International Journal of Molecular and Clinical Microbiology 2 (2013) 289-294

International Journal of
Molecular and Clinical Microbiology

Antagonistic activity of *Streptomyces gresiofuscus* PTCC1628 against isolated Gram negative bacteria from Urinary Tract Infections

Rezvaneh Mansori[1](M.Sc), Khosro Issazadeh[1]*(Ph.D), Mohammad Reza Majid Khoshkholgh Pahlaviani[2](Ph.D), Mirsasan mirpour[1](Ph.D)

1 Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, Lahijan Branch, Lahijan, Iran,
2 Department of Biotechnology, Faculty of Basic Sciences, Islamic Azad University, Lahijan Branch, Lahijan, Iran, P.O.Box:1616

ARTICLE INFO

Article history:
Received 27 May 2013
Accepted 7 Agust 2013
Available online 1 December 2013

Keywords:
S. griseofuscus,
Urinary Tract Infections,
Antagonistic Activity

ABSTRACT

Antibiotics are the best known products of actinomycete. Over 5,000 antibiotics have been identified from the cultures of Gram positive and Gram-negative organisms, and filamentous fungi, but only about 100 antibiotics have commercially been used to treat human, animal and plant diseases. The genus, Streptomycete, is responsible for the formation of more than 60% of known antibiotics. Bacterial urinary tract infections are frequent in the outpatient as well as in the nosocomial setting. The common bacteria from UTI_s were isolated from hospital and laboratory samples. The present study was designed to isolate Gram-negative bacteria from urinary tract infections and evaluate *Streptomyces gresiofuscus* PTCC1628 antimicrobial activity against pathogenic bacteria. Bacterial isolates were identified as *Acinetobater* spp. and *Pseudomonas aeruginosa*. The antimicrobial activity was examined by the agar Well diffusion method. The cell free supernatants of the *S.gresiofuscus* were able to inhibit the growth of all human pathogens (*Acinetobacter* spp. and *P.aeruginosa*) isolated in this study in Well diffusion method. The isolates also showed very promising activities against multi drug resistant human pathogens. Concentrations of produced compounds by *S.griseofuscus* PTCC1628 were determined by GC-MS method.

1. Introduction

The genus *Streptomyces* belongs to order Actinomycetales.These bacteria are filamentous, aerobic, gram positive and spread mainly in soil and considered as a good source for more than half of all antibiotics (Vaurnakis et al., 1983). Also it is known for production of many

Other products like extra cellular enzymes and inhibitors (Good Fellow et al., 1983, Chater et al., 1979, Yang 1999, Kuzhadhaivel et al., 2000, Nicole et al., 2002). Antibiotic resistant pathogens have widely and continuously been reported.

In consequence, novel antibiotics have been investigated intensively. At present, 4,000 antibiotic substances obtained from bacteria and fungi have been applied in medicine of whichabout 75% are produced from Gram-positive actinomycetes bacteria such as *Streptomyces* sp. (Miyadoh, 1993). Various antimicrobial substances from *Streptomyces* sp. and actinomycetes bacteria have been isolated and characterized including amino glycosides,

*Corresponding author. Dr.Khosro Issazadeh
Tel: 09391225570
E-mail address: Issa_kaam@yahoo.com

anthracyclins, glycopeptides, b-lactams, macrolides, nucleosides, peptides, polyenes, polyester, polypeptides, actinomycins and tetracycline's (Good fellow et al., 1988; Okami and Hotta,1988; Baltz, 1998). Therefore, this study aimed to assess the antimicrobial capability of the active substance from *S.griseofuscus* against human pathogenic bacteria. Screening of microorganisms for the production of novel antibiotics has intensively been pursued for many years by scientists. Antibiotics have been used in many fields including agriculture, veterinary and pharmaceutical industry. Actinomycetes have the capability to synthesize many different biologically active secondary metabolites such as antibiotics, herbicides, pesticides, anti-parasitic, and enzymes like cellulase and xylanase used in wastetreatment. Of these compounds, antibiotics predominate in therapeutic and commercial importance (Lacey, 1973; McCarthy and Williams, 1990; Waksman, 1961). Based on several studies among bacteria, the actinomycetes are noteworthy as antibiotic producers, making three quarters of all known products; the *Streptomyces* are especially prolific (Lacey, 1973; Lechevalier, 1989; Locci, 1989). Urinary tract infection is an infection in the urinary tract. Bacteria are the most common cause of UTI_s.They are the common and occasional recurrent bacterial illness with an increasing resistance to antimicrobials agents. Antibiotic resistance in UTI is a growing public health problem in the world (Rahman et al., 2009). Resistance in Gram-negative bacteria has been increasing, particularly over the last 6 year (Pallet and Hand, 2010).

2. Material and Methods

2.1. Isolation of Bacterial strains from UTIS samples

Urine samples were collected from UTI_S in North of Iran (Rasht hospitals). *Acinetobacter* spp. and *Pseudomonas aeruginosa* were isolated from patients with urinary tract infections on basis of morphological, cultural and biochemical characteristics according to Berg's Manual Systematic of Bacteriology.

2.2. Antibiotic Sensitivity Test

Antibiotic resistance profile was determined by Kirby Bauer disc diffusion method on Mueller Hinton (MH) agar plates (Hi-media, Mumbai). Discs were consistently tested for efficacy against isolates recommended by National Committee for Clinical Laboratory Standards (NCCLS) as well as others with known antimicrobial susceptibility pattern. The microorganism suspensions used for inoculation were prepared at 10^8cfu (colony forming units)/ml by diluting fresh cultures at McFarland 0.5. Ten antibiotics (Hi-media) were used for the antibiotic sensitivity test. Standardization of the technique controls variation in results and interpretation was based on comparison of inhibition zones with published criteria for zone diameters.

2.3. Test organisms

The selective microorganisms used for antagonistic activity were *Acinetobacter* spp. And *Pseudomonas aeruginosa* isolated from UTI_s.

2.4. Antagonistic activity

The antimicrobial activity was examined by the agar Well diffusion method. *S.griseofuscus* PTCC1628 was grown in 50 ml of starch casein broth by submerged culture in 250 ml flasks by incubating at 28°C in a shaker (150 rpm) for 7 days and centrifuged at 4000 rpm for 10 min and the clear supernatant broth samples were tested for their antagonistic activity against the isolated pathogens by agar well diffusion method. Wells of 6 mm diameter were prepared in the nutrient agar plates. Isolated pathogenic bacteria were swabbed on to the nutrient agar surface and the wells were filled with 70 µl of culture supernatant and the diameter of inhibition zones were measured after incubation for 24 h at 37°C.

2.5. Isolation of antibacterial metabolites

The filtrate was subjected for solvent extraction method to recover antibacterial metabolites in pure form Ethyl acetate was

added to the filtrate in the ratio of 1:1(v/v) and shaken vigorously for 1 h for complete extraction. The ethyl acetate phase containing Antibiotic substances were separated from aqueous phase. It was evaporated to dryness in water bath and the residue obtained was used to determine antimicrobial activity.

2.6. GC- MS analysis

The culture filtrate was extracted three times by the same volume of ethyl acetate. After removal of cell mass, supernatant was analyzed by spectrophotometer at 220nm. It was carried out for GC-MS analysis Culture filtrate extracted by ethyl acetate was analyzed by GC-MS. In order to purify the active fraction, methanol: chloroform (20: 80) ratio extract was used and chloroform was added gradually (drop wise) until formation of the first precipitate. The precipitate was separated by centrifugation. Each fraction was tested in inhibition test. Ammonium sulphate was also used for the extraction. Ammonium sulfate was grind in a glassmortar to fine powder to be easily dissolved.

The Powder was added gradually to the antagonistic solution. The solution was shaked using a vortex. The formed precipitate was separated by centrifugation. The amount of ammonium sulfate was noted for each precipitate and calculated as saturation percent. Each precipitate was dissolved in water and re-precipitated again by ammonium sulfate as a purification step. Each precipitate was dissolved in methanol and centrifuged to remove any residue of ammonium sulfate. This step was repeatedseveral timesuntil removing all ammonium sulfate.

Each precipitate was used for the inhibition test and analyzed by GC-Muslin this technique the Gas Chromatography and Mass Spectrometry were analyzed by GC-MS electron impact ionization (EI) method on GC-8000 gas chromatograph (FISONS Instruments). Compound identification was done by comparing the NIST (National Institute of Standards and Technology- Chemistry web book by WILEY) library data of the peaks with those reported in literature.

3. Results

3.1. Isolation and screening of bacteria from UTIS

Acinetobacter spp. and *P.aeruginosa* were isolated from UTI_s samples following standard protocols. The best strain was characterized by biochemical, morphological and physiological tests. According to Bergey' s manual of determinative bacteriology and the laboratory manual for identification bacteria. The identified isolates are shown in the Tables 1 and 2.

3.2. Antibiotic Sensitivity Test (AST)

The isolates with high antibiotic resistance were identified and tested against 10 antibiotics from different groups. The isolates showed high level of resistance to multiple antibiotics and were resistant to most of the antibiotics. Resistance level was low to Tetracycline as compared to other antibiotics of this group (Figure 1).

3.3. Determination of Antimicrobial activity

Streptomyces griseofuscus PTCC1628 showed potential antagonistic activity against *Acinetobacter* spp. and *P.aeruginosa* in thepresent study. The inhibition zone diameters (mm) obtained by experimental trials was found to be 5.6 mm and 10 mm for *P.aeruginosa* and *Acinetobacter* spp., respectively.

3.4. GC- MS analysis

In GC-MS analysis a total of 7 compounds were identified. The Mass Spectrometry Indices of the major peaks are shown In Figure 2. The GC-MS analysis showed1 major peaks. The highest peak area was by 1, 1-Diethoxyethane = Diethyl acetyl with retention time 8.06 min (Table 3).

Table 1. Biochemical characters of *Acinetobacter* spp.

Characteristics	Gram stain	Motility	Oxidase	Catalase	Urease	OF medium
Acinetobacter spp.	-	-	-	+	-	+

+ positive; - negative

Table 2. Biochemical characters of *Pseudomonas aeruginosa*

Characteristics	Gram stain	Motility	Indole	SH_2	OF	Oxidase	Catalase	Citrate	MR	VP
Pseudomonas aeruginosa	-	+	-	-	+	+	+	+	-	-

+ Positive; - negative

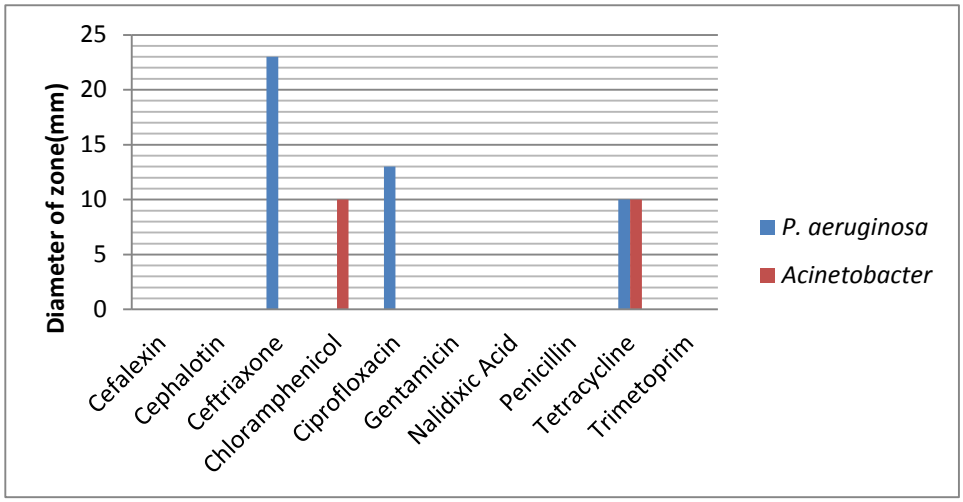

Figure 1. Sensitivity of isolated bacteria from UTI_S to antibiotics in mm

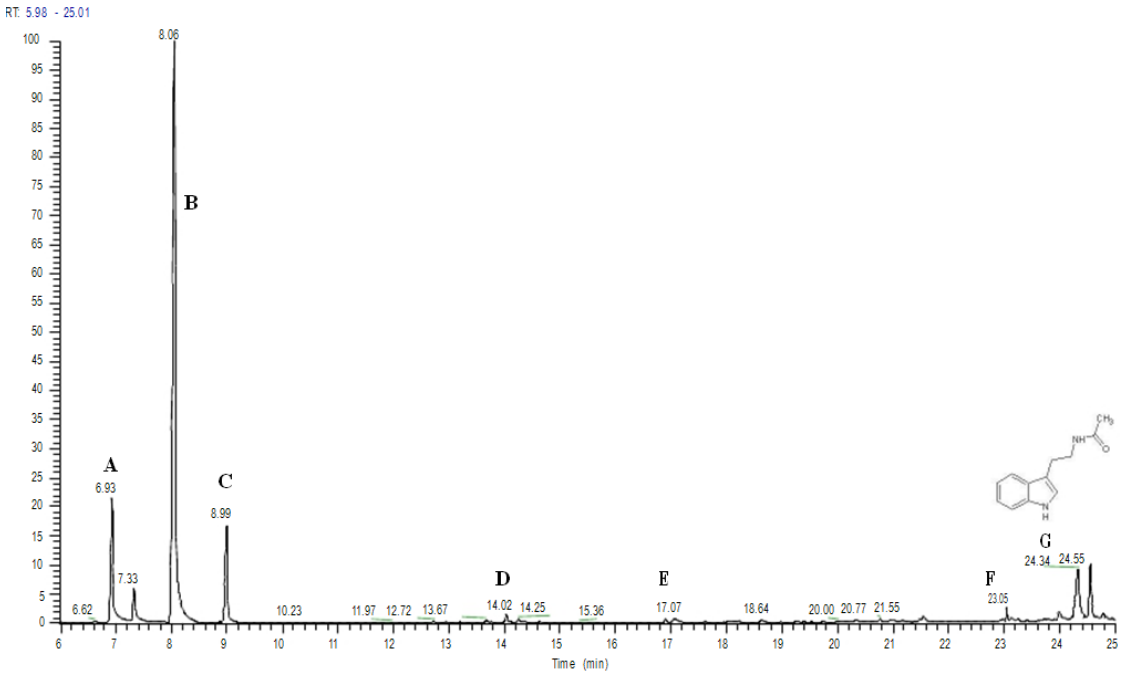

Figure 2. Spectrum of determined compounds in *S.griseofuscus* PTCC1628 by GC-MS method

Table 3. Concentrations of determined compounds in *S.griseofuscus*PTCC1628by GC-MS method

No	R_t (min)	Name of Compound	%
A	6.93	3-hexene-2-one	2.27
B	8.06	1,1-Diethoxyethane = Diethyl acetal	1.65
C	8.99	1-Propoxy-2-propanol	1.99
D	14.02	2,6-dimethyl-2,5, Heptadien-4-one	1.75
E	17.07	2-Heptanol	2.25
F	23.05	10-methyl elcosane (alkane hydrocarbon)	1.68
G	24.30	Eicosane	2.01

4. Discussion

The main objective of this study was to determine the antagonistic activity of *S.griseofuscus* against bacteria isolated from Urinary tract infections. Antagonistic effects of the *S.griseofuscus* have been studied to some extent. Numerous studies have shown that when starch-casein (SC) agar is used to isolate actinomycetes from soil, the majority of colonies developed are *Streptomyces* spp. (Hayakawa & Nonomura, 1987). The abundance of *Streptomyces* spp. on SC agar was observed during the isolation of actinomycetes from Malaysian mangrove mud and intertidal sediments (Getha et al., 2004). It was also observed in the present study (*S.griseofuscus* was grown on this medium). Inhibition zone diameters of Ceftriaxone, Tetracycline and Ciprofloxacin against Pseudomonas aeruginosa were 23, 10 and 13 mm, respectively, but Inhibition zone diameter of *S.griseofuscus* a gainst *P.aeruginosa* was 5.6mm. Inhibition zone diameters of Tetracycline and Chloramphenicol against *Acinetobacter* were 10 mm and Inhibition zone diameter of *S.griseofuscus* against *Acinetobacter* was 10 mm too. According to the findings It can be concluded that in some cases there are secondary metabolites produced by Streptomycin that can be used to treat urinary tract infections like antibiotics.

We propose these tests to be done in vivo. A study carried out by Endogen and et al. in 2010 (Soković et al., 2010) showed that essential oils of Labiatae and Umbelliferae have significant antiviral and antibacterial effects against bacteria and viruses including HSV-1, *Staphylococcus aureus*, *P.aeruginosa*, *Candida albicans* and *Klebsiella pnemoniae*. They were determined Heptanol asactive component in the essential Oils. In our study, Heptanol was identified by GC-MS analysis too. The antimicrobial properties of S. griseofuscus almost seems pertaining to Heptanol. Most of the antibiotics are extracellular-secondary metabolites, (Vilches et al., 1990). They have been used as herbicides, anticancer agents, drugs, immunoregulators and antiparasitic agents (Thomson and Bialphos, 1955).

For instance, the culture supernatant of Streptomyces sp. No. 87 previously isolated from agricultural soil from Sakonnakhon Province, Thailand, was partially purified and characterized. Its antimicrobial activity against the various plant pathogens has been investigated and presented. The results show that the active substance from *Streptomyces* sp. No. 87 is not proteinacious and greatly inhibits growth of the plant pathogens including Gram-negative bacteria. This compound might be useful for use as biocontrol in plants (Purichinawut et. al., 2004). S. griseofuscus PTCC1628 exhibited antibacterial activity against isolates and showed promising activity in vitro condition.

Acknowledgement

Financial support from the Islamic Azad university, Iran, to carry out this work is greatly acknowledged by all the authors.

References

Baltz, R.H., 1998. Genetic manipulation of antibiotic producing Streptomyces.Trends Microbiol. 6, 76-83.

Chater, K.F., Merrick, M.J., 1979. Streptomyces in: Development Biology of Prokaryotes (ed.J.H. Parish).Blackwell Scientific Publication oxford.93-114.

Getha, K., et al. 2004. Characterization of selected isolates of indigenous Streptomyces species and evaluation of their antifungal activity against selected plant pathogenic fungi. Malaysian Journal of Science. 23, 37–47.

Good fellow, M., et al., 1988 Actinomycetes in biotechnology. London, Academic Press.

Good Fellow, M., Gross, T., 1983. Classification in the Biology of the Acrimony (eds. M. Good Fellow; M. Mordarski and S.T. Williams).

Hayakawa, M., Nonomura, H., 1987. Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. J. Ferment. Technol. 65, 501–509.

Kuzhadhaivel, S., Vetrivel, K., 2000. Isolation of a chitinase overproducing mutant of Strepomyces peucetius defective in daunorubicin biosynthesis. J. Microbiol., 46(10), 959-960.

Lacey, J., 1973. Actinomycetales: Characteristics and Practical Importance. Edited by G. Sykes and F. Skinner.The Society for Applied Bacteriology Symposium Series.Academic Press London- New York No. 2.

Lechevalier, H.A., 1989. The Actinomycetes III, A Practical Guide to Generic Identification of Actinomycetes. Bergey's Manual of Systematic Bacteriology. Williams & Wilkins Company, Baltimore. 4, 2344-2347.

Locci, R., 1989. Streptomyces and related Genera.Bergey' s Manual of Systematic Bacteriology. Williams & Wilkins Company, Baltimore. 4, 2451-2508.

McCarthy, A.J., Williams, S.T., 1990. Methods for Studying the Ecology of Actinomycetes. Methods in Microbiology.Ed. by. R. Grigorova and JR Norris, Academic Press Limited, London, 22, 533-363.

Miyadoh, S., 1993. Research on antibiotic screening in Japan over the last decade.A producing microorganism approach.Actinomycetologica. 9, 100-106.

Nicole, K., et al. 2002. The positive activator of cephamycin C and clavulanic acid production in Streptomyces clavuliqerus is mistranslated in a bldA mutant .Microbiology. 148, 643-656.

Okami, Y., Hotta, K., 1988. Search and discovery of new antibiotics, actinomyces in Biotechnology. London, Academic. 33-67.

Pallet, A., Hand, K., 2010. Complicated urinary tract infections: Practical solutions for the treatment of multiresistant Gram- negative bacteria. J antimicrob chemother. 65(3), 25-33.

Purichinawut, P., Thummabenjapone, P., 2004. Hydrolytic enzymes and secondary metabolites from Streptomyces ssp. antagonistic of bacteria Acidovorax avenae subsp. curtly and fungus Pidymellabryoniae. Abstract: The 17th Seminar of the THAI Biotechnology Society with the title of “Innovative Biotechnology: The Opportunity to be Kitchen to the World” during 12-. December 2004, Pitsanulok, Thailand. 111.

Rahman, F., et al. 2009. Antimicrobial resistance pattern of Gram- negative bacteria causing urinary tract infection. Stamford journal of pharmaceutical sciences. 2(1), 44-50.

Soković, M., Glamočlija, J., Marin, P.D. et al., 2010. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. Molecules. 15, 7532-7546.

Thomson, C.J., Bialphos, S.H., 1955. Genetics and Biochemistry of antibiotic production. L.C. Vining, C. Stuttardeds, pp. 197-222.

Vaurnakis, J.N., Blander, R.P., 1983. Genetic Manipulation on of Antibiotic Producing Microorganisms. Science 219, 703-709.

Vilches, C., et al. 1990. Biosynthesis of oleandomycin by Streptomyces antibioticus: Influence of nutritional conditions and development of resistance. J. Gen. Microbiol. 136, 1447-1454.

Waksman, S.A., 1961. the Actinomycetes, Classification, Identification and Description of Genera and Species. Baltimore: The Williams and Wilkins Company. 2, 61-62.

Yang, J., 1999. Protease and amylase production of Streptomyces rimosus in submerged and Solid State cultivation bot.bull.acad.sin. 40, 259-265.