E. Coli* and *Saccharomyces cerevisiae*.

Taxonomical identification: following the **Bergey's Manual**[2,4] and surveying the literature on description of *S.* species in authers[5-9], isolates No. R16, R.102 and R.114 were identified to be belonging to *Streptomyces flavovirens*[27].

- Additional characteristics of the isolates of sub-group RDS A-II.B:

The results in Tale (2) indicate that the isolates No. R.16 and R.102 showed close similarities in their physiological and biochemical properties and they were, therefore considered to be two identical strains belonging to *Streptomyces flavovirens*[27]. Isolate No. R.114 was considered to be another strain of the same species.

Subsection RDS.B :

The 6 isolates of this subsection show certain distinct variations in the pigmentation of the substrate mycelium and they has been differentiated into two groups; group RDS.B-I (4 isolates) which was characterized by reddish-brown, pink or brown (not pH sensitive) substrate mycelium and group RDS.B-ii which includes two isolates and showed no distinctive substrate mycelium.

Diagnostic characteristics of the isolates of group RDS, B-I of the red colour series:

-Spore chain morphology: section Rectiflexibiles (Fig.7)

-Spore surface: smooth (Fig. 8).

-Colour of colony: whitish-red, whitish-brownish-red, pinkish-brown to grayish-red coloured aerial mycelium is produced on all the media used (Table5.).

-Reverse side of colony: The results in Table (5) show reddish-brown, pink or purple to brown substrate mycelium on the media employed. The substrate mycelium pigment is not pH indicator.

-Colour in medium: Melanoid pigments are not formed in tyrosine agar, peptone- yeast iron agar or tryptone-yeast broth. No pigment other than melanoids was detected (Table 5).

-Utilization of different carbon sources: Glucose, arabinose, xylose, rhaminose, fructose, inositol, are all utilized for the growth of the 4 isolates.

-Growth on czapek's medium :Moderate growth (Table5).

-Sodium chloride tolerance: > 4% - ≤ 6%

Zagazig J. Pharm. Sci., December 1995,
Vol. 4, No. 2, pp. 98-108

Table (3) : Cultural characteristics of 7-14 days old cultures of isolates of subgroup RDS.A ii.a

Medium	Colour of colony	Colour of reverse side of colony*	Colour in medium	Growth
Inorganic salts-starch agar	Yellowish-red	Greenish-yellow	Non-pigmented	+++
Glycerol-asparagine agar	Yellowish-red	Greenish-yellow	Non-pigmented	++
Malt-yeast-extract agar	Red	Greenish-brown	Non-pigmented	+++
Oat meal agar	Yellowish-red	Olive brown	Non-pigmented	++
Czapek's solution agar	Red	Greenish-yellow	Non-pigmented	+
Starch-nitrate agar	Yellowish-red	Greenish-yellow	Non-pigmented	+
Glucose-nitrate agar	Greyish-red	Greenish-brown	Non-pigmented	+
Glycerol-nitrate agar	Greyish-red	Greenish-brown	Non-pigmented	+
Potato agar	Greyish-red	Greenish-brown	Non-pigmented	++
Fish meal agar	Greyish-red	Greenish-brown	Non-pigmented	++

+++ = Good growth ++ = Moderate growth + = Scanty growth
* The pigment of substrate mycelium was not pH-sensitive, when tested with 0.05N HCl or 0.05N NaOH.

Table (4) : Cultural characteristics of 7-14 days old cultures of isolate of subgroup RDS.A ii.b.

Medium	Colour of colony	Colour of reverse side of colony*	Colour in medium	Growth
Inorganic salts-starch agar	Greyish red	Greyish-yellow	Non-pigmented	++
Glycerol-asparagine agar	Greyish red	Pale yellow to greyish yellow	Non-pigmented	++
Malt-yeast-extract agar	Greyish red	Pale yellow	Non-pigmented	+++
Oat meal agar	Greyish red	Pale yellow	Non-pigmented	++
Czapek's solution agar	Greyish red	Pale yellow	Non-pigmented	+
Starch-nitrate agar	Greyish red	Greyish-yellow	Non-pigmented	+++
Glucose-nitrate agar	Greyish red	Greyish-yellow	Non-pigmented	++
Glycerol-nitrate agar	Greyish red	Pale yellow	Non-pigmented	++
Potato agar	Greyish red	Pale yellow	Non-pigmented	++
Fish meal agar	Greyish red	Greyish-yellow	Non-pigmented	+++

+++ = Good growth ++ = Moderate growth + = Scanty growth
* The substrate mycelium pigment are not pH-sensitive, when tested with 0.05N HCl or 0.05N NaOH.

Table (5) : Cultural characteristics of 7-14 days old cultures of isolate of group RDS.B i.

Medium	Colour of colony	Colour of reverse side of colony*	Colour in medium**	Growth
Inorganic salts-starch agar	Whitish red to greyish red	Reddish brown	Non pigment or traces of brown	+++
Glycerol-asparagine agar	Whitish red to greyish red	Reddish brown	Non pigment or traces of brown	++
Malt-yeast-extract agar	Whitish red to greyish red	Reddish brown	Non pigment or traces of brown	++
Oat meal agar	Whitish-red to greyish red	Pink	Traces of reddish brown	++
Czapek's solution agar	Whitish-red to greyish red	Brown	Non-pigmented	++
Starch-nitrate agar	Whitish red to greyish red	Purple	Non-pigmented	+++
Glucose-nitrate agar	Whitish-red to greyish red	Brown	Traces of brown	++
Glycerol-nitrate agar	Whitish-red to brown	Brown	Non-pigmented	++
Potato agar	Whitish-brownish-red	Brown	Non-pigmented	+
Fish meal agar	Whitish-pinkish brown	Brown	Non-pigmented	+++

+++ = Good growth ++ = Moderate growth + = Scanty growth

* The substrate mycelium pigment are not pH sensitive, when tested with 0.02N HaOH or 0.02N HCl.

** Such types of soluble pigment are not pH sensitive, when tested with 0.02N HaOH or 0.02N HCl.

Table (6) : Cultural characteristics of 7-14 days old cultures of Group RDS.B ii.

Medium	Colour of colony	Colour of reverse side of colony*	Colour in medium	Growth
Inorganic salts-starch agar	Greyish-red	Pale yellow to greyish yellow	Non-pigmented	+++
Glycerol-asparagine agar	Greyish-red	Pale yellow	Non-pigmented	++
Malt-yeast-extract agar	Greyish-red	Pale yellow	Non-pigmented	++
Oat meal agar	Greyish-red	Greyish-yellow	Non-pigmented	++
Czapek's solution agar	Greyish-red	Pale yellow	Non-pigmented	++
Starch-nitrate agar	Greyish-red	Greyish-yellow	Non-pigmented	++
Glucose-nitrate agar	Pinkish-brown	Pale yellow	Non-pigmented	++
Glycerol-nitrate agar	Pinkish-brown	Greyish-yellow	Non-pigmented	+
Potato agar	Pinkish brown	Greyish-yellow	Non-pigmented	+
Fish meal agar	Pinkish-brown	Pale yellow to greyish yellow	Non-pigmented	++

+++ = Good growth ++ = Moderate growth + = Scanty growth

* The substrate mycelium pigment are not pH sensitive, when tested with 0.02N HCl or 0.02N HaOH

Zagazig J. Pharm. Sci., December 1995,
Vol. 4, No. 2, pp. 98-108

Fig. 1 : Microphotograph of spore chains of *S.Phaeopurpureus*

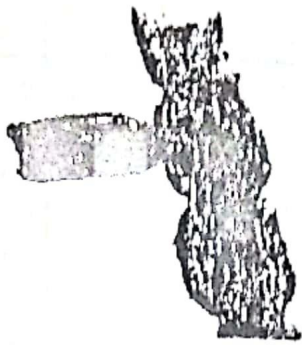
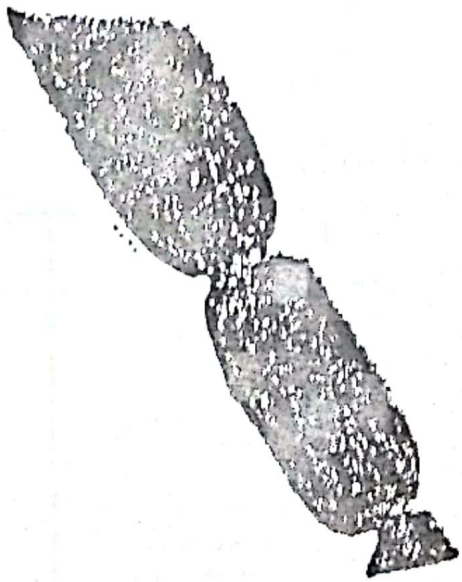


Fig. 2 : Electron micrograph of *S.Phaeopurpureus*

Fig. 3 : Microphotograph of spore chains of *S.roseoviridis.*



Figr. 4 : Electron micrograph of *S.roseoviridis.*

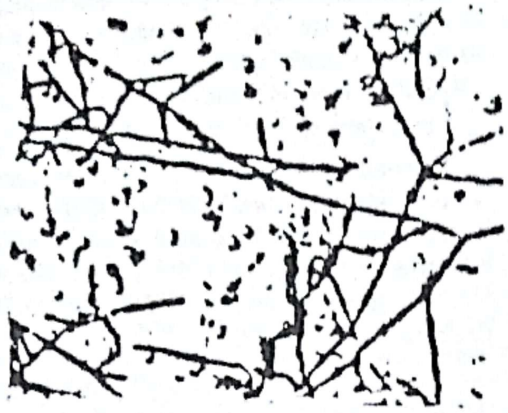


Fig. 5 : Microphotograph of spor chains of *S. flavotricini.*

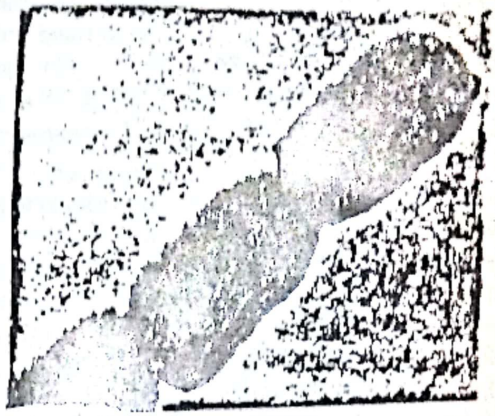


Fig. 6 : Electron micrograph of *S. flavotricini.*

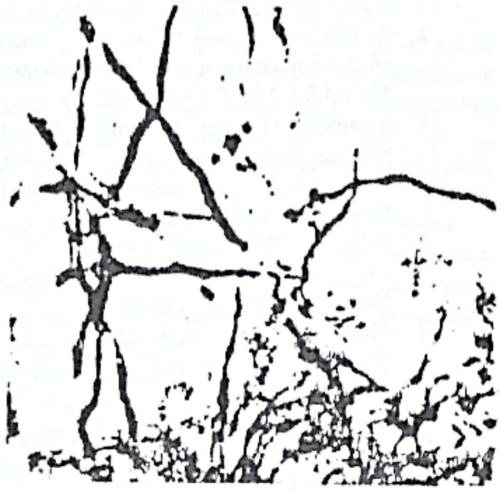


Fig. 7 : Microphotograph of spore chains of *S. prunicolor.*

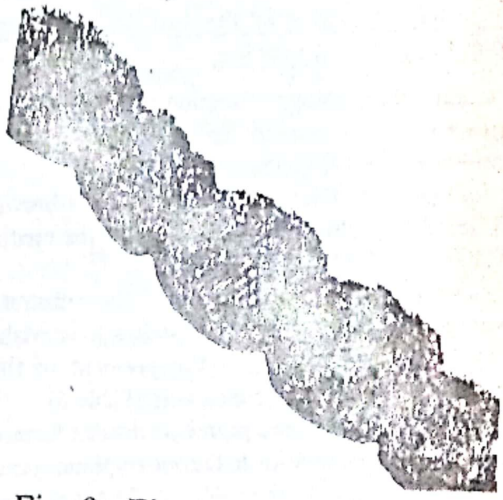


Fig. 8 : Electron micrograph of *S. pruniclor.*

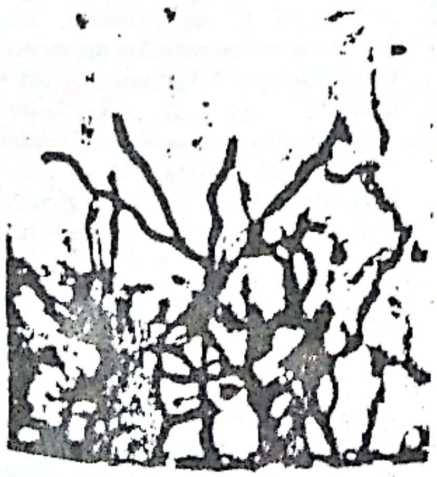


Fig. 9 : Microphotograph of spore chains of *S. exfololiatus.*

Fig. 10 : Electron micrograph of *S. exfoliatus.*

-Antagonistic activities: the 4 isolates of this group exhibit strong activities against gram-positive bacteria, *E.coli* and *Saccharomyces cerevisiae*.

-**Taxonomical identification**: According to the **Bergey's** Manual key[24] and surveying the literature on description of *Streptomyces* species. On the articles of ISP[4-8], isolates No. R. 8, R. 86, R. 28 and R85 were identified to be belonging to *Streptomyces prunicolor*[28].

Additional characteristics for isolates of the group RDS B-i :

The results manifested in Table (2)indicate that the two isolates (No R. 8 and R. 86) are quite similar in their physiological and biochemical behavior but they are not substantially different from the isolates number R. 28 and R. 85.

Therefore, each of them had been considered to be two identical strains of the species *Streptomyces prunicolor*[28].

Diagnostic characteristics of the isolates of group RDS.B-ii of the red colour series:

-*Spore* chain morphology: section Rectiflexibiles (Fig.9)

-*Spore* surface: smooth (Fig.10).

-*Colour* of colony: Grayish-red or pinkish-brown colored aerial mycelium is produced on the media used (Table6).

-*Reverse* side of colony: the colour of the substrate mycelium is not distinctive (pale yellow to grayish-yellow) on all the media used the pigment of the substrate mycelium is not pH-indicator (Table 6).

-*Colour* in medium: melanoid pigments are not formed in tyrosine agar, peptone-iron agar or tryptone -yeast broth. No pigment other than melanoids was noticed in the same media (Table 6).

-*Utilization* of different carbon sources: glucose, arabinose, xylose, rhaminose, fructose, rafinose and sucrose are utilized for growth of the two isolates, but no growth is observed on inositol and mannitol.

-*Growth* on Czapek's medium: the two isolates of this group produce moderate growth on this medium (Table 6).

-Antagonistic properties: the two isolates No. R. 27 and R. 56 exhibit slight activity against *B. subtilis* and *Staph. aureus* only.

-**Taxonomic identification**: Following the diagnostic key[24] and surveying the literatures on description of *S. species* in authers[4-8], isolates No. R.27 and R.59 were identified as strains of *S. exfoliatus*[29].

-Additional characteristics of isolates of the group RDS.B-ii:

The data presented in Tables (2) indicate that the isolates No. R. 27 and R.59 show quite similarity towards their physiological and biochemical properties and they are therefore considered to be two identical strains belonging to *S. exfoliatus*[29].

In the present investigation the additional physiological and biochemical properties of the studied organisms were used for further description and for identification of the species-group or strains. However, certain tests notably, starch hydrolysis, cellulose decomposition, gelatin liquifaction, coagulation and peptonization of milk, reduction of nitrates to nitrites, production of H_2S, utilization of oxalate, hydrolysis of hippurate, tolerance to sodium chloride, utilization of organic acid salts, hydrolysis of urea and utilization of different nitrogen sources were employed herein for differentiation among *Streptomyces* species or strains. This is conformety with the results recommended by mamy authers[21-34].

REFERENCES

1. Cummins, C.S. and Harris, H.: Studies on cell-wall composition and taxonomy of Actinomycetales and related groups **Gen. J. Microbial**. 18, 173-189. (1958)
2. Goodfellow, M. and Alderson, G.: Numerical taxonomy of actinomadura and related actinomycetes. **Gen. J. Microbiol**. 112, P.95-111. (1979)
3. Sermonti, G. and Hopwiid, D.A: Genetic recombination in *Streptomyces* In Gunsalus, I.C. and Stanier, R.Y. (eds.). The Bacteria Vol. 5: Here-dity. Acad Press, New York 223. (1964)
4. Shirling, E.B. and Gottlieb, D.: Methods for characterization of Streptomyces species **Int. J. Syst Bact**. 16, P.313-340. (1966)
5. Shirling, E.B. and Gottlieb, D.: Co-operative description of type cultures of *Streptomyces*. II. Specie description fromm first study. **Int. J. Syst. Bact**. 18. 69-189. (1968a)
6. Shirling, E.B.and gottlieb, D.: Co-operative III. Additional species descriptions from first and second studies **Int. J. Syst. Bact.** 18, P.279- 391. (1968b)
7. Shirling, E.B. and Gottlieb, D.: Co -operative description of type cultures of *Streptomyces* IV species descriptions from the second, third and fourth studies **Int. J. Syst.Bact.** 19,.391-512. (1969)
8. Shirling, E.B. and Gottlieb, D. Co-operative description of type straind of Streptomyc. V. Additional descriptions **Int. J. Syst. pact**. 22, 265-394. (1972)
9. Kuster, E.: Simple working key for the classe- fication and identification of named taxa included in ISP **Int. J. Syst. Bact**. 22, 139-148. (1972)
10. Nonomura, H.: Key for classification and identification of 458species of the Streptomy-cetes included in ISP. J. **Ferment.** 52, 78-92. (1974)
11. Szabo, I.M., Marton, M., Buti, I. and Fernandez, C. Adiagnostic key for the tne identification of "species" of *Streptomyces* and Streptoverti-cillium included in ISP. **Acta Bacteria Acad. Scient. Hungaricae** 21,.387-418. (1975)
12. El- Sayed, A. El-S., Mansour, F.A., A.Ismail and Enan, G.: Biological characteristics and taxonomy of bacteria belonging to the genus Streptonyces IV Streptomyce-tes weth white coloured aerial mycelium **Egypt. J. Microbiol**. (Under publication)
13. Johnson, L.F., Curs, E.A.,Bond, J.a and fnioaurg H.A.: Methods for studying soil microflora plant

disease relationship. Burgess publishing Co. Minneapolis 15, Minn. U.S.A. (1959)
14. Pridhan, T.G. and Gottlieb, D.: The utiliza- tion of carbon compounds by some Actinomycetales as an aid for species identification. **J. Bact.** 56, 107-114. (1948)
15. Harrigan, W.F. and Mc Cane M.E.:**Laboratory Methos in Microbiology**. Academ. Press. London & New york. (1966)
16. Waksman, S.A.: The Actinomycetes. Vol. I. The Wolliams & Wilkins Co., Baltimore. (1959)
17. Williams, S.T. Goodfellow, M.,Alderson, G., Wellington, E.M., Sneath, P.H. and Sookl, M. J. Numerical classification of Strepto-myces and related genera. **Gen. J. Microbiol. 129**, 1743. (1983)
18. Crawford, D.L. and Mc Coy, E.: Cllulases of Themomonospora fusca and *Streptomyces* thermo-diastaticus. **App. Microbiol** . 24, 150-152. (1972)
19. Allen, O.M. : Experiments in soil Bacteriology, Ist. Ed. Burgess publ. Co. (1953)
20. Kuster, E. and Williams, S.T.: Production of hydrogen sulphide by ***Streptomycetes*** and methods for its detection .**Appl. Microbiol**. 12, 46-52. (1964)
21. Nitsch, B.and Kutzner, H.J.:Decomposition of oxalic acid and other organic acids by strep-tomycetes as a taxonomic aid . **Zeitschreft fur Allegemeing Microbiol**. 9,.613-632. (1969A)
22. **Nitsch, B.and kutzner, H.J.:** Harnstoff-zersetzung und Harnstoffverwertung durch strepto- myceten. **Zentrasblatt fur bakteriol.**123, 380-398. (1969b)
23. Ziegler, P and Kutzner, H. J.: Hippurate hydrolysis as a taxonomic criterian in the genus *Streptomyces* (order Actmomycetales). **Zeitschreft fur allaemeine Mikrobiolgie** 13. 263- 270. (1973)
24. **Bergey's Manual of Systematic Bacteriology** : 1 st ed. Vol. 4. Williams and Wilkins, Baltimore, Hongkong, London, Sydney. (1989).
25. Shinobu, R.: Two new species of *Streptomyces*. Mim osaka, Unev. lib. Arts and Educ., **B. Natur, Sci.** 6, 63-73. (1957)
26. Preobrazhenskaya,T.P.:Characteristics of antagonistec actenomycetes of the fradiae series (in Russian)P. 51-57. In Gauze, G.F., preobra-zhenskaya, T.p., Kudrina, F.S., Blinov, N.O., zhenskaya, T.P., Kudrina, F.S., Blinov, N. O., Ryabova, I.D. and Sveshnikova, M.A.(ed.) problems of classefecation of actinomycetes antiagonises. House of Medical literature, Medgiz, Moscow. (1957)
27. Preobrazhenskaya, T.P. and Sveshnikova, M.A.: Charactreistics of antagonistec actinomycetes of the lavendulae-Rose series (in Russian). P. 31-50. In Gaarze et al .(1957). **House of Medical litera ture Medgiz**, Moscow. (1957)
28. Ryabova, I.D. and Preobrazhenskaya, T.P.: Characteristics antagonistec of actinomycetes of the Veolaceus series (in Russian). p. 278-197. In Gauze et al.,(1957): **Problems of Classifica-midical Literature. Medg iz** , Moscow. (1957)
29. Waksman, S.A. and Henrici, A.T.: Family Actino-mycetaceae, Buchanan and Family streptomycetaceae Waksman and Henrici. In Bergey's Manual of determinative bacteriology 6th ed, 892-980 (1948)
30. Burkholder, P.R. Sun, S.H., Anderson, L.E. and Ehrlich J. Criteria of Specification in the Genus *Streptomyces*. **Ann. N. Y. Acad. Sci**. 60, 102-123. (1954).
31. Tresber, H.D. Hayes, J.A.and Backus, E.J.: Differential tolerance of *Streptomycetes* to sodium chloride as a taxonomic aid. **Appl. Microbiol**. 16, 1134-1136. (1968)
32. Iegler, P. and Kutzner. H.J. : *Streptomyces* taxonomy: hydrolys is of hippuratet and inability to utilize binzoic acid naturwisserschaften 59, 123. (1972)
33. Ziegler, P. and Kutzner, H.J.: Hippurate hydrolysis as a taxonomic criterian in the genus *Streptomyces* (order Actinomycetases). Zeitschrift fur Allqeneine Mikrobiolgie 13,.263-270. (1973)
34. Kurnwe, H.J. Bottiger, V. and Heitzer, R.D: The use of physiologycal citeria in the taxonomy of *Streptomyces* and *Streptovorticillium* sp. 25-29. In Mordarski, M., Kurylowicz, W. andmyces. proceedings of the Internateor. al *Streptomyces* on Nocardea and *Streptomyces*. Warsaw (1976) Stuttgart, New York. Gustav Fischer Verlag. (1978)

الخواص البيولوجية وتصنيف البكتريا التابعة لجنس ستربتوميسيس الميسليوم الهوائى الاحمر اللون

أحمد اسماعيل عبدالقادر وفتحى عواد منصور*

قسم النبات – كلية العلوم – جامعتى الزقازيق والمنصورة* – مصر

استهدف هذا البحث تصنيف "١٣" معزولا ذات ميسليوم هوائى أحمر اللون تم عزلها جميعا من مناطق مختلفة تمثل الجزء الشمالى لمصر قسموا جميعا فى قسم واحد وصنفوا على حسب مقدرتهم على انتاج صبغة الميلانين إلى تحت قسمين. الأول ويشمل سبع معزولات وجميعهم ينتجوا صبغ الميلانين ولأن المعزولات تبدى أختلافا فيما بينها من حيث لون الميسليوم المنبتى فقد تميزت إلى شعبتين. أولهما تضم معزولتان ذات ميسليوم منبتى بنى محمر وقد عرفا على أنهما سلالاتين متشابهتين تتبعا ستربتوميسيس فايوبوربوريس. وثانيهما تضم خمس معزولات ذات ميسليوم منبتى لونه الفعلى غير مميز وقد صنفت الخمس معزولات بدورهم إلى تحت شعبتين حسب قدرتها على استخدام المصادر الكربونية. الأولى منهما وتشمل معزولتان صنفوا على أنهما سلالاتان مختلفتان تتبعا ستربتوميسيس روزيوفيربايس. والثانية منهما تشمل ثلاث معزولات ، أثنين منهم صنفا على أنهما سلالتان متشابهتان تتبعا ستربتوميسيس فلا فوتريشيى ، أما المعزول الثالث فقد صنف على أنه سلالة مختلفة عن الاثنتين السابقتين وتتبع نفس النوع.

أما تحت القسم الثانى ، ويشمل ست معزولات تتميز جميعها بعدم قدرتها على انتاج صبغات الميلانين وبما أنها تبدى اختلافا فيما بينها من حيث لون الميسليوم المتنى فقد تميزت إلى شعبتين. الأولى تضم أربعة معزولات ذات ميسليوم منبتى رمادى محمر إلى أحمر وعنابت غذائية غير ملونة وقد صنفا على أنهما مجموعتان مختلفتان من السلالات المتشابهة تتبع ستربتوميسيس بروتيكولور. والثانية تضم معزولين يتميز كل منهما بميسليوم منبتى أصفر مشوب بظلال رمادية وقد صنفا على أنهما سلالتان متشابهتان تتبعان ستربتوميسيس اكسفولياتس.