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Antimicrobial activity of *Streptomyces* sp. VITBT7 and its synthesized silver nanoparticles against medically important fungal and bacterial pathogens

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ABSTRACT

In the present study an attempt was made to evaluate the antimicrobial potential of culture filtrate of Streptomyces sp. VITBT7 and biologically synthesized silver nanoparticles using the culture filtrate of VITBT7. Actinomycetes isolates obtained from soil samples were screened for antimicrobial activity against selected fungal and bacterial pathogens by well diffusion method. The potential isolate was identified by molecular taxonomic characterization. The culture filtrate of the potential isolate was assessed for the synthesis of silver nanoparticles. The synthesized silver nanoparticles were characterized for surface plasma resonance (SPR) peak, shape and size distribution by TEM analysis. Out of 240 actinomycetes colonies recovered, 19 isolates showed mild to moderate antimicrobial activity and one potential isolate showed broad spectrum of activity against tested microbial pathogens with higher zone of inhibition against Pseudomonas aeruginosa and Aspergillus niger. The isolate was identified to be belonged to the genus Streptomyces and designated as Streptomyces sp. VITBT7 (accession number JX188053). The culture filtrate synthesized silver naoparticles (AgNPs) within 24 h. The biologically synthesized AgNPs showed SPR peak at 420 nm and were found to be spherical in shape with the size range of 20-70 nm. Synthesized AgNPs also exhibited antimicrobial activity against fungal and bacterial pathogens. Synthesized AgNPs by Streptomyces sp. VITBT7 could be used effectively to control fungal and bacterial pathogens. The secondary metabolites produced by Streptomyces sp. VITBT7 could be responsible for observed antimicrobial activity.

Keywords: *Streptomyces* sp. VITBT7, silver nanoparticles, fungal pathogens, bacterial pathogens, antimicrobial activity.

INTRODUCTION

Infectious diseases caused by human systemic pathogens are life threatening and cause leading health problems in the developing countries [1]. Repeated use of antibacterial drugs had resulted in the development of resistance against them. In particular, multiple drugs resistance is one of the major problem and new bioactive compounds are required to overcome this multi drug resistance [2]. Antifungal drugs available in the market are very limited but there is a steady increase in variations among opportunistic mycotic infections. *A. niger* is one of the most common causes of mycosis in humans. The occurrence of invasive aspergillosis was increasing in immuno compromised patients in the recent fast. Reports on the drug resistant isolates of *Aspergillus* species due to repeated exposure to antifungals have emerged in recent years [3].

Antibiotic resistance is a complex, continually evolving problem which is often difficult to put into perspective [4]. The resistance problem is due to increased use of antibiotic and the presence resistance gene in the microbial strains.

Hence there is a need for development of new drugs that act on new targets and those that block resistance mechanisms [5]. Bacteria use several mechanisms to become antibiotic-resistant which include inactivation of the antibiotic, efflux pumping of the antibiotic, modification of the antibiotic target, alteration of the pathway [6]. Multidrug resistance occurs due to accumulation of multiple resistance genes typically on plasmids and also due to the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs [7].

Secondary metabolites from actinomycetes exhibit tremendous novelty. Actinomycetes are Gram-positive filamentous spore formers with high G+C (>55%) content of DNA. They are free living saprophytic bacteria forming a major group of soil population. *Actinobacteria* are widely distributed in terrestrial and aquatic ecosystems, especially in soil, where they decompose complex mixtures of polymers in dead plant. They also play main role in recycling of organic matter [8]. Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported and actinomycetes alone produce 10,000 of these compounds. Many of these secondary metabolites are potent antibiotics, which has made *Streptomycetes* the primary antibiotic-producing organisms exploited by the pharmaceutical industry [9]. It is estimated that about two-thirds of naturally occurring antibiotics have been isolated and majority of the antibiotics have been isolated from the genus *Streptomyces* [10, 11]. Other industrially important bioactive compounds from *Streptomyces* includes anti-tumor agents [12], novel thiol-antioxidants [13], immune enhancers [14], immune suppressive agents [15], enzyme inhibitors [16] etc. Potential antibacterial and antifungal activity of marine *Streptomyces* species has widely been reported. Similarly *Streptomyces* species isolated from soil samples have also been reported to contain various biological activities. They play crucial role in biodegradation by recycling nutrients associated with recalcitrant polymers such as keratin, lignocelluloses and chitin [17]. Hence, un-exploited habitats are promising source for novel actinomycetes and for discovery of novel bioactive compounds. Considering the rise of resistant pathogens that ruin current antimicrobial therapeutic options, the necessity to explore the unexplored areas in a ecofriendly, and safe manner to isolate and to identify potent compounds is very crucial. In the present study, the antimicrobial potential of culture filtrates and synthesized nanoparticles of *Streptomyces* sp. VITBT7 was studied against medically important fungal and bacterial pathogens.

MATERIALS AND METHODS

Sample collection and isolation of actinomycetes

Soil samples were collected at 5 different locations with varying soil texture. Over layered soil was removed using sterile spoon and samples were collected in sterile air lock bags at 15cm depth as reported earlier [18]. Pretreatment was done to target selective isolation, one set of samples were kept at 45°C, 2 days while the other batch was air dried at 28°C for a week [19]. Soil sample (10 grams) mixed with 100 ml of sterile distilled water (10:1 ratio) was kept in the orbital shaker overnight for thorough mixing [20]. About 0.1ml of each dilution was spread plated over actinomycetes isolation agar (AIA) (Himedia, Mumbai, India) and incubated at 28°C for 7 days. Colonies recognized based on their colony morphology were selectively sub-cultured on starch casein agar and, Kuster's agar [21]. The growth media supplemented with antibiotics cycloheximide (25 µg/ml), and nalidixic acid (25 µg/ml) (Himedia, India) to avoid bacterial contamination and incubated at 28°C for 7 days [22]. The isolate was stored as slant cultures at 4°C and 20% (v/v) glycerol stocks were stored at -80°C for future use.

Bacterial and fungal pathogens

Gram positive bacterial pathogens such as *Staphylococcus aureus* (MTCC 739), *Klebsiella pneumonia* (ATCC 700603) and Gram negative pathogens, *Pseudomonas aeruginosa* (MTCC 424), *Bacillus cerus* (MTCC 1168) and *E.coli* (ATCC 25922) were used. Fungal pathogens *Aspergillus niger* (MTCC 1344), *Candida albicans* (MTCC 227), *Aspergillus fumigates* (MTCC 3002), and *Aspergillus flavus* (MTCC 1973) were used for the study.

Screening for antibacterial activity

Screening for antibacterial and antifungal activity was carried out by well diffusion method. Well grown matured pure colonies were selected for screening antimicrobial activity. For bacteria, the turbidity of suspension was adjusted to 0.5 Mac-farelands standards in 0.85% saline. Lawn culture was spread using sterile swabs. Wells (8 mm diameter, 2 cm apart) were bored on Muller Hinton Agar (Hi-media) plate [23]. The fungal cultures were maintained in 0.2% dextrose medium and the optical density was adjusted to 0.10 at 530 nm using spectrophotometer and lawn culture was spread over sabaurauds dextrose agar media [24]. Cell free culture supernatant was obtained from the isolate by filtering the culture broth through Whatman no-1 filter paper. Cell-free supernatant (100 µl) was carefully loaded into each well and pre-incubated at 4°C for half an hour to facilitate uniform diffusion into the agar. The plates were incubated at 37°C for 24 h. Presence of inhibition zones surrounding each well evidenced antimicrobial

and antifungal activity. Their activity was evaluated by measuring the inhibition zones diameter in millimeters. Each experiment was repeated three times and the mean of inhibitory zones was recorded.

Molecular taxonomic characterization of the isolate

Macroscopic observations including morphological, cultural, physiological and biochemical characterization was carried out as per International Streptomyces Project (ISP) protocol [25]. Diffused pigmentations were recorded on (ISP-3), Oat meal agar [26]. Inorganic salt starch agar (ISP 4) and Glycerol asparagine agar base (ISP 5) were tested. The reverse side pigments of the colony, distinctive (+), non distinctive (-) was tested using peptone yeast extract iron agar (ISP 6) [27]. Melanoid pigments were tested on ISP 1 and ISP 7. Direct examination of spores under oil immersion (100x), spore bearing hyphae, spore chain and their branching pattern were observed under light microscope. Spore surface morphology was observed under Scanning Electron Microscope (Hitachi, S-3400N) [28, 29].

In order to optimize cultural conditions and harvest maximal yield of secondary metabolites, different media ISP-1 to ISP-7, ISP-9, SCA, AIA, MHA, and NA were tested. As per International Streptomyces project recommendations, the ability of the isolate to utilize carbon sources 1% (w/v) D-glucose, D-mannitol, fructose, xylose, sucrose, raffinose, inositol, D-galactose, arabinose and rhamnose as its sole source were tested on carbon utilization agar (ISP) supplemented with 1% carbon sources. Nitrogen utilization was tested with leucine, histidine, tryptophan, serine, glutamic acid, lysine, arginine, methionine, and tyrosine. The other biochemical tests like utilization of citrate, urease production, mannitol motility, indole, MR, VP, and triple sugar ion tests were carried out. Salt concentration was optimized by supplementing varying concentrations of NaCl (0, 6, 10, 15 and 25%), similarly varying temperature (15, 28, 37, and 45) and pH (4, 6, 7, 8, and 9) were recorded on ISP- 1 media [30].

The DNA was isolated by HiPurA bacterial DNA isolation and purification kit (Himedia, India) and the 16S rDNA was amplified by PCR using a master mix kit, Medomix (Medox, India) as per manufacturer's instruction. The primers and the PCR procedures were followed as reported earlier [31]. Sequencing was carried out bi-directionally using dideoxy chain termination method [32, 33]. The similarity and homology of the16 S rRNA partial gene sequence was analyzed with the similar existing sequences available in the data bank of National Center for Biotechnology Information (NCBI) using BLAST search. The DNA sequences were aligned and phylogenetic tree was constructed by neighbour joining method using ClustalW software [34]. A bootstrap analysis of 1000 replicates was carried out.

Synthesis of silver nanoparticles

The isolate *Streptomyces* sp. VITBT7 was inoculated into 800 ml of ISP1 broth culture, incubated at 28°C for 7 days under shake flask condition. The broth was then filtered through Whatman no-1 filter paper. $AgNO_3$ (1mM) 95 ml was added to 5 ml of cell free supernatant obtained from *Streptomyces* sp VITBT7 in a 250 ml conical flask at room temperature. The flask was incubated in an orbital shaker at 200rpm in a dark condition [35]. It was monitored continuously for color change. Absorption spectrum of the reaction mixture was measured between 200-800 nm using a Schimadzu UV-spectrophotometer from time to time. Broth culture of *Streptomyces* sp VITBT&7 without silver nanoparticle solution was included as a control. Transmission Electron Microscopic (TEM) analysis of synthesized nanoparticles was carried out using a JEOL JEM 2100 High Resolution Transmission Electron Microscope, operating at 200kV to measure the shape and size of silver nanoparticles.

RESULTS

The actinomycete isolate *Streptomyces* sp. VITBT7 was screened for antifungal and antibacterial activity on Sabaurauds Dextrose Agar (SDA) and Muller Hinton Agar (MHA) respectively. The cell free supernatant of the isolate exhibited antimicrobial activity against both Gram negative and Gram positive bacterial pathogens. The cell free supernatant showed fungicidal activity with the inhibition zone of 20 mm against, *A. niger* followed by *A. fumigates* 18 mm, *A. flavus* 17 mm and *C. albicans* 15 mm zone of inhibition. The cell free supernatant also showed bactericidal activity with the inhibition zone of 37 mm against *P. aeruginosa*, 25 mm against *K. pneumonia*, 23 mm against *E. coli*, 23 mm against *S. aureus* and 19 mm against *B. cerus* (Table 1).

The silver oxide/silver nanoparticles were synthesized within 24 h of incubation evidenced by change in pale yellow color to brownish color indicating the reduction of silver nitrate and formation of silver oxide/silver nanoparticles. However no color change was observed in either the culture supernatant alone or silver nitrate control experiments.

The silver nanoparticles synthesized using the cell free supernatant showed selective inhibition over fungal and bacterial pathogens tested. AgNPs exhibited 22 and 20 mm zone of inhibition against *A. niger* and *A. fumigates* respectively. Similarly it showed inhibitory activity against *P. aeruginosa* (40 mm) and *S. aureus* with the inhibition zone of 40 and 28 mm respectively.

Table: 1 Antibacterial activity of *Streptomyces* sp. VITBT7 **against fungal and bacterial pathogens**

Selected Pathogens	Cell free culture supernatant	Silver nanoparticle synthesized mixture
	Zone of inhibition (mm)	
Fungus		
Aspergillus fumigates (MTCC3002)	18	20
Aspergillus niger (MTCC1344)	20	22
Aspergillus flavus (MTCC1973)	17	-
Candida albicans (MTCC227)	15	-
Gram negative bacteria		
Pseudomonas aeruginosa (MTCC 424)	37	40
Klebsiella pneumoniae (ATCC 700603)	25	-
E.coli (ATCC 25922)	23	-
Gram positive bacteria		
Staphylococcus aureus (MTCC739)	26	28
Bacillus cerus (MTCC1168)	19	-

Macroscopic observations revealed dull white creamy colonies on ISP 1 media. No characteristic pigment was produced in ISP6, ISP 7, and ISP 9. Glycerol asparagine agar base (ISP 5) showed cream powdery to dull white colonies. Optimal culturing conditions for the target isolate was found to be temperature 28°C, 7 days, pH-8, and 10 % NaCl concentration (Table 2).

Enhanced growth was observed when D-Glucose, D-mannitol, and D-galactose were used as carbon source. Under optimized conditions, the isolate *Streptomyces* sp. VITBT7 produced abundant growth with matured colonies on production media ISP 1. Observation under SEM analysis displayed spores was spherical in shape, arranged in long chains and each chain contains 10-25 spores (Fig. 1). The mature spores are 0.5-1.0 mm in diameter and the length is between 0.8 and 1.0 mm.

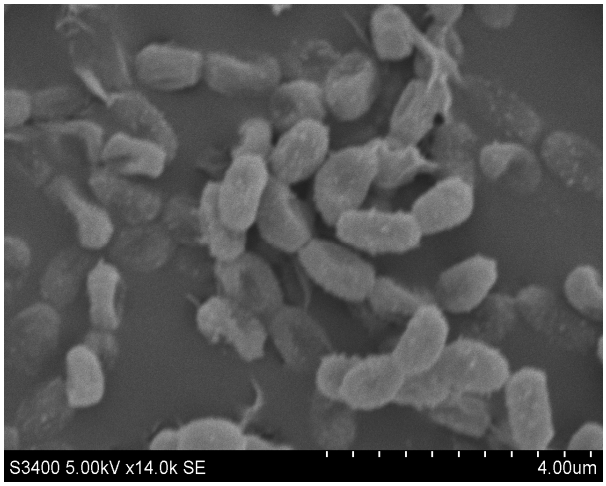

Figure 1: *Streptomyces* sp. VITBT7 observed under Scanning Electron Microscope (3400N Hitachi) showing matured smooth surfaced spore chains.

Phylogenetic analysis revealed that the organism belongs to genus *Streptomyces* and *designated as Streotomyces* sp. VITBT7 (Fig.2) 16S rRNA partial gene sequence was submitted under the accession number JX188053.

Silver nanopartilces synthesized was characterized by UV –visible spectrum, a strong and broad peak was observed at 420 nm, indicating the presence of silver oxide/silver nanoparticles. The SPR bands of silver oxide/silver nanoparticle solution remain close to 420 nm throughout the reaction period (Fig. 3). The appearance of SPR peak at

420 nm provides a convenient spectroscopic signature for the formation of silver nanoparticles. TEM analysis measured AgNPs in the size range of 20-70 nm and spherical in shape (Fig. 4).

Table: 2 Biochemical characteristics of *Streptomyces* sp. VITBT7

BIOCHEMICAL TESTS	*Streptomyces* sp. VITBT7
Gran stain	+++
Wet mount	+
Aerial mycelium	+++
Substrate mycelium	+
Motility	Non-motile
Spores	+
Optimum incubation time	7 days
AIA	Very Good
SCA	Good
Diffused pigment test	Pink pigment, Non-diffusible
Melanin production	-
6%	+/_
10%	++
15%	+
ISP1 broth	Very Good, bead appearance, light brown color
Kusters broth	Good, bead texture, light brown/orange pigment.
Starch casein nitrate broth	Good, light brown pigment
Nitreint broth	Good, cream turbid.
Citrate	++
Urease	++
Mannitol	+/_
Triple sugar ion	Alkaline slant and Butt, NO Gas
Indole	-
H2S production	-
MR and VP	_
Catalase	+
Oxidase	-
Glucose	_
Lactose	+
Sucrose	_
Fructose	++
Mannose	_
Galactose	+
Arabinose and Cellobiose	-
Raffinose and Inositol	-
15°C	_
28°C	+++
37°C	+
45°C	+
4	_
6	++
7	+++
8	++
9	_

Note: +++ Positive within two days, ++ Positive in 7 days, + Positive in 10 days

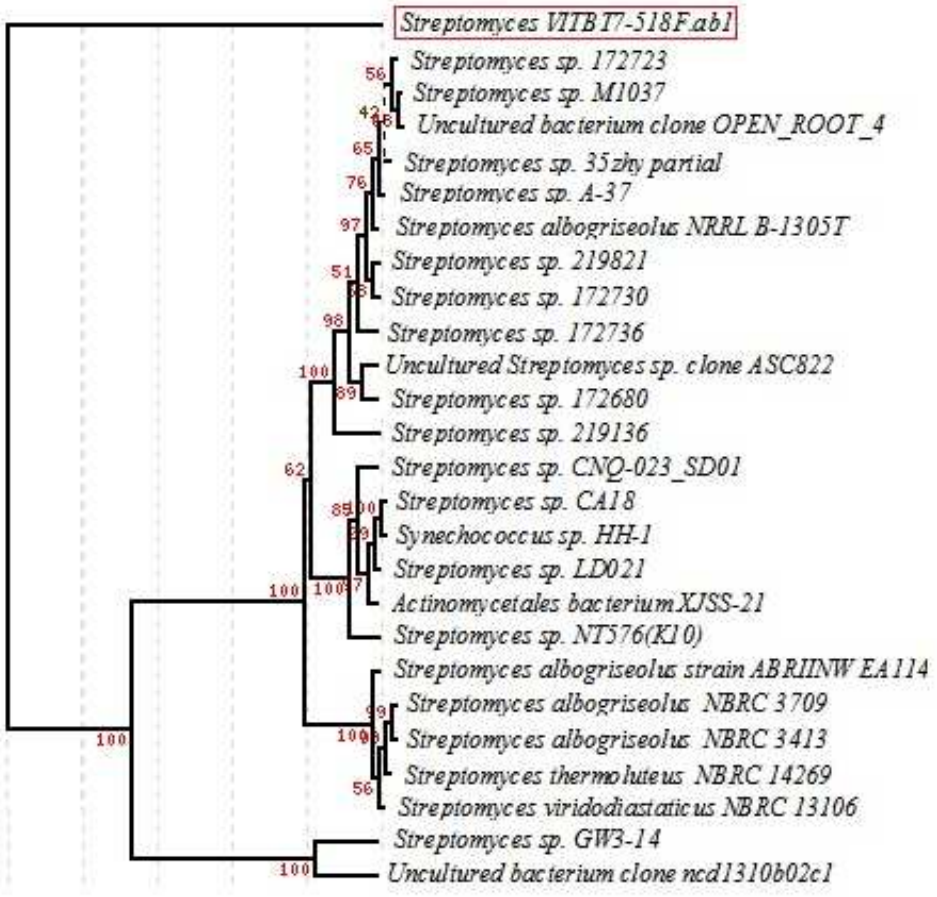

Figure 2: The phylogram of *Streptomyces* sp. VITBT7 compared to other *Streptomyces* species based on 16S rDNA sequences.

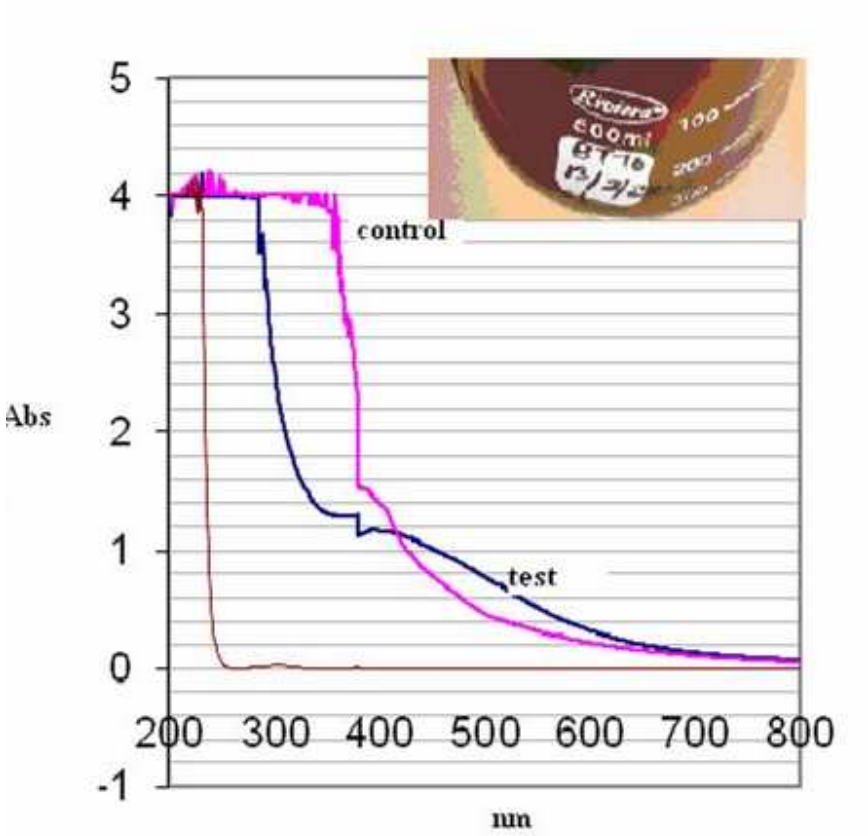

Figure 3: UV-absorption spectra of synthesized silver nanoparticles, (insert) Dark brown colour indicates reduction of silver.

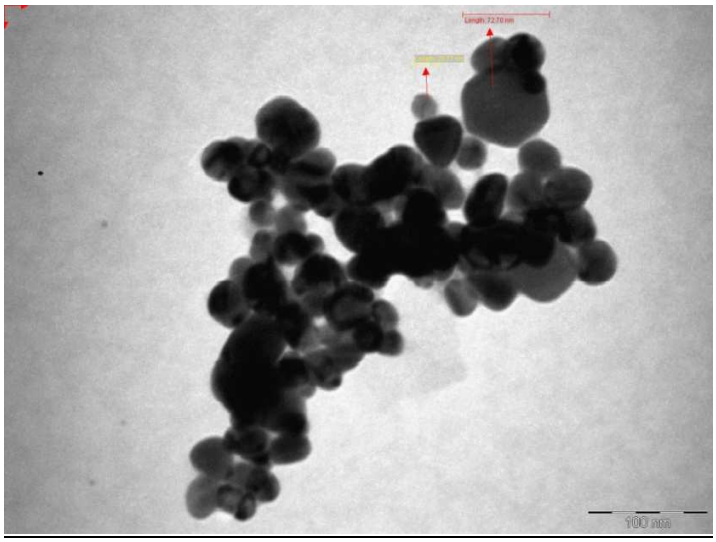

Figure 4: TEM image of silver nanoparticles (size 20-70 nm).

DISCUSSION

Streotomyces sp. VITBT7 isolated from under explored environment displayed significant antifungal and antibacterial activity to harmful pathogens suggest that the isolate produces antagonistic bioactive compound. Actinomycetes, “the reservoir of secondary metabolites and enzymes”, dominant and significant microbes inhabiting

soil environment comprises about 50% of the uncultivable soil microbes. The immense source of novel metabolites and their therapeutic applications, specifically as drug lead molecules serves as a natural blue print for developing new drugs [36]. Fungal drug resistance is a growing problem worldwide and search for new and effective antifungals to overcome drug resistance is of current importance [37]. Actinomycetes isolate producing polyene type of metabolite has been reported to be effective against pathogenic bacteria and fungi [38]. A polyketide antibiotic extracted from *Streptomyces* sp. AP-123 have been shown to be very effective against *C. albicans* and filamentous fungi *A. niger* [39]. New triazole antifungal drugs and combination drugs are underway to overcome invasive fungal infections and emergence of resistance [40].

Pennicillin, Tetracycline, cephalosporins are important soil derived antibiotics. Vancomycin (isolated in 1956) from actinomycete species found in Indian and Indonesian soils, is very powerful and currently serves as the last line of defense to treat bacterial infections [41]. Actinomycetes (C11 and C12) isolated from marine environment has been shown to be effective against group of bacterial pathogens [42]. Marine actinobacteria isolated from salt pan environment, SRB25 has been reported to be effective against multidrug resistant *Staphylococcus aureus* (MDRSA) [43]. At present there has been considerable attention towards novel strains and their compounds as therapeutic agents to control bacterial and fungal pathogens.

Over the past few decades, microbial assisted synthesis of nanoparticles from noble metals such as silver contribute in the development of many potential applications in different fields. They form clean, nontoxic, and environmentally acceptable ‘‘green chemistry’’ [44]. Several reports are available on extracellular synthesis of different nanoparticles by *Streptomyces* species [45-48]. Synthesis of silver nanoparticles (AgNPs) by extra cellular components of *Streptomyces albogriseolus* and activity against bacterial food pathogens has been reported [49].

CONCLUSION

In this study, *Streptomyces* isolated from Brahmapuram, Vellore city, Tamilnadu were evaluated for its potential antimicrobial activity against infectious pathogens and their ability to synthesize silver nanoparticles. The isolate *Streptomyces* sp. VITBT7 can be exploited for isolation of bioactive compound and bulk production of nanoparticles using a green approach for future commercial applications.

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REFERENCES

[1] JE. Stach, AT. Bull, *Antonie van Leeuwenhoek*, **2005**, 87, 3-9.
[2] R.Black, K D Brown, S Becker, M Yunus, *Am. J. Epidemiol.*, **1982**, 115, 305 -314.
[3] PH. Chandrasekar,. *Med. Mycol.*, **2005,** 43, 295-298.
[4] SB. Levy, *J. Antimicrob. Chemother.*, **2002,** 49, 25-30.
[5] SB Levy, *Trends Microbiol*., **1994,** 2, 341–342.
[6] FC. Tenover. *Am. J. Med.*, **2006,** 119, S3–S10.
[7] H. Nikaido, *Annual Rev. Biochem*., **2009,** 78,119-146.
[8] Z. Cwala, EO. Igbinosa , AI. Okoh, *Afr. J. Pharm. Pharmacol*., **2011**, 5,118-124.
[9] J.Berdy, *J. Antibiot.*, **2005,** 58,1-26.
[10] Atta H M, MH. El-Sehrawi, NM. Awny, NI. El-Mesady, *Researcher*, **2011**, 3, 2.
[11] KS Lam, *Curr. Opin. Microbiol.*, **2006,** 9, 245–225.
[12] GM. Cragg, DGI. Kingston, DJ. Newman, Anticancer agents from natural products. Taylor & Francis, **2005**.
[13] GL. Newton, K. Arnold, MS. Price, C. Sherrill, SB. Delcardayre, Y. Aharonowitz, G. Cohen, J. Davies, RC. Fahey, C. Davis, *J. Bacteriol*., **1996**, 178, 1990-1995.
[14] E. Morou-Bermudez, RA. Bume, *Infect. Immun.*, **2000**, 68, 6670–6676.
[15] GM. Garrity, K. Arnold, MS Price, C Sherrill, SB Delcardayre, Y Aharonowitz, G Cohen, J Davies, RC. Fahey, C. Davis, *Indian J. Microbiol*.,**1993,** 12:42-47.
[16] J. Mann, *Nat. Prod. Rep.*, **2001**,18:417-430.
[17] W. Pecznska-Czoch, M. Mordarski. Actinomycetes in biotechnology. M.Goodfellow, ST. Williams, M. Mordarski, editors, London: Academic Press; **1988.** p. 219-283.
[18] V. Vimal, BM. Rajan, K. Kannabiran, *Asian J. Med. Sci.*, **2009**; 1: 57-63.

[19] ZY. Ng, S. Amsaveni S, *J. Adv. Biomed. Pathobiol.*, **2012**, 2, 96-107.
[20] K. Sourav, K. Kannabiran, *J. Nat. Env. Sci.*, **2010**,1,56-65.
[21] E. Kuster, C.Williams. *Nature*, **1964**, 202, 928-929.
[22] R. Baskaran, R. Vijayakumar, P. Mohan, *Malaysian J. Microbiol.*, **2011**, 7,26-32
[23] JH. Jorgensen, MJ. Ferraro. *Clin. Infect. Dis.*, **2007**, 49, 1749-1755.
[24] L. Karthick, K. Gaurav, KVB. Rao, *Int. J. Pharm. Pharm. Sci*., **2010**, 2, 199-203.
[25] EB. Shirling, D. Gottlieb, *Int. J. Syst.Evol. Microbiol.*, **1996**, 16, 313-340.
[26] H. Nonomura, *J. Ferment. Technol.*, **1974,** 52, 78-92.
[27] S. Das, PS. Lyla, SA. *Khan. Natl. Acad. Sci. Lett.*, **2008,** 31,149-60.
[28] A. Gniadek, AB. Macura, *Adv.Med. Sci.*, **2007**, 52,283–587.
[29] HA. Lechevalier, The Actinomycetes III, A practical guide to generic identification of actinomycetes. Bergey's Manual of Systematic Bacteriology, Williams & Wilkins Company, Baltimore **1989, 4,** p. 2344-2347.
[30] M. Thenmozhim, K. Kannabiran, *J. Nat. Env. Sci.*, **2011, ,** 2,1-8.
[31] FA. Rainey NL. Ward-Rainey, RM. Kroppenstedt, E. Stackebrandt, *Int. J. Syst. Bacteriol.*, **1996,** 46,1088-1092.
[32] TJ. Mincer, PR Jensen, CA Kauffman, W. Fenical, *Appl. Environ.*, **2002**, 68, 5005-5011.
[33] NA. Magarvey, JM. Keller, V. Bernan, M. Dowerkin, DH. Sherman, *Appl. Environ. Microbiol.*, **2004**, 70, 7520-7529.
[34] N. Saitou, M. Nei, *Mol. Biol. Evol.*, **1987**, 24,189-204.
[35] K. Kathiresan K, S. Manivannan, MA Nabeel, B. Dhivya, *Colloids Surf. B.*, **2009,** 71,133–13.
[36] R. Subramani, W. Aalbersberg. *Microbio. Res.*, **2012**, 167,571-80.
[37]. A. Goffeau, *Nature*, **2008**, 452,541-542.
[38] M. Valan, K. Asha, V. Duraipandiyan, S. Ignacimuthu, P. Agastian, *Asian Pac. J. Trop. Biome.*,.. **2012,** 2, 803-810.
[39] MV. Arasu,V. Duraipandiyan, S. Ignacimuthu, *Chemosphere*, **2013,** 90,479-487.
[40] G. Aperis, E. Mylonakis, *Expert Opin. Investig. Drugs*., **2006**,15, 579-602.
[41] S. Velayudham, K. Murugan, *J. Microbiol. Res.*, **2012,** 2, 41- 46.
[42] S. Valli, SS. Suvathi, O. Aysha, P. Nirmala, KP. Vinoth, A. Reena. *Asian Pac. J. Trop.Biomed.*, **2012,** 2,469-473.
[43] KS. Sathish, VB. Kokati. *Asian Pac. J. Trop. Biomed.*, **2012,** 2,787-792.
[44] L. Xiangqian, X. Huizhong, C. Zhe-Sheng, C. Guofang. *J. Nanomaterials*. **2011,** Article ID. 270974.
[45] VK. Prabhu, C. Sundaramoorthi, S. Devarasu, *J.Pharm. Res.*, **2011**, 4,820-22**.**
[46] S. Sadhasivam, P. Shanmugam, K.Yun, *Colloids Surf. B: Biointerfaces*, **2010**, 81:358-362.
[47] PR. Shetty, BS. Kumar, YS. Kumar, GG. Shankar, *J. Microbiol. Biotechnol.*, **2012,** 22,614-621.
[48] NF. Zonooz, M. Salouti,. *Scientia Eranica*, **2011,**18,1631-1635.
[49] A. Samundeeswari, SP. Dhas, J. Nirmala, SP. John, A. Mukherjee, N. Chandrasekaran, *Biotechnol. Appl. Biochem.*, **2012,** 59,503-507.