JOURNAL OF CULTURE COLLECTIONS
Volume 5, 2006-2007, pp. 10-15

TAXONOMIC STUDY OF *STREPTOMYCES* SP. STRAIN 34-1

Stefka Antonova-Nikolova[1]*, Vanya Stefanova[1] and Lyubomira Yocheva[2]

[1]Sofia University, Biological Faculty, Department of General and Industrial Microbiology, 8 Dragan Tzankov st., 1164 Sofia, Bulgaria;
[2]Institute for Cryobiology and Food Technology, 53 Cherni vrah blvd., 1407 Sofia, Bulgaria

*Corresponding author, e-mail: antonova@biofac.uni-sofia.bg

Summary

The present work is a part of a complex study of Streptomyces sp. strain 34-1 - a producer of trypsin and trypsin-like protease inhibitors with antiviral effect. The taxonomically significant properties of the strain were examined according to the International Streptomycetes Project (ISP). The identification of the strain was based on Nonomura's key. The morphological, cultural, physiological and biochemical characteristics of Streptomyces sp. strain 34-1 were compared with the references for similar streptomycetes species. The strain was identified as Streptomyces chromofuscus and designated as S. chromofuscus 34-1.

Key words: *streptomycetes, taxonomy, protease inhibitor.*

Introduction

Microorganisms of the genus *Streptomyces* produce a wide spectrum of bioactive substances (antibiotics, pigments, and enzymes) with application in pharmaceutical and food industries, in biotechnology and laboratory practice [2].

The ability of the streptomycetes to synthesize enzyme inhibitors reveals a new aspect of microbial antagonism.

It is known that proper proteolytic enzymes of the infected cells play decisive role in the replication of numerous viruses including the influenza viruses. To become infectious, the viruses have to undergo posttranscriptional cleavage of the basic antigen – hemagglutinin. This reaction is carried out by the trypsin-like proteases of the host [7, 13]. The inhibition of these proteases leads to the inhibition of the viral replication.

The proved ability to synthesize a substance which inhibits the activity of trypsin and trypsin-like proteases and viruses makes *Streptomyces* sp. strain 34-1 a perspective producer of the protease inhibitor [1].

This work is a part of a complex study of *Streptomyces* sp. strain 34-1 – producer of a protease inhibitor and aims its characterization and taxonomic identification.

Materials and Methods

The soil isolate *Streptomyces* sp. strain 34-1 from the collection of the Department of General and Industrial Microbiology at Sofia University was the subject in this study. The taxonomic properties of the strain were determined according to the International Streptomycetes Project (ISP) [9] and some procedures described by Gause et al. [5].

The strain morphology was observed on the 7th, the 14th, and the 21st day for mature cultures. The morphology of the spore-bearing aerial hyphae was determined by direct microscopic examination of the culture surface. The length of the spore chain and the spore surface ornamentation were observed by an electron microscope. The electron microscope investigation was made on grids coated with Formvar without shadowing.

The colour of the aerial mass, the substrate mycelium and the soluble pigment was

determined using the Bondartsev colour scale [3]. The production of a melanin pigment was observed after 2 and 4 days.

The ability of the strain to use different carbon sources was determined according to the ISP recommendation [9]. The starch hydrolysis and the growth on gelatin and skim milk were tested according to Gauze et al. [5].

The sensibility to antibiotics was examined by the paper discs method [6]. The following antibiotics ("BulBio") were used: erythromycin (15 µg/disc), ampicillin (10 µg/disc), streptomycin (10 µg/disc), gentamycin (10 µg/disc), penicillin (10 E/disc), chloramphenicol (30 µg/disc), tetracyclin (15 µg/disc). The diameters of the growth inhibition zone were measured after incubation for 48-72 h.

The taxonomic identification of *Streptomyces* sp. 34-1 was based on Nonomura's key [8] and the species description according to ISP [10, 11, 12], Gauze et al. [5] and Bergey's Manual [4].

Results and Discussion

Micromorphological characteristics. The substrate mycelium of strain *Streptomyces* sp. 34-1 did not fragment. The aerial mycelium formed monopodially branched spore-bearing hyphae with the shape of hooks, loops, open or compact spirals with 3-6 curves (Fig. 1). The spore chains contained 10-50 spores. They were ellipsoidal with spiny surface (Fig. 2).

Macromorphological characteristics. The strain was clearly polymorph. It formed 7 morphological types of colonies on Gauze I mineral medium. The types of colonies differed in shape (round or ellipsoidal), profile (flat or slightly raised, umbonate or with craters), surface (smooth or wrinkled), edges (undulating or rhizoid). The colonies were completely covered by aerial my-

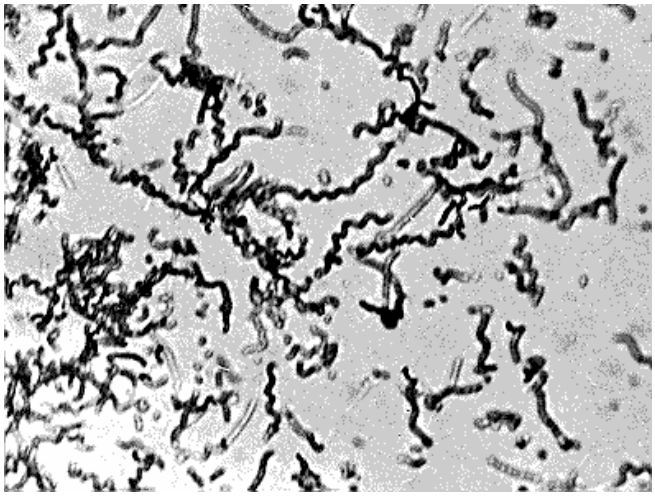

Fig. 1. Morphology of the spore-bearing aerial hyphae of *Streptomyces* sp. 34-1 (ISP-3, 800x).

A **B**

Fig. 2. Spore chain (**A**, 13 400x) and spore surface ornamentation (**B**, 25 000x) of *Streptomyces* sp. 34-1 (ISP-3).

celium or it formed concentric or radial lines (Fig. 3). The dominant type was presented by round, umbonate colonies, completely covered by pale grey aerial mycelium with slightly undulating edges.

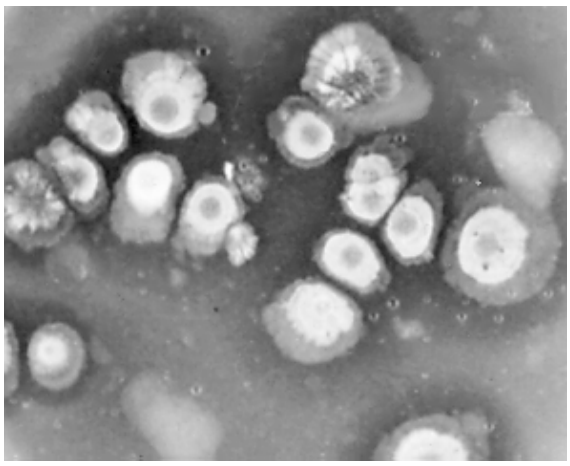

Fig. 3. Morphological types of colonies of *Streptomyces* sp. 34-1 (Gauze I mineral medium).

Streptomyces sp. 34-1 grew on all of the used media. The abundance and the colour of the aerial mycelium depended on the medium composition and the age of the culture. The aerial mass colour varied from white and pale blue to different nuances of grey (from pale grey to green-grey), therefore it could be assigned to the grey series (Table 1).

The substrate mycelium colour varied from pale grey to dark grey depending on the medium composition and the age of the culture. It could be accepted as not distinctive according to Nonomura's key [8]. pH changes did not affect the reverse colour.

On most of the media used *Streptomyces* sp. 34-1 produced a soluble pigment whose colour varied from beige-olive (on Gauze I mineral medium, ISP-3 and ISP-5 media) to dark brown (ISP-2 medium). The strain did not produce any diffusible pigment on ISP-4 medium (Table 1).

Table 1. Cultural characteristics of *Streptomyces* sp. 34-1.

Medium	Growth	Age (days)	Colour of aerial mycelium	Colour of substrate mycelium	Colour of soluble pigment
ISP-2	abundant	7	white; dark ash-grey	dark brown-green	dark brown-green
		14	white; mouse-grey	dark ash-grey	brown
		21	mouse-grey	brown-yellow	dark brown
ISP-3	abundant	7	pale blue; blue	dark ash-grey	-
		14	pale blue; blue	dark grey	beige-olive
		21	green-grey	dark grey	dark beige-olive
ISP-4	abundant	7	white; pale blue	ash-grey	-
		14	white	dark ash-grey	-
		21	mouse-grey	pale grey-violet	-
ISP-5	moderate	7	white; pale grey-violet	dark grey	-
		14	white; pale grey-violet	dark ash-grey	beige-olive
		21	grey-violet	grey-violet	dark beige-olive

Physiological and biochemical properties. The strain produced melanoid pigments on pepton iron agar (ISP-6). Melanin production was not observed on tyrosine agar (ISP-7).

Streptomyces sp. 34-1 grew poorly in the presence of D-xylose and I-inositol as a sole carbon source. The strain formed abundant mycelium on the media with D-glucose, D-fructose, L-arabinose, L-rhamnose and D-mannitol, but its growth was doubtful on the media with sucrose and raffinose (Table 2).

Strain 34-1 grew on gelatin without liquefying it. It also grew on skim milk but did not cause coagulation and peptonization. It hydrolysed starch.

The strain was sensitive to the action of antibiotics such as tetracyclin, gentamycin and erythromycin (Table 3).

Table 2. Carbon utilization by *Streptomyces* sp. 34-1.

Carbon source	Utilization
No carbon source (negative control)	-
L-Arabinose	++
D-Xylose	+
i-Inositol	+
D-Mannitol	++
D-Fructose	++
L-Rhamnose	++
Sucrose	±
Raffinose	±
Cellulose	-
D-Glucose (positive control)	++

Legend: utilization of the tested carbon is equal to or grater than that of the positive control (++); weaker utilization in comparison to the positive control (+); doubtful utilization (±); no utilization (-).

Table 3. Sensibility of *Streptomyces* sp. 34-1 to different antibiotics.

Antibiotics	Sterile zone [mm]
Tetraciclin	22
Streptomycin	-
Gentamycin	12
Erythromycin	21
Ampicillin	-
Penicillin	-
Chloramphenicol	-

Identification of *Streptomyces sp*. 34-1. Morphological, cultural, physiological and biochemical properties of the strain 34-1 were compared to *Streptomyces* species included in Nonomura's key [8]. Using references given by ISP [10, 11, 12] and Bergey's Manual [4], the strain was compared with the most similar species (Table 4).

Streptomyces sp. 34-1 showed greatest similarity to *S. chromofuscus* [11] in morphology of the sporophores (spiral), ornamenttation of the spore surface (spiny), cultural properties (grey aerial mass colour, substrate mycelium without distinctive colour, beige-olive to yellow soluble pigment) and by the absence of melanin production on the medium ISP-7. The strain 34-1 differed from *S. chromofuscus* in carbon utilization. It utilized D-fructose and its growth was doubtful in the presence of sucrose. The spore-bearing hyphae of *Streptomyces* sp. 34-1 formed besides the compact spirals single open spirals, and hooks and loops (RA). It could be concluded on this base that *Streptomyces* sp. 34-1 is identical or very similar to *S. chromofuscus*.

Differences in the "secondary" taxonomic features such as carbon utilization were the reason to identify the strain as *S. chromofuscus* 34-1.

Short characterization of *Streptomyces chromofuscus* 34-1. The strain forms monopodial branched sporophores with the shape of hooks, loops, imperfect and perfect spirals with 3-6 curves (SRA). Spore surface is spiny. The spore chain length is between 10 and 50 spores. The aerial mass colour is of the grey series (from pale grey to grey-violet), and it is white to pale blue on some media and in young cultures. The colour of substrate mycelium is not distinctive (greyish or brownish nuance). The culture is polymorph. It produces olive or brownish soluble pigment on all of

Table 4. Basic taxonomical characteristics of *Streptomyces* sp. 34-1 and related streptomycetes species.

Species name	Aerial mass colour	Melanoid pigment[1]	Reverse side pigment	Soluble pigment	Spore chain[2]	Spore surface	L-arabinose	D-xylose	I-inositol	D-mannitol	D-fructose	L-rhamnose	Sucrose	Raffinose	D-glucose
Streptomyces sp. 34-1	grey	+–	–	beige-olive dark brown	SRA	spiny	+	+	+	+	+	+	±	±	+
S. echinatus	grey	++	–	–	SRA	spiny	+	+	+	+	+	+	–	+	+
S. chromofuscus	grey	+–	–	pale yellow	SRA[3]	spiny	+	+	+	+	±	+	–	±	+
S. griseochromogenes	grey	++	–	–	SRA	spiny	+	+	+	+	+	–	+	+	+
S. gannamycicus	grey	++	–	–	S	spiny	±	+	+	+	+	+	±	+	+
S. flavoviridis	grey	++	–	–	SRA	hairy	+	+	+	+	+	+	–	–	+

Legend: [1] Melanin production on both ISP-6 and ISP-7 media (++); melanin production only on ISP-6 (+–);
[2] Hooks, loops and open spirals (RA); spirals (S);
[3] 11 % of the strains belonging to the species *S. chromofuscus* form RA spore-bearing aerial hyphae [6].

the used media with the exception of the ISP-4 medium. It synthesizes melanin on the ISP-6 medium only. It does not possess tyrosinase activity. The strain grows on gelatin and liquefies it scarcely perceptibly, inclining to negative. It grows on skim milk without causing its peptonization or coagulation. It hydrolyses starch. The culture utilizes D-glucose, D-fructose, L-arabinose, D-xylose, L-rhamnose, I-inositol and D-mannitol. The utilization of sucrose and raffinose is doubtful. The strain produces trypsin and trypsin-like proteases inhibitor [1]. It has antiviral activity. It is sensitive to tetracyclin, gentamycin and erythromycin.

Acknowledgments. This study was supported by the research grant No L-11006/2001 from The National Science Fund of The Bulgarian Ministry of Education and Science. We express our thanks to Vassilis Kapurdov from the National Center of Hygiene, Sofia for the generous access to electron microscope.

References

1. Angelova, L., E. Ivanova, J. Serkedjieva, I. Ivanova, 2003. *Biotechnol. Biotechnol. Eq.*, **17** (2), 110-114.
2. Antonova-Nikolova, S., I. Ivanova, 1999. *Biology of Streptomycetes – producers of antibiotics*, Sofia: St. Kl. Ohridski, 107 (in Bulgarian).
3. Bondartsev, A., 1954. *Colour Scale*, Moskva-Leningrad: Acad. Sci. USSR, 27 (in Russian).
4. Cross, T., H. Lechevalier, 1994. Actinomycetes: Group 22-29. In: *Bergey's manual of determinative bacteriology*, J. Holt, N. Krieg, P. Sneath, J. Staley, S. Williams (Eds), Ninth Edition, Baltimore-Philadelphia-Hong Kong: Williams & Wilkins, 2344-2347.
5. Gauze, G., T. Preobrazhenskaja, M. Sveshnikova, L. Terehova, T. Maksimova, 1983. *Guide of Actinomycetes*, Moskva: Nauka, 248 (in Russian).
6. Gushterov, G., P. Andonov, Ts. Todorov, L. Kominkov, M. Gincheva-Starcheva, 1977. *Manual of microbiology and virology*, Sofia: Nauka i Izkustvo, 393 (in Bulgarian).
7. Lozitsky, V., A. Fedchuk, L. Puzis, V. Buiko, I. Girlia, 1987. *Voprosi Virusologii*, **32**, 413-419 (in Russian).
8. Nonomura, H., 1974. *J. Ferment. Technol*., **52** (7), 78-92.
9. Shirling, E., D. Gottlieb, 1966. *Intern. J. System. Bacteriol*., I, **16** (3), 313-340.
10. Shirling, E., D. Gottlieb, 1968. *Intern. J. System. Bacteriol*., II, **18** (2), 106-118.
11. Shirling, E., D. Gottlieb, 1968. *Intern. J. System. Bacteriol*., III, **18** (4), 307.
12. Shirling, E., D. Gottlieb, 1969. *Intern. J. System. Bacteriol*., V, **19,** 300-303.
13. Zhirnov, O., A. Ovcharenko, A. Bukrinskaja, 1985. *Voprosi Virusologii*, **30,** 207-214 (in Russian).

ТАКСОНОМИЧНО ИЗСЛЕДВАНЕ НА ЩАМ *STREPTOMYCES* SP. 34-1

Стефка Антонова-Николова[1]*, Ваня Стефанова[1], Любомира Йочева[2]

Резюме

Настоящата работа е част от комплексно проучване на щам Streptomyces sp. 34-1, продуцент на инхибитор на трипсин и трипсин-подобни протеази с антивирусна активност. Представени са данни за таксономичните му свойства, получени чрез методите на Международния стрептомицетен проект (ISP). При идентифицирането му е използван ключът на Nonomura. Морфологичните, културалните и физиолого-биохимичните характеристики на Streptomyces sp. 34-1 са сравнени с литературните данни за сходни стрептомицетни видове. Щамът е отнесен към вида Streptomyces chromofuscus и е означен като S. chromofuscus 34-1.