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6

Az-SA-501: A New Antibiotic with Antiviral Inducing Effect Produced by a Strain of *Streptoverticillium lavenduligriseum*. 1—Taxonomic Discussion of the Producing Strain

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A strain of *Streptoverticillium lavenduligriseum* which proved to produce an antiviral-inducing peptide was isolated from a soil sample collected from Al-Ezaziah, Makkah, Saudi Arabia. The isolate belonged to the "grey colour series" of the ISP classified *Streptomyces* but showing occasional verticils, Bergey Table 17.48 was consulted. From the taxonomic studies, the strain resembles *Streptomyces bottropensis, S. umbrosus, S. rameus, S. achromogenes subsp. rubradinis, S. galbus, S. reticuli* and *Streptoverticillium lavenduligriseum* in some morphological, cultural and physiological properties. It exhibits differences from the previous *Streptomyces* and/or *Streptoverticillium* species. It is at present considered as a strain of *St. lavenduligriseum* due to its verticillate sporophores, and it is at present given the name *Streptoverticillium lavenduligriseum Az-SA*-501.

In the course of a screening for new biologically active metabolites, an agent inducing antiviral effect was detected in the culture broth of an actinomycete isolate No. 501. This actinomycete was isolated from a soil sample obtained from Al-Ezaziah locality, Makkah, Saudi Arabia. This paper reports the characterization and the taxonomic position of the isolate posing the difficulty of its identification that could lead—in further work—to consider it a new species.

Material and Methods

An actinomycete isolate No. 501 was characterized by its grey aerial mass colour and its formation of yellow brown, light olive brown, deep orange yellow, brownish

orange, brilliant orange yellow and deep orange yellow reverse colour on the ISP (International Streptomyces Project) media described by Shirling and Gottlieb (1966) and Waksman (1961). A yellow soluble pigment was detected in all media used. These studies were carried out at 30 °C for 14 days.

The generic identification of the isolate was made on the basis of the morphological, cultural, physiological characteristics and chemical analysis of a whole cell hydrolysate. The chemical analysis was carried out according to the method of Becker *et al.* (1964) and Yamaguchi (1965). The tests used to characterize strain 501 were those described by Shirling and Gottlieb (1966) and Waksman (1961). The keys of Waksman (1961), Kuster (1972), Pridham and Tresner (1974), Nonomura (1974) and Szabo *et al.* (1975) together with the ISP descriptions of the *Streptomyces* species (Shirling and Gottlieb 1968a, 1968b, 1969, 1972; Waksman 1961) were used to identify the isolate 501 to the species level.

Results

The results of the characterization of the actinomycete isolate No. 501 according to the methods previously mentioned are as follows.

Cell wall analysis

A whole cell hydrolysate analysis revealed the presence of LL-di-aminopimelic acid, for which the isolate was assigned to the Family Streptomycetaceae (Bergey 1974, 74).

Spore chain morphology

Morphological observations were carried out on cultures with phase contrast and transmission electron microscopes. The culture was grown at 30 °C for 14 days on yeast extract-malt extract agar, inorganic salts starch agar or oatmeal agar. Abundant, good or moderate growth of aerial mycelium was observed on all tested media. The photographs of sporophores (Fig. 1) show simple verticils carried on sterile straight sphorophores. The spirals (Bergey 1974, 748 and 880) were of the section RF (Rectus Flexibilis) to RA (Retinaculum Apertum).

Spore surface

The spores are spherical or oval with smooth spore surface (Fig. 2).

Fig. 1. Phase contrast or aerial mycelium of strain No 501(X2,000 × 3)

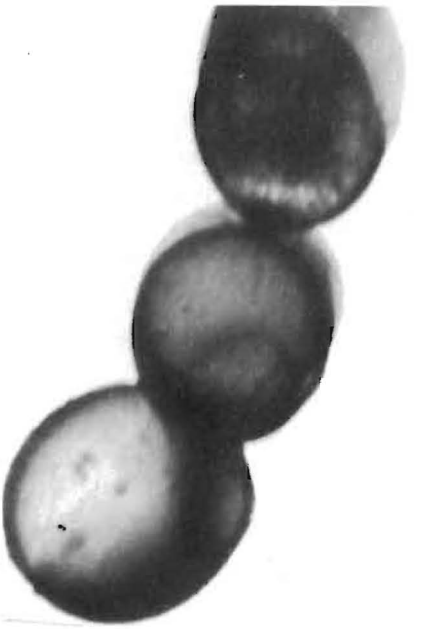

Fig. 2. Spore surface of strain No. 501 by transmission elecron microscope (X20,000 × 3)

Table 1. Cultural characteristics of strain Az-SA-501.

Media	Growth	Aerial mycelium	Reverse colour	Soluble pigment
Oatmeal agar	Abundant	Grey	Yellowish brown	Yellow
Malt extract-yeast	Abundant	Grey	Yellowish brown	Yellow
Inorganic salts-starch agar	Abundant	Light brownish grey	Light olive brown	Yellow
Sucrose-nitrate agar	Good	Light yellow	Brownish orange	Yellow
Glycerol-asparagine	Good	Brilliant yellow	Deep orange yellow	Yellow
Glucose-nitrate agar	Abundant	Pale greenish yellow	Brilliant orange yellow	Yellow
Starch-nitrate agar	Abundant	Greyish yellow	Orange yellow	Yellow
Glycerol-nitrate agar	Good	Light orange yellow	Deep orange yellow	Yellow
Glucose-asparagine agar	Good	Brilliant yellow	Deep orange yellow	Yellow
Nutrient agar	Moderate	Yellowish white yellow	Deep orange yellow	Yellow

Colour of colony

The mature aerial mass colours are in the grey colour series on the ISP (Shirling and Gottlieb 1966) and Waksman (1961) media (Table 1). The colour names (Kelly 1965) were used for colour determination of both aerial and substrate mycelia.

Colour of reverse side of colony

The reverse sides of colonies were yellowish brown, light brown, deep orange yellow, brownish orange, brilliant orange and deep yellow on the ISP and Waksman (1961) media.

Colour in medium

Melanoid pigments were formed in peptone-yeast extract-iron agar tyrosine agar and tryptone-yeast broth. The water soluble pigments produced were yellow in all tested media. The produced pigments were found to be insensitive to both acids and alkalies, i.e. not pH indicators.

Physiological characteristics and carbon sources utilization

Physiological properties and utilization of carbon sources of strain 501 are summarized in Tables 2 and 3. Coagulation of milk, liquefication of gelatin and blood

Table 2. Physiological characteristics of strain Az-SA-501.

Test	Result	Test	Result
Production of melanoid pigment	Positive	Gelatin liquefaction	Negative
Tyrosinase reaction	Positive	Blood agar	No haemolysis
Milk coagulation	Negative	Hydrogen sulfide	Positive
Milk peptonization	Positive	Nitrate reduction	Positive
Starch hydrolysis	Positive		

Table 3. Carbon sources utilization by strain Az-SA-501.

Sugar	Result	Sugar	Result	Sugar	Result
D-Glucose	+ + + +	D-Xylose	+ +	Rhamnose	+ +
L-Arabinose	+ + +	D-Fructose	+ + +	i-Inositol	−
Sucrose	+ +	D-Mannitol	+ + +	Raffinose	±

+ + + +, = Abundant growth; + + +, = Good growth; + +, = Moderate growth; ±, = doubtful.

haemolysis are negative; peptonization of milk, starch hydrolysis, tyrosinase, hydrogen sulphide and nitrate reduction are positive.

Utilization of carbon sources was examined using Pridham and Gottlieb's (1966) basal medium. D-glucose, L-arbinose, sucrose, D-xylose D-fructose, D-mannitol and rhamnose were utilized; raffinose was weakly utilized and inositol was not utilized.

Sensitivity to streptomycin.

Strain 501 was inhibited by streptomycin.

Growth on Czapek's solution.

Growth on Czapek's solution was good.

Identification of strain Az-SA-501

Following descriptions of strain 501 and the use of the identification keys for *Streptomyces* and *Streptoverticillium* species leads to the following:

Kuster key entry as distinctive reverse pigments (p. 41) leads to *S. bottropensis*, but when entry as no distinctive pigment, it leads to *S. umbrosus* (p. 142).

Pridham and Tresner (Bergey 1974, Table 17.42, p. 764) leads to *S. rameus* and *S. achromogenes* subsp. *rubradinis.*

Being verticillate, entry in Nonomura should have been: Section Verticillati (p. 91). Nonomura key entry as GY and Spirales (111) leads to *S. bottropensis* and when entry as GY and SRA (101) "two types of spore chain morphology i.e. Spirales and Retanculiaperti" leads to *S. umbrosus* and *S. galbus* (F).

Szabo *et al.* (p. 409) leads to *S. lavenduligriseus.*

Waksman key, entry as sporophores in aerial mycelium form verticils, melanin positive, sporophores are straight or spiral shaped leads to *S. reticuli.*

Discussion

Consulting Shirling and Gottlieb (1968, 1969, 1972), Bergey (1974) and/or Waksman (1961) descriptions, the present isolate shows the following variations from the previous related *Streptomyces* species.

S. bottropensis failed to produce soluble pigment on glucose-asparagine agar, produce brown soluble pigment on sucrose-nitrate agar, pink growth first on starch-agar, later darker (pH sensitive; acid-pink, alkaline-blue).

S. rameus failed to utilize rhamnose, produce the antibiotic streptomycin.

S. ambrosus, melanin pigment may be less distinct in tyrosine agar, inositol was utilized as a carbon source and no or only trace of growth with sucrose.

S. achromogenes subsp. *rubradinis,* showed poor growth in Czapek's agar and produce the antibiotic rubracidin.

S. galbus, sporophores monopodially branched, weak growth on glucose asparagine agar, give yellowish to yellow green soluble pigment on glucose-nitrate agar, inositol was utilized, no or only trace of growth with sucrose or rhamnose and produce neomycin.

Questionable *S. lavenduligriseus* (Szabo *et al.* 1975 but not valid pub. Bergey 1974, p. 841, both considering ISP), spore chain morphology, section RF to RA, one observer recorded monoverticillate sporophores (ISP V p. 302). Aerial mycelium is poorly developed or absent on glycerol-asparagine agar. Soluble pigment is a pH sensitive.

S. reticuli, the verticils are primary and secondary; no spirals were observed (Ettlinger *et al.* 1958). They also report the species to be melanin negative.

The descriptions of the above species found in Shirling and Gottlieb (1968, 1969, 1972), in Bergey (1974) and in Waksman (1961) show that the present isolate is

near to *S. lavenduligrieseus* and *S. reticuli.* The liquefaction of gelatin and production of neomycin by some strains of *S. reticuli* justify the present isolate to be more near to *S. lavenduligriseus* than *S. reticuli.* Consequently, the isolate Az-SA-501 is felt better (on the basis of morphological, cultural and physiological characters) considered as *S. lavenduligriseus* although it differs in sporophore, in the development of poor aerial mycelium or absent on glycerol-asparagine agar and the production of a pH sensitive soluble pigment. The previous characters were not detected in the verticillate sporophore isolate under investigation. For this, it is considered a strain of *S. lavenduligriseus* and was given the name *Streptomyces lavenduligriseus* Az-SA-501.

However, being verticillate and because the questionable name *S. lavenduligriseus* (Szabo *et al.* 1975) is not val. Pub: (Bergey 1974, p. 841), the name now considered is *Streptoverticillium lavenduligriseum* given the strain symbol Az-SA-501. Deviations from the species justify considering its taxonomy in further work. At present, interest is directed towards its antibiotic antiviral-inducing effect. This is the object of the second paper in this series.

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أز ـ س ع ـ ٥٠١ مضاد أحيائي جديد يستحث صفه ضد الفيروسية يفرز بواسطة كائن ستربتوفرتسليوم لايفينديوليجرزيوم . ١ ـ شرح الوضع التقسيمي للكائن

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لقد تم عزل مضاد حيوي ببتيدي جديد يستحث صفه ضد الفيروسية من سلالة ستربتوفرتسليوم معزولة من عينة ترابية من منطقة العزيزية بمكة المكرمة بالمملكة العربية السعودية .

وقد وجد ان الكائن يتبع المجموعة الرمادية في المشروع الدولي لتقسيم جنس ستربتوميسيس .

وقد اتضح من الدراسات التقسيمية ان الكائن المستخدم يماثل في بعض الصفات المورفولوجية ، والمزروعية ، والفسيولوجية كائنات الستربتوميسيس التالية : بوتروبينسيس ، أميروزس ، جاليس ، راميس أكروموجينز تحت جنس روبرادينس ، وربتيكيولاى وقد نوقشت الاعتبارات التصنيفية في ظل المفاتيح الحديثة .

وقد وجد ان الكائن محل الدراسة يختلف في بعض الصفات عن الكائنات الستربتوميسينية السابقة وبالتالي فقد اعتبر في الوقت الحاضر سلالة جديدة من نوع استربتوفرتسليوم لافينديوليجرزيوم نظرا لبعض الاعتبارات المشروحة في معظم الصفات وبالتالي فقد أعطى اسم ستربتوفرتسليوم لافينديوليجرزيوم ـ أز ـ س ع ـ ٥٠١ في الوقت الحاضر ، واتجه الجهد لدراسة نشاطه المذكور الذي سينشر في بحث تالي .