Isolation, Sequencing and Annotation of three genes putatively involved in capreomycidine biosynthesis in *Streptomyces ribosidificus* NRRL B-11466

Khaled Mohamed Anwar Aboshanab *
Microbiology and Immunology Department, Faculty of Pharmacy, Ain Shams University, Organization of African Unity St., POB: 11566, Abbassia, Cairo, Egypt.

ABSTRACT

Capreomycidine is a nonproteinogenic amino acid that is biosynthesized and condensed with other amino acids via a nonribosomal peptide synthase mechanism to form various antibiotics of tuberactinomycin family. This family of peptide antibiotics is of enormous activity against Mycobacterium tuberculosis infections and is particularly used for the treatment of multidrug-resistant tuberculosis, methicillin-resistant Staphylococcus aureus strains and vancomycin-resistant enterococci. In this research, isolation, sequencing and annotation of three genes putatively involved in capreomycidine biosynthesis from Streptomyces ribosidificus NRRL B-11466 were carried out. Various heterologous and homologous primers were designed and used in PCR to amplify various homologous DNA fragments. The PCR products were sequenced and the obtained DNA sequences were assembled using the Staden-Package programme into a continuous DNA segment of 3484 bp. The obtained DNA segment was analysed and submitted to the EMBL database under the accession code HQ327309. Analysis of the respective DNA segment revealed three completed open reading frames where the respective gene products showed good amino acid identities to homologous gene products of viomycin-biosynthetic gene cluster. These gene products were putative L-arginine hydroxylase (86% identity), putative capreomycidine synthase (82% identity), and putative permease (85% identity). This is the first report about gene products putatively involved in capreomycidine biosynthesis in Streptomyces ribosidificus. Results obtained from this study will contribute to explore the production of a new peptide antibiotic by Streptomyces ribosidificus, the producer of the aminoglycoside antibiotic ribostamycin.

***Key words**: tuberactinomycins, capreomycidine biosynthesis, viomycin, Streptomyces ribosidificus NRRL B-11466.*

INTRODUCTION

Tuberactinomycins (TUBs) are peptide antibiotics characterized by the presence of an amino acid with a 6-membered cyclic guanidine side chain (capreomycidine) and two or more 2,3-diaminopropionate residues[(1)]. TUBs include various antibiotics such as viomycin, tuberctinomycins, streptothricin and capreomycins produced by different *Streptomyces* strains[(2-6)]. The antibiotic viomycin (tuberactinomycin B), the well-studied antibiotic contain nonproteinogenic amino acids such as L-capreomycidine, 2,3-diaminopropionate, β-ureidodehydroalanine, and β-lysine (Fig. 1)[(1,6)]. The full biosynthetic pathway of these antibiotics still not completely identified however it was anticipated that they are synthesized via a nonribosomal peptide synthase (NRPS) mechanism[(6,7)]. The full biosynthetic gene cluster of viomycin antibiotic from *Streptomyces* strain ATCC 11861 was completely isolated and analyzed[(1,6)]. The unusual nonproteinogenic amino acids were anticipated to be synthesized from normal amino acids in cell such as 2,3-diaminopropionate from L-serine and L-ornithine, 2,3-diaminopropionate would be further modified to form, β-ureidodehydroalanine, L-capreomycidine from L-arginine, and β-lysine from L-lysine [(6)]. These amino acids would be condensed to produce these antibiotics via nonribosomal peptide synthases (NRPSs) whose respective genes were also located with the identified biosynthetic gene clusters.

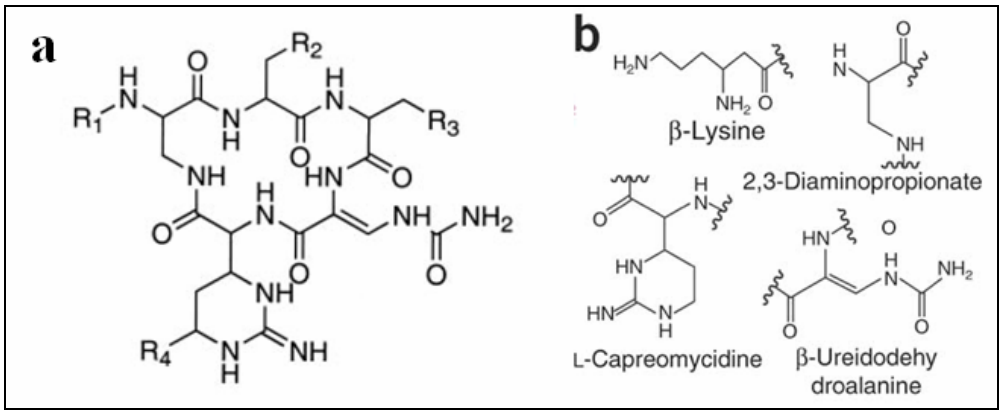

Fig. 1 Basic chemical structures of: a) TUB family of antibiotics, b) nonproteinogenic amino acids from which TUB antibiotics are built[6,13].

Moreover, conversion of (2S)-arginine to (2S,3R)-capreomycidine by VioC and VioD from the viomycin biosynthetic pathway of *Streptomyces* sp. strain ATCC11861 was determined[22]. TUBs are of great importance in treatment of various sever bacterial infections particularly the multidrug-resistance tubercle bacilli (MDR-TB) infections, methicillin-resistant *Staphylococcus aureus* strains (MRSA) and vancomycin-resistant enterococci (VRE) [6,8,9,10]. TUBs also have antiviral activities since they target the catalytic RNAs involved in viral replication[11,12]. Interestingly, some members of TUBs family are listed in the Wold Health Organization's model drug list 2002. Recently, it was investigated that tuberactinomycins inhibit translocation on 70S ribosome by stabilizing the tRNA in the A site in the pretranslocation state[13]. It was also mentioned that these agents bind adjacent to the binding sites for the some 2-deoxystreptamine aminocyclitol aminoglycoside antibiotics (2DOS-ACAGA) such as paromomycin and hygromycin B[13].

Streptmyces ribosidificus NRRL B-11466 is a producer of ribostamycin, a 2DOS-ACAGA. The ribostamycin biosynthetic gene cluster was completely sequenced and analysed[14]. Analysis of the submitted DNA segment harbouring the ribostamycin biosynthetic gene cluster showed the presence of three ORFs with a very good amino acid identities (about 80%) to those located in the viomycin biosynthetic gene cluster of *S. vinaceus*. These three ORFs were putative type II thioestrase and two NRPSs however their exact biosythetic roles in *S. ribosidificus* were not yet known. Whether a full viomycin-related biosynthetic gene cluster is located in *S. ribosidificus* has to be explored. Therefore, in the present work, heterologous and homologous primers were designed and used in PCR to amplify and sequence gene(s) from *S. ribosidificus* homologous to those in the viomycin gene cluster. The primers were designed base on gene products putatively involved in the biosynthesis of capreomycidine, the main nonproteinogenic amino acids in TUBs antibiotics. The obtained DNA sequences were assembled using Staden-package programme followed by gene analysis and annotation.

MATERIAL & METHODS

Bacterial strains, culture media

Streptomyces (S.) ribosidificus NRRL B-11466 (ribostamycin producer) was cultured on tryptic soy broth (TSB)[15,16] or on M65 (DSMZ, Braunschweig, Germany) at 28°C.

Oligonucleotides used in this study

Table 1: Homologous primers

Primer designation	Target gene	Primer sequences [1]	Annealing temperature (T), Annealing time (t)
PSriC-F	*sriC*	5' AGCCGGTATGCGATGGGTG 3'	60°C, 1 min
PSriC-R	(0.9 kb)	5' TCACCGCTGCCCCAAGAC 3'	
PSriD-F	*sriD*	5' CAGCGGTGACCGGCCCGCTC 3'	65°C, 1 min
PSriD-R	(1.2 kb)	5' CGAGGTCATCGCGTCCCCCTGC 3'	
PSriE-F	*sriE*	5' ACGCGATGACCTCGCCC 3'	57°C, 1 min
PSriE-R	(1.4 kb)	5' GGTCGGGTCAGCGATTGG 3'	

Table 2: Heterologous primers

Target genes (examples)	Designation and sequence of the heterologous primer	Expected size of PCR product (kb)
Putative L-arginine hydroxylase	PHC- F: 5' CACCGGGSCGACTACGTAGGC 3' PHC- R: 5' CACCGCTGGCSCGTATCTC 3'	0.5
Putative L-capreomycidine synthase	PHD- F: 5' GTGCACAACTACACCKTTGGGGA 3' PHD- R: 5' CCGTATGMCTTGGACAGGGTG 3'	0.6
Putative permease	PHE- F: 5' GAAAGCSCGCGTCGCTGTGGC 3' PHE- R: 5' GMCGACCACGSCGGTCCGC 3'	0.86
L-arginine hydroxylase-permease conserved domaine	PHCE- F: 5' CATCACCSGGGATTTGCACCGGTCGC 3' PHCE- R: 5' CGGSCTCGAAGATCAGCACTGTGC 3'	2.5
L-capreomycidine synthase-permease conserved domaine	PHDE- F: 5' CTTCCAGGCGAAGASCGACATCAGCAGC 3' PHDE- R: 5' CGGSCTCGAAGATCAGCACTGTGC 3'	1.8

The following letters in the primer sequences indicate: S = (C or G); K = (T or G); M = (A or C)

Extraction of genomic DNA

Chromosomal DNA of *S. ribosidificus* was prepared according to the method of Pospiech and Neumann (1995) with the following modifications[17]. Strain inoculation was done in 25 ml TSB in 250 ml-volume flask and grown at 28°C on a shaker (180 rpm) for 72 hs. The mycelia was then harvested by centrifugation at 4,000 rpm for 7 min and washed twice with 10.3% sucrose, resuspended in 20 ml SET buffer with 1.5 mg/ml lysozyme and incubated for 1 hr at 37°C. About, 1/10 volume SDS 10% and proteinase K to the final concentration of 0.5 mg/ml were added and incubated at 55°C for 1 - 2 hrs with frequent gentle inversion. About 1/3 volume 5 M NaCl was added and an equal volume phenol/chloroform was added and incubated at room temperature for 20 min with gentle inversion. The mixture was then centrifuged at 4,000 rpm for 10 min and the aqueous phase was further extracted with an equal volume of chloroform/isoamyl alcohol (24:1), incubated at room temperature for 20 min with gentle inversion, centrifuged at 4,000 rpm for 10 min. The DNA was precipitated by the addition of an equal volume of isopropanol, centrifuged at 4,000 rpm for 5 min. DNA was then washed using 70% ice cold ethanol, dried and finally dissolved in 1000 µl TE buffer with RNase 100 µg/ml.

Agarose gel electrophoresis

Agarose gel electrophoresis was carried out essentially as described by Sambrook and Russell (2001) using 0.8% agarose gels containing 0.1 µg/ml ethidium bromide[18]. DNA fragment size was determined by

comparison to conventionally 1 Kb DNA ladder (Sigma-Aldrich co, Egypt).

Recovery of DNA fragments from agarose gels

The PCR products obtained on agarose gel were excised and subsequently purified using QIA quick Gel Extraction Kit (Qiagen, Hilden) according to the manufacture's specifications.

Polymerase chain reaction (PCR)

Amplification of different probes by PCR was performed using 200 - 400 ng of the genomic DNA as a template and the selected primers for each probe (Tabs. 1 & 2). PCR was performed in a Nyx-Technik Inc. Personal Cycler (ATC401, USA). Each assay (50 µl) consisted of 200 ng chromosomal DNA, 100 pmole of each appropriate primer, 0.2 mM dNTPs (Invitrogen, Karlsruhe, Germany), 3 mM $MgCl_2$, 10% DMSO to improve the denaturation of the template DNA and 2 U *Taq* DNA polymerase (Sigma, USA). PCR general conditions were: 98°C for 5 min; then 30 cycles [95°C for 1 min; annealing temperatures and time according to Tab. 1 & 2; 72°C for 1 min (normally 1 min for 1 kb)]; and 72°C for 5 min (ramping rate 1°C/sec).

DNA sequencing

DNA sequencing of the purified PCR products was basically done by AGOWA company (Berlin, Germany) and Promega company. (Lab Technology, Cairo, Egypt) according to the method described by Sanger et al. (1977)[19].

Nucleotide accession codes

The nucleotide sequence reported in this study in the EMBL/GenBank databases under the accession codes HQ327309.

Computer-assisted analysis of DNA sequences

The programs used for computer-assisted analysis of nucleotide and protein sequences were Staden package[20], FramePlot[21], Online analysis tools (http://molbiol-tools.ca/), ClustalW2[22]. Restriction analysis was carried out using: Restriction Enzyme Site Mapper version 3 (http://www.restrictionmapper.org/). PCR amplification temperatures were computed using pDRAW32 (http://www.acaclone.com).

RESULTS

As shown in Fig. 2, PCR products of 0.6, 0.5, 0.85, 1.8 and 2.5 kb were obtained corresponding to the expected size of PCR products using the following heterologous primer pairs, HD- F/PHD- R, PHC- F/ PHC- R, PHE- F/ PHE- R, PHDE- F/ PHDE- R, PHCE- F/PHCE- R, respectively. These PCR products were excised from agarose gel and subsequently purified and sent for DNA sequencing.

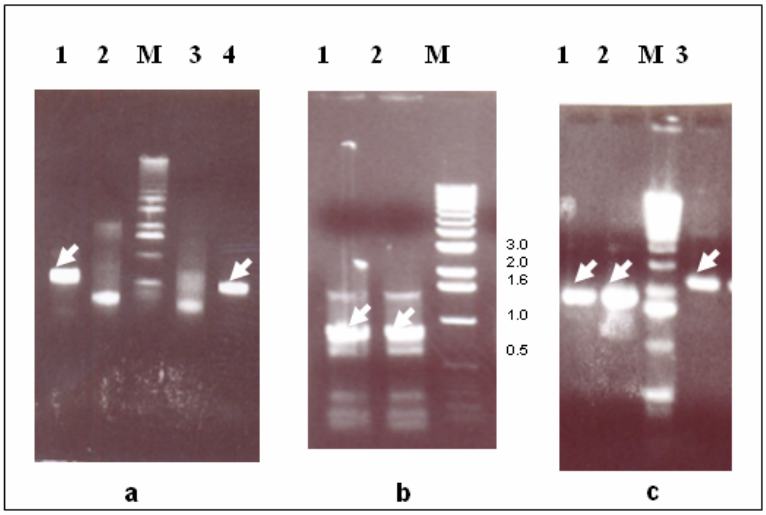

Fig. 2 Agarose gel electrophoresis (0.8%) showing different PCR products using the following heterologous primer(s): a: Lane 1: PHD- F/PHD- R; Lane 2: PHD- F; Lane 3: PHD- R; Lane 4: PHC- F/ PHC- R, **b:** Lanes 1 & 2: PHE- F/ PHE- R, **C**: Lanes 1 & 2: PHDE- F/ PHDE- R, Lane 3: PHCE- F/PHCE- R. Arrows indicate the expected PCR products. Lane "M" is 1.0 kb DNA ladder (Invitogen, Karlsruhe, Germany).

Moreover, homologous primers were designed and used for PCR. The obtained PCR products were also sequenced and used for further verification of the entire DNA sequences of the continuous DNA fragment (Fig. 3). The continuous DNA fragment (3484 bp) was analysed and submitted to the EMBL GenBank under the accession code HQ327309. Frame analysis of the respective DNA fragment revealed the presence of three genes namely *sriC*, *sriD*, and *sriE*, of 894, 1176, and 1386 bp, respectively. The ORFs of the three genes were found to be within the same direction (located on the parent DNA strand).

Blast protein analysis and amino acid sequences alignments using ClustalW2 programme of the respective ORFs revealed the presence of high percentage of amino acid identities (about 80%) to those present in the viomycin and capreomycin biosynthetic gene clusters. The three ORFs were putative L-arginine hydroxlase (SriC, 297 aa), putative L-capreomycidine synthase (SriD; 391 aa), and putative permease (SriE; 461 aa; Figs. 5, 6 & 7).

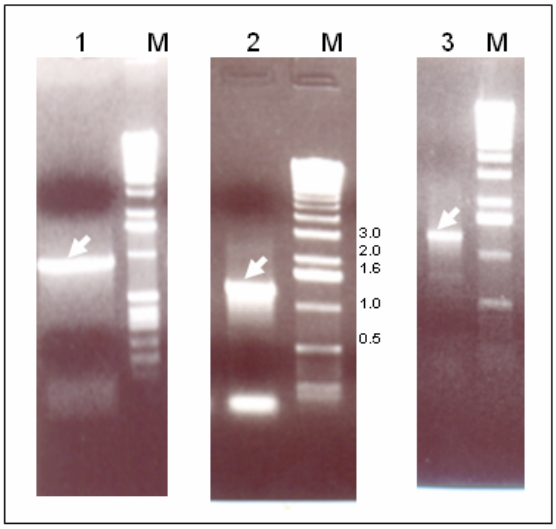

Fig. 3 Agarose gel electrophoresis (0.8%) showing different PCR products using the following homologous primers: Lane 1: PSriC-F/PSriC-R; Lane 2: PSriD-F/PSriD-R; Lane 3: PSriE-F/ PSriE-R. Arrows indicate the expected PCR products. Lane "M" is 1.0 kb DNA ladder (Invitogen, Karlsruhe, Germany).

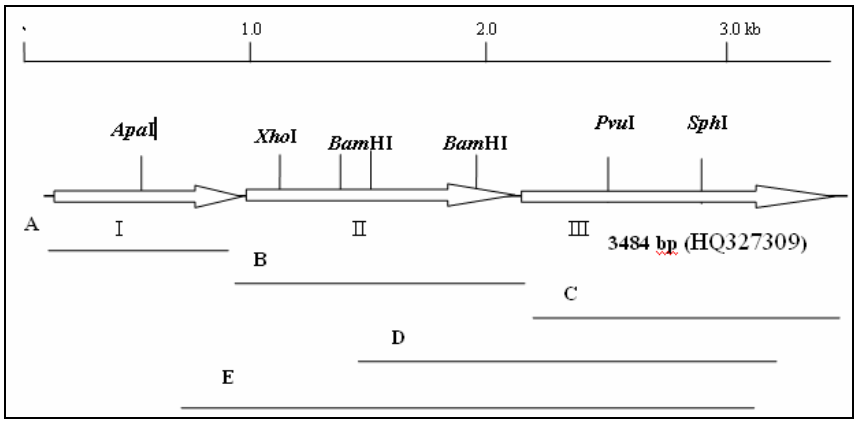

Fig. 4 Restriction analysis profile of the DNA segment submitted to EMBL database (accession code: HQ327309). ORF-I = putative L-arginine hydroxylase (SriC; 297 aa), ORF-II = putative L-capreomycidine synthase (SriD; 391 aa), ORF-III= putative permease (SriE; 461 aa). A, B, C, D, and E represent sizes of different PCR products obtained using the following primer pairs PSriC-F/PSriC-R; PSriD-F/PSriD-R; PSriE-F/PSriC-R; PHDE-F/PHDE-R; PHCE-F/PHCE-R, respectively. Arrows indicate direction of the ORFs. Abbreviations: ORF = Open Reading frame; aa = amino acid.

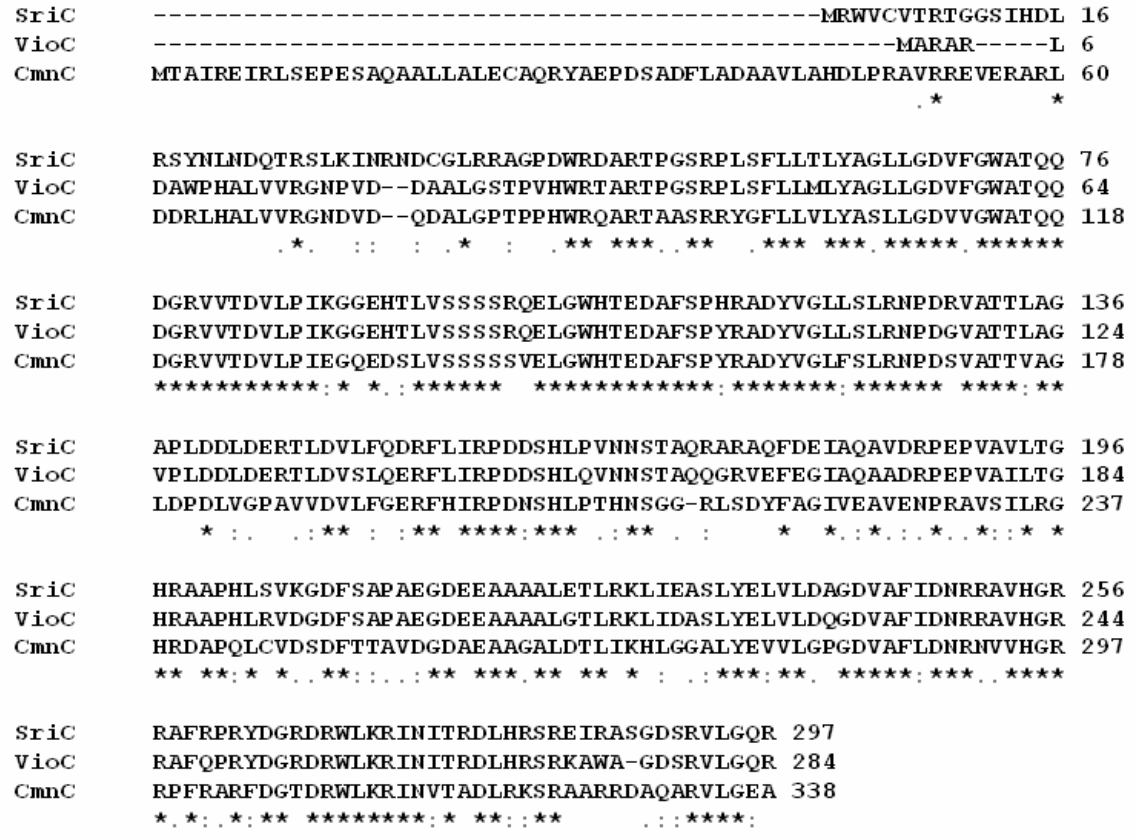

Fig. 5 Alignment of putative L-arginine hydroxylase homologous proteins using multiple amino acid sequence alignment (ClustalW2). The numbers indicate the position within the corresponding proteins. SriC = *S. ribosidificus*, VioC = *S. vinaceus* (viomycin producer; accession code, AAO66427), CmnC = *Saccharothrix mutabilis* subsp. *capreolus* (capreomycin producer; accession code, ABR67746).

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SriD        VTGPLGAGARKLPPAPLEDWLRERYFQAKTDISSSGVHNYTFGELRALDPALLGTEELDR 60
VioD        MTGPLGAGPQALPAAPLEDWLRERYFQAKTDISSSGVHNYTFGELRALDPALLGTRELDQ 60
CmnD        ------MMVRDLPAAALEDWLRERYFTARVDVSSSGVADHRLADLRRIG--GITVEELDA 52
                     : **.*.********** *:.*:***** :: :.:** *.   : ..***

SriD        LMFRDGPSLGDERLRAAVAVRVRPGPGHTVMTTHGSSEALFLAFTALVRPGDEVVVATPA 120
VioD        LMFRDGPSLGDERLRAAVAARVRPGPGHVVMTTHGSSEALYLAFAALVRPGDEVVVATPA 120
CmnD        VVFRDGPSLGAERLRAALADRLRPGPDHVVMTAHGSSEALFLAMTALVRPGDEVVVPDPA 112
            ::******** ******:* *:****.*.***:*******:**::***********. **

SriD        YHSLSALAVTAGAVLRPWPLRQENGFVPDLDDLRAVLTARTRLVVVNFPHNPSGACVDPR 180
VioD        YHSLSGLATAAGASLRPWPLRPENGFAPDLDDLRAVLSDRTRLVVVNFPHNPSGACVDPR 180
CmnD        YHSLSALARACGAVLRPWPVL---GAAPDPADLRALLTPRTRLVVVNFPHNPTGVTVDAA 169
            *****.** :.** *****:    * .**  ****:*: **************:*. **.

SriD        TRADLLDLVAGSGATLVWDGAFTDLTYEHPPLADPSQDLDRVLSFGTLSKAYGLPGLRVG 240
VioD        GRTELLDLVANSQAVLLWDGAFTDLVHDHPPLAEPSQDLDRVLSFGTLSKAYGLPGLRVG 240
CmnD        VQAELLDVVGRSGAYLLWDNAFRDLVYDAPPLPEPTALGGRVLSTGTLSKAHGLPGLRVG 229
             :::***:*. * * *:**.** **.:: ***.:*:   .**** ******:********

SriD        WCVVPRGLVPDLVRIRDYLTLTLSPLTERVAAVAVDHAHTDALIAPRLANARNNRERDAA 300
VioD        WCVVPQDLVSELVRIRDYLTLSLSPLVERVAAVAVEHA--DALITPRLTEARHNRRRVLE 298
CmnD        WCVLPADLAPELVRVRDYLTLSLSPLTELLAAVAVEHA--DELIAPRLAEATANRRRLLD 287
            ***:* .*..:***:******:****.* :*****:**  * **:***::*  **.*

SriD        VGSGNPGAVELPVPRGGVTAFPRFTGHADVTGPCERLLSEHGVLVVPGRVFGHADRIRIG 360
VioD        WAAASEGAIDCPVPRGGVTAFPRFTAHTDVTDLCERLLARHGVLVVPGRVFGQADRMRIG 358
CmnD        WAAAHG--VDCPAPGGGVTAFPRFPGVADVTPLCDRLMSEHGVLTVPGGCFGFPDRMRIG 345
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SriD        FSCPRAELERGLAALAEELGTPARGRRRGTR 391
VioD        FSCPRPELERGLAAISEELGTHARGRRRGTG 389
CmnD        FGCDPAVFAAGLTALGAVLAEKRLPAKL--- 373
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Fig. 6 Alignment of putative L-capreomycidine synthase homologous proteins using multiple amino acid sequence alignment (ClustalW2). The numbers indicate the position within the corresponding proteins. SriD = *S. ribosidificus*, VioD = *S. vinaceus* (viomycin producer; accession code, AAO66428), CmnD = *Saccharothrix mutabilis* subsp. *capreolus* (capreomycin producer, accession code, ABR67747).

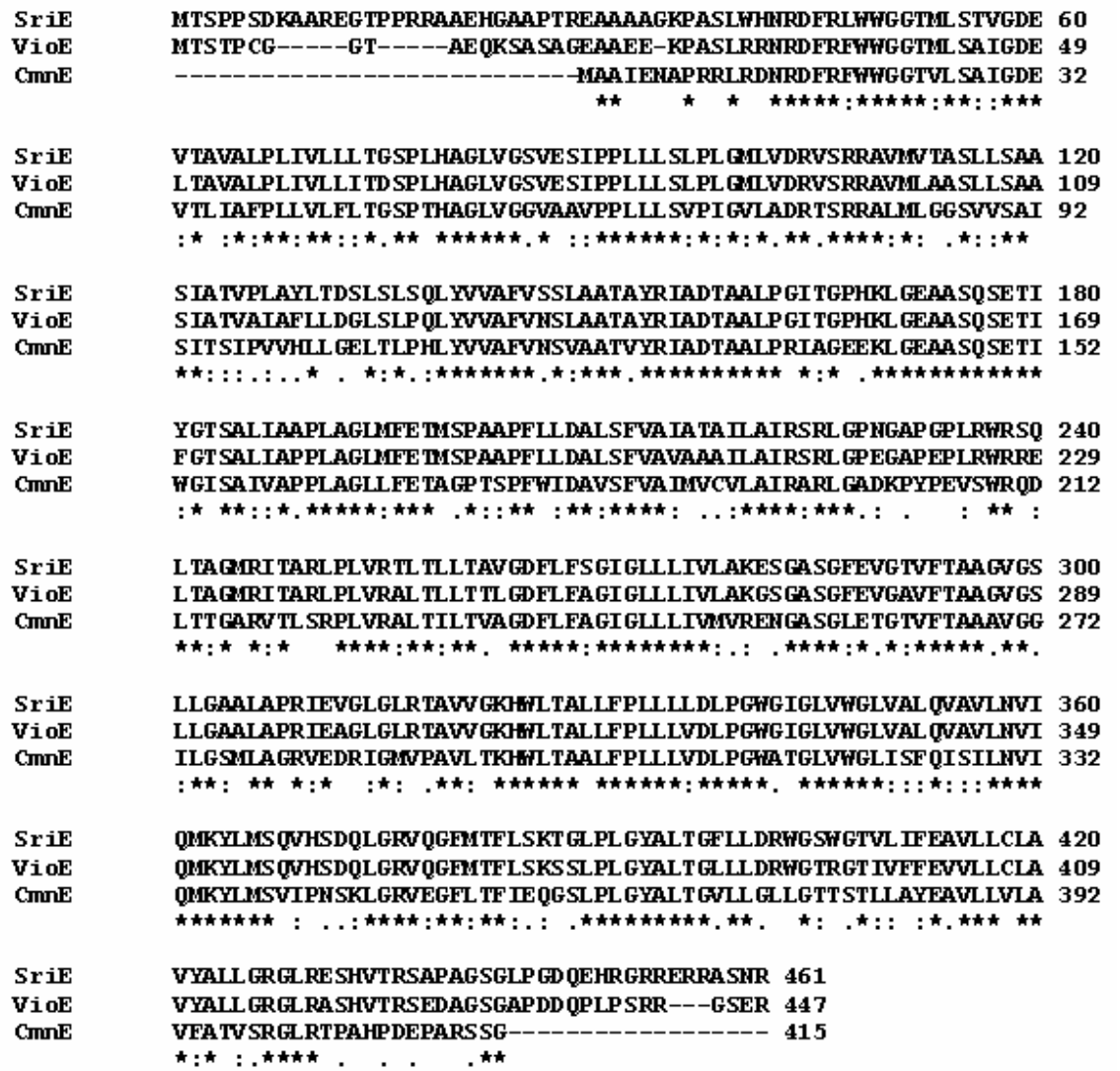

Fig. 7: Alignment of putative permease homologous proteins using multiple amino acid sequence alignment (ClustalW2). The numbers indicate the position within the corresponding proteins. SriE = *S. ribosidificus*, VioE = *S. vinaceus* (viomycin producer; accession code, AAP92495), CmnE = *Saccharothrix mutabilis* subsp. *capreolus* (capreomycin producer, accession code, ABR67748).

DISCUSSION

The full biosynthetic pathways of viomycin, tuberctinomycins, streptothricin and capreomycins, the main members of TUBs still not biochemically identified. They are composed of several nonproteinogenic residues such as L-capreomycidine, 2,3-diaminopropionate, β-ureidodehydroalanine, and β-lysine that are biosynthesized from various amino acids through a nonribosomal peptide synthase mechanism[2-6]. The biosynthesis of L-capreomycidine, the most important nonproteinogenic residue in these antibiotics was determined in the viomycin producer *Streptomyes* sp. strain ATCC11861 where both VioC and VioD proteins were involved[23].

Analysis of the submitted DNA segment (accession code: AJ744850) harbouring the ribostamycin biosynthetic gene cluster had revealed the presence of three ORFs (SribL03.14c, SribL03.15c, SribL03.16c) with high amino acid identities to homologous ORFs (AAP92496.1, AAP92497.1, AAP92498.1) in the viomycin biosynthetic gene cluster (Thomas et al., 2003; Aboshanab, 2005). Therefore, the aim of this study was to detect whether a full

viomycin-related biosynthetic gene cluster is located in *S. ribosidificus* or not. Accordingly, various heterologous and homologous primers were designed based on available nucleotide and protein sequences in the GenBank database. The selection criteria for construction of heterologous primers were based on alignment of homologous proteins, responsible for the synthesis of main core unit in the TUB family of antibiotics such as L-capreomycidine. Moreover, for construction of heterologous primers, two areas of maximum conservation of the amino acid sequences have been selected and thereof back translated into their corresponding nucleotide sequences taking into consideration the codon usage for Actinomycetes[24]. Five heterologous primer pairs were constructed and used in PCR for amplification of target DNA fragment (cf. Materials & methods; Tab. 2). Results showed that the expected size of the PCR products was obtained on agarose gel electrophoresis (Fig. 2). The respective PCR products were recovered, purified and sequenced. The obtained sequences were assembled using the Staden Package Programme version 2 into a continuous DNA fragment of 3484 bp (cf. Fig. 4). Furthermore, homologous primers were constructed for verification based on the obtained sequence and the expected sizes of PCR products were obtained and sequenced (cf. Fig. 3). Accordingly, analysis of this DNA segment revealed the presence of three ORFs with high amino acid identities (about 80%) to respective ORFs of the viomycin and capreomycin gene clusters[6,25]. These ORFs were putative L-arginine hydroxlase (SriC, 297 aa; 86% identity), putative L-capreomycidine synthase (SriD; 391 aa), and putative permease (SriE; 461 aa; cf. Figs. 5, 6 & 7)[6,10,25]. Amino acid alignment of the respective ORFs to those of viomycin and capreomycin gene clusters showed about 86, 82, 85% amino acid identities which mean their possible involvement in similar biosynthetic steps. Interestingly, conversion of (2S)-arginine to (2S,3R)-capreomycidine was biochemically identified where both VioC and VioD gene products from the viomycin biosynthetic pathway of *Streptomyces* sp. strain ATCC11861 were involved (Fig. 8)[23].

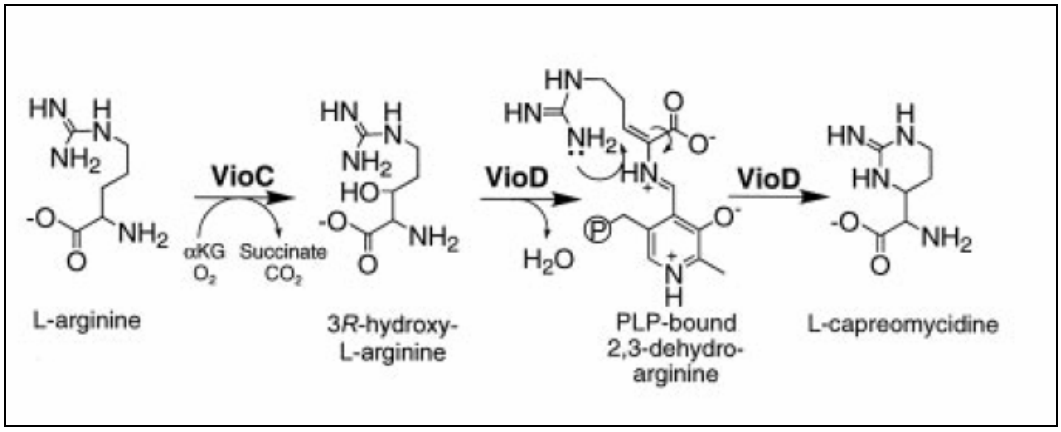

Fig. 8. Roles of VioC (L-argining hydroxylase) and VioD (L-capreomycidine synthase) in the biosynthesis of L-capreomycidine biosynthesis [6].

Therefore, both SriC and SriD proteins would be expected to be involved in the biosynthesis of L-capreomycidine moiety in *S. ribosidificus* however, this has to be confirmed biochemically.

On the other hand, the structures of viomycin and capreomycin bound to the 70S ribosome was invesitgaed and it was found they inhibit translocation by stabilizing the tRNA in the A site in the pretranslocation state[13]. In addition, these structures show that the tuberactinomycins bind adjacent to the binding sites for the paromomycin and hygromycin B antibiotics. This result may signify the presence of a common resistance genes for both antibiotics as previously prooved[25]. It was found that resistance to capreomycin was mediated via an rRNA-modifying enzyme that mediated also resistant to the aminoglycoside antibiotic kanamycin in *Saccharothrix mutabilis* subsp. *capreolus* ATCC 23892. Therefore, the prospective of this study include: i) explore the

production of a peptide antibiotic of TUBs family by the *S. ribosidifics*, the producer of the aminoglycoside antibiotic ribostamycin; ii) biochemical analysis of the respective putative capreomycidine-biosynthetic homologous proteins; and iii) construction of knock-out mutant of the different genes obtained in this study followed by recording the different phenotypic changs that will occur on the mutant strain.

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فصل وتحديد التتابع النيكلوتيدى و النتاج البروتينى لثلاثة جينات المفترض ضلوعهم فى التكوين الحيوى لمركب الكابروميسيدين داخل سلالة الستربتومايسس ريبوزيديفكس NRRL B-11466

خالد محمد أنور أبوشنب

قسم الميكروبيولوجيا و المناعة- كليه الصيدلة- جامعة عين شمس

يعتبر مركب الكابروميسيدين من الاحماض غير امينية التى تتكون وتتحد مع احماض امينية اخرى عن طريق آلية تكوين الببتيد غير ريبوزومية (NRPS) وذلك لتكوين مضادات حيوية عديده من عائلة التيوبراكتينومايسن. و تعتبر هذه العائلة من المضادات الحيوية ذات فاعلية فائقة ضد ميكروب الدرن و بالاخص عديد المقاومة للمضادات الاخرى و كذلك ضد ميكروب الستافيلوكوكس اوريس المقاومة لمضاد الميسيللن وايضا ضد المكورات المعوية المقاومة لمضاد الفنكوميسن. لذلك تناول هذا البحث فصل وتحديد التتابع النيكلوتيدى و النتاج البروتينى لثلاثة جينات المفترض ضلوعهم فى التكوين الحيوى لمركب الكابروميسيدين داخل سلالة الستربتومايسس ريبوزيديفكس NRRL B-11466. تم تصميم بادءات غير متجانسة و متجانسة واستخدامها فى تكبير اجزاء عديده من الحمض النووى من خلال تفاعل البلمرة المتسلسل واتبع ذلك معرفة التتابع النيكلوتيدى لهذه الاجزاء المكبره وعمل دمج لهم للحصول على جزء متصل من الحمض النووى بطول ٣٤٨٤ ثنائى النيكلوتيد ثم اتبعة ادخالة فى البنك الجينى الاوربى تحت رقم HQ327309.

التحليل النووى لهذا الجزء المتصل من الحمض النووى تبين وجود ثلاثة جينات كاملة ووجد ان النتاج البروتينى لهذه الجينات له نسبة عالية جدا من التماثل على مستوى الاحماض الامينية تقدر بحوالى ٨٦% وذلك للنتاج البروتينى لثلاثة جينات اخرى داخل المجموعة الجينية المسئولة عن التكوين الحيوى لمضاد الفيومايسن. و هؤلاء الثلاثة هم بروتين ال-أرجينين هيدروكسلاز(86%) والبروتين المسئول عن التكوين الحيوى لمركب الكابروميسيدين (82%) وبروتين المسئول عن النفاذ الخلوى (85%). وهذا هو أول تقرير عن وجود جينات مفترض ضلوعهم فى التكوين الحيوى لمركب الكابروميسيدين داخل سلالة الستربتومايسس ريبوزيديفكس NRRL B-11466. والجدير بالذكر ان نتائج هذا البحث سوف تساهم فى اكتشاف مضاد حيوى بروتينى جديد فى سلالة الستربتومايسس ريبوزيديفكس NRRL B-11466 المنتجة لمضاد الريبوستاميسن التابع لمجموعة الامينوجليكوزيد.