Sequence-based differentiation of strains in the *Streptomyces cyaneus* species-group

Ehsan Rashidian¹ and Michael Goodfellow²

¹Department of Microbiology, School of Veterinary Medicine, Lorestan University, Khorramabad, I.R. Iran. ²School of Biology, University of Newcastle, Newcastle Upon Tyne, NE1 7RU, United Kingdom.

Abstract

In an extensive numerical phenetic survey, a number of blue, red and grav spored streptomycete strains were grouped together as Streptomyces cyaneus species-group, a taxon which encompasses strains known to produce antitumor antibiotics, notably anthracyclines. In the present investigation these and related streptomycetes were the subject of morphological and 16S rRNA sequencing studies designed to clarify their taxonomic relationships. It is evident from these results that the Streptomyces cyaneus species-group encompasses misclassified strains and members of several distinct species. However, some of the red-spored strains (e.g. Streptomyces janthinus ISP 5206^T, Streptomyces roseoviolaceus ISP 5277^T and Streptomyces violatus ISP 5209^T) formed a distinct clade. In contrast, most of the blue and gray-spored strains showed much less sequence homology between and with one another and were scattered throughout the 16S rRNA Streptomyces tree. The improved classification of this group of streptomycetes provides an essential basis for establishing their industrial and economical significance.

Keywords: 16S rDNA sequencing; Streptomycetes; Streptomyces cyaneus.

INTRODUCTION

Traditional streptomycete taxonomy was dependent on morphological characteristics such as aerial spore mass

Correspondence to: Ehsan Rashidian, Ph.D Telefax: +98 661 4203470 E-mail: er117kh@yahoo.co.uk colouration, diffusible pigments and spore chain morphology. The subjective nature of such tests, the high natural morphological diversity and intense economic interest in these organisms led to a massive overspeciation of the genus (Trejo, 1970). The second largest phenon (cluster 18) delineated in the numerical phenetic survey of the genus Streptomyces carried out by Williams et al. (1983) and encompassed the type strains of 31 validly described species. This taxon was designated the Streptomyces cyaneus species-group as the earliest validly described species it contained was Streptomyces cyaneus (Krassilnikov, 1941) Waksman 1953. A taxon which encompasses strains known to produce antitumour antibiotics, notably anthracyclines (Brockmann and Bauer, 1950; Oki, 1977; Omura et al., 1978; Yoshimoto et al., 1980; Oki et al., 1981; Kawai et al., 1987; Fedorova et al., 1996). Strains assigned to this species group still showed heterogeneity in respect to the coloures the aerial spore mass produced by them. The taxonomic integrity of cluster 18 was further brought into doubt by the results of fatty acid analysis (Saddler et al., 1987), total protein pattern (Manchester et al., 1990), DNA: DNA relatedness (Labeda and Lyons, 1991), numerical phenetic survey (Goodfellow et al., 1992) and the LFRFA (Beyazova and Lechevalier, 1993) studies. It is evident from the results of the studies outlined above that the Streptomyces cyaneus species-group recognized by Williams et al. (1983) encompasses a taxonomically diverse group of strains. The DNA: DNA relatedness and associated studies show that some of these strains can be considered as synonyms of Streptomyces coeruleorubidus and Streptomyces purpurascens (Labeda and Lyons, 1991). However, it is difficult in

light of the inconsistences in some of the other datasets to know which of the remaining strains might be sufficiently closely related to be included in any additional DNA: DNA pairing studies. It is evident that the current classification of streptomycetes can be critical in establishing taxonomic relationships between strains which produce commercially significant bioactive compounds, notably antibiotics. This aspect is exemplified in the present study which was designed to establish taxonomic relationships between streptomycetes belonging to the *Streptomyces cyaneus* species-group (Williams *et al.*, 1983) based on 16S rDNA sequencing data.

MATERIALS AND METHODS

Organisms and growth conditions: Thirty seven strains of Streptomysetes were obtained from different sources (Table 1). Working stock cultures were maintained on yeast extract-malt extract agar slants at 4°C. Biomass used for the extraction of DNA was grown on non-sporulating agar plates for 3-5 days at 28°C. Approximately 50 mg (one loopful or rise grain size of biomass) was needed for DNA extraction.

Colour grouping: The strains were examined for aerial spore mass colour, substrate mycelium colour and soluble pigment production following incubation on oatmeal agar at 28°C for up to 21 days. Peptone-yeast extract- iron agar plates were used to detect the formation of melanin pigments following incubation at 28°C for up to 4 days.

Morphology: The Spore chain morphology was observed by light and in some cases by scanning electron microscopy (SEM) of 21-day-old cultures grown at 28°C on oatmeal agar plates.

Isolation of genomic DNA: Chromosomal DNA was extracted and purified using Genomic G2 Kits (Helena BioScience, Sunderland, UK) with some minor modifications in the recommended procedure; like lyses by lysozyme.

PCR amplification of 16S rDNA: The 16S rRNA genes of the test strains were amplified using conserved primers (Lane, 1991). PCR was performed for 30 cycles at 95°C for 45 seconds, 45°C for 45 seconds, and 72°C for 1.5 minutes.

16S rRNA sequencing: Approximately 10 μ l of each of the purified PCR product alongwith 10 μ l of each of the oligonucleotide primers specifically designed for hybridising to conserved sites in the 16S rRNA molecule (Lane, 1991) were sequenced using the dideoxy method. Sequencing reactions were run on an ABI Prism 377 automated sequencer.

Data analysis: The 16S rRNA sequences were aligned automatically or manually by using the CLUSTAL X (Thompson *et al.*, 1997) and PHYDIT (Chun, 1995) programs against those of reference streptomycete nucleotide sequences retrieved from the RDP (Maidak *et al.*, 2001) and EMBL-GenBank (Benson *et al.*, 2000, Stoesser *et al.*, 2001) databases. Phylogenetic trees were inferred by using the neighbour-joining (Saitou and Nei, 1987), maximum-parsimony, least-squares algorithms and generated by either the PHYLIP software package (Felsenstein, 1993) or the TREECON program (Van de Peer and De Wachter, 1994).

RESULTS

The tested strains were divided into four groups on the basis of the production of aerial spore mass pigments on oatmeal agar (Table 2). Nearly all of the organisms assigned to these groups can be distinguished from one another on the basis of diffusible pigment and substrate mycelial colour and by their ability to produce melanin pigments on peptone yeast iron agar. The tested strains showed a range of spore chain morphologies and spore surface ornamentations when grown on oatmeal agar plates at 28°C for 21 days. Many of the strains formed chains of spiral, spiny spores (Fig. 1).

Almost complete 16S rDNA sequences (>1,400 nucleotides) were obtained for all of the strains. The data was compared with 27 corresponding and almost complete nucleotide sequences of representatives of the genus *Streptomyces* retrieved from the RDP database. The accession numbers of all of the strains are given in figure 2. The strains can be assigned to four loose multimembered aggregate groups, clades A to D, based on the results of the neighbour-joining analysis. Generally the 16S rDNA sequencing data support the grouping of the red-spored strains viz, *S. violatus, S. roseoviolaceus, S. janthinus, S. violarus, S. fumanus* and *S. pallidus* in one group. On the other hand, the blue spored strains had much less 16S sequences homology with each other and are widely dispersed on

ISP or code	Name	Source and strain history
5228 ^T	Streptomyces afghaniensis	DSM 40212< ISP 5228< M. Shimo, 772; soil
5293^{T}	Streptomyces arenae	DSM 40293< ISP 5293 <t. 269-m2;="" abbott="" j.="" laboratories.,="" na="" oliver,="" soil<="" td=""></t.>
5106^{T}	Streptomyces azureus	DSM 40106
5185^{T}	Streptomyces bellus	S.T. Williams, A106
5084^{T}	Streptomyces caelestis	DSM 40084< ISP 5084< NRRL 2418< A. Dietz, Upjohn Co. UC 2011; soil
5085^{T}	Streptomyces chartreusis	H. J. Kutzner, DSM 40085
5467^{T}	Streptomyces cimabarinus	DSM 40467< ISP 5467 <g. 1242;="" f.="" gauze,="" ina="" soil<="" td=""></g.>
5144^{T}	Streptomyces coeruleofuscus	DSM 40144< ISP 5144 <t. 2922="" 57;="" ina="" p.="" preobrazhenskaya,="" soil<="" td=""></t.>
5145^{T}	Streptomyces coeruleorubidus	DSM 40145< ISP 5145 <t.p. 12531="" 54;="" ina="" preobrazhenskaya,="" soil<="" td=""></t.p.>
5146^{T}	Streptomyces coerulescens	NCIMB 9616
5129^{T}	Streptomyces collinus	DSM 40129< ATCC 19743 < ISP 5129< P. Wilde, 1st 301; soil
5107^{T}	Streptomyces curacoi	DSM 40107
5108^{T}	Streptomyces cyaneus	S. T. Williams, A13
5013^{T}	Streptomyces echinatus	S. T. Williams, A77
5154^{T}	Streptomyces fumamus	A. Seino, KCC S-0497
5499^{T}	Streptomyces griseochromogenes	DSM 40499< ISP 5499< ATCC 14511< Fukunaga 2A-327; soil
5469^{T}	Streptomyces griseorubiginosus	DSM 40469< ISP 5469< G. F. Gauze, INA 7712; soil
5042^{T}	Streptomyces hawaiiensis	DSM 40042< ISP 5042< K. E. Crook, Bristol Laboratories, 678 506; soil
5206^{T}	Streptomyces janthirus	A. Seino, KCC S-0387
5090^{T}	Streptomyces lanatus	DSM 40090< ISP 5090< NRRL B-2291< W. Frommer, SV 1944; soil
5166^{T}	Streptomyces longisporus	DSM 40166< ISP 5166< T. P. Preobrazhenskaya, INA 4417/56; soil

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ISP or code number	Name	Source and strain history
5482 ^T	Streptomyces iakyrus	A. Seino, KCC S-0773
5483^{T}	Streptomyces luteogriseus	A. Seino, KCC S-0796
5588^{T}	Streptomyces neyagawaensis	DSM 40588< ISP 5588< IFO 13477, IFO 3784; soil
MAV^{T}	Streptomyces paradoxus	DSM 43350< S. T. Williams< KCC A-0052< V. D. Kutznetsov, RIA 655< INMI 3180; soil
5212^{T}	Streptomyces pseudovenezuelae	DSM 40212 <isp 1158,="" 5212<="" 742<="" a.="" krassilnikov,="" n.="" ria="" td=""></isp>
5310^{T}	Streptomyces purpurascens	DSM 40310 <isp 515;="" 5310<="" maria="" p.="" soil<="" td="" wilde,=""></isp>
5133	Streptomyces resistomycificus	DSM 40133 <isp 23893;="" 262.="" 32680];="" 5133<="" [eth="" eth="" p.="" pürk="" soil<="" td="" wilde,=""></isp>
5277^{T}	Streptomyces roseoviolaceus	A. Seino, KCC S-0513
5205^{T}	Streptomyces violarus	DSM 40205 <isp 1221.="" 493];="" 5205<="" [nihj="" inmi="" krassilnikov,="" n.a.="" soil<="" td=""></isp>
5209^{T}	Streptomyces violatus	A. Seino, KCC S-0851
5140	Streptomyces sp.	DSM 40140< ISP 5140 ('Streptomyces bicolor'') <t.p. 5140;="" ina="" preobrazhenskaya,="" soil<="" td=""></t.p.>
5207	Streptomyces sp.	S. Kurylowicz, ATCC 15893
5227	Streptomyces sp.	A. Seino; KCC S-0519
5432	Streptomyces sp.	DSM 40432< ISP 5432 (' <i>Streptomyces indigocolor</i> ')< N.A. Krassilnikov, INMI 206
5531	Streptomyces sp.	A. Seino, KCC S-0810 ('Streptomyces patlidus')
5592	Streptomyces sp.	DSM 40592< ISP 5592 ('Streptomyces peruviensis')< NRRL 2775< Rhone-Poulenc, 6227

Mascheroder Weg 1b, D-38124, Braunschweig, Germany, ETH, Eidgenössische Technische Hochschule, Institut für spezuekke Botanik, Zurich, Switzerland, IFO, Institute för Fermentation, 17-85 Juso-Honmachi, 2-chome, Yodogawa- ku, Osaka, Japan; INA, Culture Collection of the Institute of New Antibiotics, Academy of Medical Sciences; Moscow, Russia; ISP, International *Streptomyces* Project, Ohio Wesleyan University, Delaware, Ohio, 43015, U.S.A.; KCC, Culture Collection of Actinomycetes, Kaken Pharmaceutical Co. Ltd., Tokyo, Japan; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland, U.K.; NRRL, Culture Collection of the Northern Research Laboratories, US. Department of Agriculture, Peoria, Illinois, U.S.A. Abbreviations: ATCC, American Type Culture Collection, 1081 University Boulevard, Manassas, U.S.A.; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen,

ISP or lebonatomy	Name	Oatn	ieal agar	Peptone yeast	Oatm	eal agar
number		Substrate mycelium colour	Diffusible pigment		Spore chain morphology	Spore ornamentation ^a
A. Aerial spore	e mass colour-Blue					
5228^{T}	S. afghaniens is	Red	Red	+	Spiral ^a	${\rm Spiny}^{a}$
5106^{T}	S. azureus	No distinctive	None	+	Spiral	Smooth ^a
5185^{T}	S. bellus	colour Yellowish white	None	+	Open loops	${ m Spiny}^{a}$
5140	'S. bicolor'	No distinctive	Yellow	+	$Spiral^{a}$	${ m Spiny}^{a}$
5084^{T}	S. caelestis	No distinctive	None	+	Open loops	Smooth
5085^{T}	S. chartreusis	colour No distinctive	None	+	Spiral ^a	${ m Spiny}^{a}$
5144^{T}	S. coeruleofuscus	colour Light grayish brown	None	+	Spiral ^a	${ m Spiny}^a$
5145 ^T	S. coeruleorubidus	Cream/dark grayish	None	+	Spiral	${ m Spiny}^{ m a}$
5146^{T}	S. coerulescens	No distinctive	None	+	Spiral	${ m Spiny}^{a}$
5107^{T}	S. curacoi	No distinctive	None	+	Spiral	${ m Spiny}^{a}$
5090^{T}	S. lanatus	Corour Orange yellow	Yellow	+	Spiral ^a	Spiny^a
B. Aerial spore) mass colour-Gray					
5293^{T}	S. arenae	No distinctive	None	+	Spiral ^a	${ m Spiny}^{ m a}$
5129	S. collinus	colour No distinctive	None	+	Spiral	Smooth ^a
5108^{T}	S. cyaneus	Corour Dark grayish blue	Pale violet	+	Spiral/open loops	${ m Spiny}^{a}$
5013^{T}	S. echinatus	Brown	Yellow brown	+	Spiral	${ m Spiny}^a$
5499^{T}	S. griseochromogenes	Grayish greenish	None	+	Spiral/ open loops	${ m Spiny}^a$
5469^{T}	S. griseorubiginosus	yenow Light olive brown	Light grayish yellow hrown	+	Straight	Smooth ^a
5482^{T}	S. iakyrus	Olive grey	Yellow	+	Spiral	${ m Spiny}^a$

Table 2. Colour grouping, spore chain morphology and spore ornamentation of strains assigned to the *Streptomyces cyaneus* species-group *sensu* Williams *et al.* (1983)

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ISP or	Name	Oatm	eal agar	Peptone yeast	Oatm	eal agar
laboratorynu mber		Substrate	Diffusible pigment	iron agar Melanin	Spore chain	Spore
5432	'S. indigocolor'	Grayish red	Pale violet	+	Spiral ^a	Spiny ^a
5483^{T}	S. luteogriseus	Olive brown	Light brown	+	Spiral	Smooth ^a
5588^{T}	S. neyagawaensis	Dark olive brown	Light gray	+	Spiral ^a	$Smooth^a$
$\mathbf{MAV}^{\mathrm{T}}$	S. paradoxus	Light brown	None	•	Spiral ^a	S piny ^a
5592	'S. peruviensis'	No distinctive	None	+	Spiral	Spiny or Smooth ^a
5212^{T}	S. pseudovenezuelae	colour Light brown	None	+	Straight ^a	Smooth ^a
5133^{T}	S. resistomycificus	Dark reddish brown	None	+	Spiral/ open loops	Smooth ^a
5227	'S. thermotolerans'	Brown	Trace of yellow	+	Spiral	Spiny
C. Aerial spore n	nass colour-Red					
5467^{T}	S. cimabarinus	Pink	Pink	+	Straight	Smooth
5154^{T}	S. fumaraus	Grayish yellow/mild	None		Spiral	Smooth ^a
5206^{T}	S. janthirus	yellow Cream	None	+	Spiral	S piny ^a
5531	'S. pallidus'	Light yellow	Trace of yellow	+	Open loops	Smooth ^a
5310^{T}	S. purpurascens	Dark yellowish pink	None	+	Spiral	Spiny^a
5277^{T}	S. roseviolaceus	Mild red	Light pink	+	Spiral	Spiny
5205^{T}	S. violarus	Mild red	Light pink	+	Spiral	Spiny
5209^{T}	S. violatus	Mild red	Light pink	+	Spiral	${ m Spiny}^{a}$
5207	'S. violochromogenes'	Deep red	Grayish pink	+	Spiral	Smooth
D. Aerial spore 1	nass colour -Yellow C. homoitoneic	Doddich humm	I inth monocontinue to the I	4	Onon Loon	C
7400	o. Hawaiterbio	TI MOTO TISTIMAN	тыдли оталуе уелом	F	sdoor IIado	funde
5166	S. longisporus	Yellow	None	+	Spiral ^a	Spiny^{a}
+, positive; -, neg ^a Spore chain mor	ative. phology and spore ornamentatic	on data taken from the ISP o	descriptions (Shirling & Gc	utlieb, 1968a, 1968b,	1969, 1972).	

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Table 2. (continued)

the tree. It is evident that *S. cyaneus*, the type strain of the cluster 18, had little 16S sequence homology in common with the other strains and was separated from the remaining strains in the cluster. Our study supports the suggestion that members of the species-group demonstrated a high degree of heterogeneity.

DISCUSSION

The 16S rDNA sequence data generated in the present investigation indicate that the Streptomyces cyaneus species-group sensu Williams et al. (1983) is markedly heterogeneous, a finding which underpins the results of earlier DNA: DNA pairing (Labeda and Lyons, 1991), fatty acid (Saddler et al., 1987) and total protein pattern (Manchester et al., 1990) studies. However, an important advantage of the 16S rDNA sequence data over the results of these earlier studies is that they provide a measure of the taxonomic diversity encompassed within the Streptomyces cyaneus species-group. In the present study, it was particularly interesting that the type strains of validly described species assigned to the taxon by Williams et al. (1983) were recovered in two out of the four 16S rDNA clades recognized in the streptomycete tree. It was also interesting that 19 out of the 31 type strains were assigned to 16S rDNA clade A together with 14 representatives of validly described Streptomyces species. It is evident from the 16S rDNA sequence data that streptomycetes which produce a blue aerial spore mass on oatmeal agar form a markedly heterogeneous group. These results although interesting are not surprising as it is now realised that streptomycete species need to be delineated not on the basis of a few subjectively chosen morphological and pigmentation properties but by using a combination of reliable genotypic and phenotypic data (Manfio et al., 1995; Atalan et al., 2000; Sembiring et al., 2000). The results of the 16S rDNA sequencing studies carried out in the present investigation have gone someway towards clarifying relationships between members of the Streptomyces cyaneus species-group and between them and representatives of validly described Streptomyces species included in the RDP database. The next step is to determine the taxonomic status of the 16S rDNA subclades by evaluating their composition in light of available genotypic and phenotypic data.

There is some evidence that only streptomycetes from certain phyletic lines can produce specific metabolites, as exemplified by the production of clavulanic acid from members of the *Streptomyces clavuligerus* subclade (Payne *et al.*, 2001). In the present study, anthracycline-like antibiotics were detected in eleven out of the thirty-seven members of the *Streptomyces cyaneus* species-group (Dr. E. Lacey,



Figure 1. Scanning electron micrographs of "*Streptomyces thermotolerans*" ISP 5227 grown on oatmeal agar plates for 21 days at 28°C: (a) Spiral spore chains; (b) Spiny spores.



Figure 2. Neighbour-joining tree based on almost complete 16S rDNA sequences showing relationships between members of the *Streptomyces cyaneus* species-group and some representative strains of the genus *Streptomyces*. The numbers at the nodes indicate the level of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets. The scale bar indicates 0.02 substitutions per nucleotide position.

Abbreviation: Letter **A** corresponds to the strains that produce anthracycline compounds (unpublished data provided by Dr. E. Lacey); B=blue, G= gray, R=red and Y=yellow refer to aerial spore mass colours.

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unpublished data). However, seven out of the eleven strains were assigned to the *Streptomyces cyaneus* and *Streptomyces purpurascens* subclades, a result which suggest that it may be possible to predict streptomycete metabolite potential from good quality taxonomic data. Additional comparative studies are needed to test this hypothesis.

In general, the results of the present study are very encouraging, especially when taken together with earlier findings, as they indicate that relationships between taxonomically complex groups, such as the *Streptomyces cyaneus* species-group *sensu* (Williams *et al.*, 1983) can be resolved using appropriate combinations of genotypic and phenotypic data.

References

- Atalan E, Manfio GP, Ward AC, Kroppenstedt RM, Goodfellow M (2000). Biosystematic studies on novel streptomycetes from soil. *Anton Leeuwenhoek* 77: 337-353.
- Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Rapp BA, Wheeler DL (2000). GenBank. *Nucleic Acid Res* 28: 15-18.
- Beyazova M, Lechevalier MP (1993). Taxonomic utility of restriction endonuclease fingerprinting of large DNA fragments from *Streptomyces* strains. *Int J Syst Bacteriol* 43: 674 682.
- Brockmann H, Bauer K (1950). Rhodomycin, a red antibiotic from actinomycetes (in German). *Naturwissenschaften* 37: 492-493.
- Chun J (1995). *Computer-assisted classification and Actinomycetes*, PhD thesis, University of Newcastle, Newcastle upon, Tync, UK.
- Fedorova GB, Golova TP, Arkhangel'skaia NM, Lazhko EI, Abramova EA, Bychkova OP, Polunin A, Katrukha G. S (1996). Rubomycin Q1-an anthracycline metabolite from *Streptomyces coeruleorubidus* 2679, a strain producing rubomycin C. *Antibiotiki i Khimioterapiia* 41: 3-8.
- Felsenstein J (1993). *PHYLIP (Phylogeny Inference Package)* version 3.5c. Department of Genetics, University of Washington, Seattle.
- Goodfellow M, Ferguson EV, Sanglier JJ (1992). Numerical classification and identification of *Streptomyces* species-a review. *Gene*, 115:225-233.
- Kawai H, Hayakawa Y, Nakagawa M, Furihata K, Shimazu A, Seto H, Otake N (1987). Arugomycin, a new anthracycline antibiotic. I. Taxonomy, fermentation, isolation and physico-chemical properties. *J Antibiot* 40:1266-1272.
- Krassilnikov NA (1941). Keys to Actinomycetales. Translated from Russian 1966 ed. Academic of

Sciences of the USSR, Institute of Microbiology, Moscow.

- Labeda DP, Lyons AJ (1991). Deoxyribonucleic acid relatedness among species of the *Streptomyces cyaneus* cluster. *Syst Appl Microbiol* 14: 158-164.
- Lane DJ (1991). 16S/23S rRNA sequencing. In Nucleic Acid Techniques in Bacterial Systematics, pp: 115-175, Edited by E. Stackebrandt & M. Goodfellow. John Wiley & Sons, New York.
- Manchester L, Pot B, Kersters K, Goodfellow M (1990). Classification of *Streptomyces* and *Streptoverticillium* species by numerical analysis of electrophoretic protein patterns. *Syst Appl Microbiol* 13: 333 - 337.
- Manfio GP, Zakreezewska-Czerwinska J, Atalan E, Goodfellow M (1995). Towards minimal standards for the description of *Streptomyces* species. *Biotechnologia* 7-8: 242-253.
- Maidak BL, Cole JR, Lilburn TG, Parker CT, Saxman PR, Farris RJ, Garrity GM, Olsen GJ, Schmidt TM, Tiedje J. M (2001). The RDP-II (Ribosomal Database Project). *Nucleic Acid Res* 29: 173-174.
- Oki T (1977). New anthracycline antibiotics. *Japanese J Antibiot* 30: 70-84.
- Oki T, Matsuzawa Y, Kiyoshima K, Yoshimoto A, Naganawa H, Takeuchi T, Umezawa H (1981). New anthracyclines, feudomycins, produced by the mutant from *Streptomyces coeruleorubidus* ME130-A4. *J Antibiot* 34: 783-90.
- Omura S, Tanaka H, Iwai Y, Nishigaki K, Awaya J, Takahashi Y, Masuma R (1978). A new antibiotic, setomimycin produced by a strain of *Streptomyces*. J Antibiot 31:1091-1098.
- Payne G, Ward, AC, Goodfellow M (2001). The Streptomyces clavuligerus clade: a home for clavulanic acid producing streptomycetes. The 12th International Symposium on the Biology of Actinomycetes (ISBA), 5-9 August 2001, Vancouver, British Columbia, Canada.
- Saddler GS, O'Donnell AG, Goodfellow M, Minnikin DE (1987). SIMCA pattern recognition in the analysis of streptomycete fatty acids. *J Gener Microbiol* 133: 1137-1147.
- Saitou N, Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecul Biol Evolu* 4: 406-425.
- Sembiring L, Ward AC, Goodfellow M (2000). Selective isolation and characterisation of members of the *Streptomyces violaceusniger* clade associated with the roots of *Paraserianthes falcataria*. *Antonie van Leeuwenhoek* 78: 353-366.
- Shirling EB, Gottlieb D.(1968a). Cooperative description of type cultures of *Streptomyces*. II. Species description from first study. *Int J Syst Bacteriol* 18: 69-189.
- Shirling EB, Gottlieb D (1968b). Cooperative description of type cultures of *Streptomyces*. III. Additional species descriptions from first and second studies. *Int J Syst*

Bacteriol 18: 279-392.

- Shirling EB, Gottlieb D (1969). Cooperative description of type cultures of *Streptomyces* IV. Species descriptions from the second, the third, and the fourth studies. *Int J Syst Bacteriol* 19: 391-512.
- Shirling EB, Gottlieb D (1972). Cooperative description of type cultures of *Streptomyces*. V. Additional descriptions. *Int J Syst Bacteriol* 22: 265-394.
- Stoesser G, Baker W, Broek AVD, Camon E, Garcia-Pastore M, Kanz C, Kulikova T, Lombard V, Lopez R, Parkinson H, Redaschi N, Sterk P, Stoehr P, Tuli MA (2001). The EMBL nucleotide sequence database. *Nucleic Acid Res* 29: 17-21.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The CLUSTAL X Windows Interface: flexible strategies for multiple sequence alignment aided by quality analysis tool. *Nucleic Acid Res* 24: 4876-4882.

- Trejo WH (1970). An evaluation of some concepts and criteria used in the speciation of streptomycetes. *Transactions of the New York Academy of Science*, Series 2, 32: 989-997.
- Van de Peer Y, De Wachter R (1994). TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Compu Applica for Biologic Sci* 10: 569-570.
- Williams ST, Goodfellow M, Alderson G, Wellington EMH, Sneath PHA, Sackin MJ (1983). Numerical classification of *Streptomyces* and related genera. *J Gener Microbiol* 129: 1743-1813.
- Yoshimoto A, Oki T, Takeuchi T, Umezawa H (1980) .Microbial conversion of anthracyclinones to daunomycin by blocked mutants of *Streptomyces coeruleorubidus*. J Antibiot 33:1158-66.