3 Biotech
https://doi.org/10.1007/s13205-017-1044-7

ORIGINAL ARTICLE

Profiling of red pigment produced by *Streptomyces* sp. JAR6 and its bioactivity

Jayanthi Abraham[1] · **Ritika Chauhan**[1]

Received: 16 May 2017 / Accepted: 8 December 2017

Abstract
Actinomycetes strain was isolated from leaf litter soil sample and was identified as *Streptomyces* sp. by conventional and molecular approaches. The biologically active compound responsible for antimicrobial and anticancer activity of the strain JAR6 was elucidated by solid state fermentation followed by subsequent chromatographic and spectroscopic analysis. Extraction, purification and structural confirmation of red pigment metabolite viz undecylprodigiosin were established on the basis of spectroscopic studies and comparing the data from the literature. The biologically active compound was tested against Gram-positive and Gram-negative clinical isolates and its minimum inhibitory concentration was recorded. The antimicrobial activity of undecylprodigiosin is more prominent against *Salmonella* sp., *Proteus mirabilis*, *Shigella* sp. and *Enterococcus* sp. whereas, it was less effective against *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli*. The anticancer activity of undecylprodigiosin was tested against HeLa cell lines and it exhibited commendable cytotoxicity effect with IC_{50} value of 145 µg/ml. The present investigation reveals that undecylprodigiosin produced by *Streptomyces* strain JAR6 is a potent bioactive metabolite with effective pharmaceutical properties.

Keywords Antimicrobial · Cytotoxicity · Actinomycetes

Introduction

Natural products and their derivatives are most conspicuous and successful source of new medicines (Newsman et al. 2003). They have been characterized as the single most productive source which leads to drug discovery and development (Harvey 2008). In developing countries, the infection caused by superbugs (multidrug-resistant bacterial strains) is a major problem. Cancer also is a leading cause of morbidity and mortality throughout the world (Barros et al. 2007). To overcome the infectious diseases and to combat cancer, there is an urgent need to develop new therapeutic agents. Microbes have been known for long as leading source of natural products of therapeutic agents. In the search for new pharmaceutical active agents, microbes with diverse habitats including tropical forests and various terrestrial regions are explored by various researchers to obtain potential bioactive compounds (Demain and Sanchez 2009). More than 22,500 biologically active compounds have been extracted from microbes, among them 45% have been produced by actinomycetes, 38% is contributed by fungi and 17% by unicellular bacteria (Jose and Jebakumar 2013; Smaoui et al. 2012). This indicates that actinomycetes are promising sources of new biologically active compounds.

Actinomycetes are Gram-positive, free living and saprophytic bacteria widely distributed in soil and without a doubt a potential source of many biologically active compounds (Conlon et al. 2004). They are considered to be the largest producers of bioactive metabolites with novel pharmaceutical background. There are few new antibiotics being introduced by the pharmaceutical industry in the last decade, none of them have improved the activity against multidrug-resistant bacteria (Solanki et al. 2008). Among actinomycetes, *Streptomyces* genus represents 50% of the total population of soil actinomycetes. The bioactive metabolites produced by actinomycetes infer a wide range of biological activities such as antibacterial, antifungal, antitumor, immunosuppressive and insecticidal properties, and enzyme inhibition (Locci 1989). The classification of actinomycetes genus is based on the combination

✉ Jayanthi Abraham
jayanthi.abraham@gmail.com

[1] 109A, Hexagon Microbial Biotechnology Laboratory, School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu 632014, India

Published online: 18 December 2017

of genotypic, phenotypic and taxonomic characteristics. Actinomycetes are also capable of producing dark brown colored substances in the culture medium. These colored substances have been long described as melanoid pigments and are essential and useful measure in taxonomic studies (Shirling and Gottlieb 1996). Actinomycetes produce discrete and variable shades of pigments either as intracellular or extracellular products. The pigment production by actinomycetes is influenced by pH of the culture medium, sole carbon and nitrogen sources. There is a dearth of literature supporting the extraction and chemical nature of pigments from *Streptomyces* genera. Hence, in the present investigation, actinomycetes were isolated from leaf litter soil sample and screened for pigment production. The isolated strain was screened for diffusible pigment, and optimization studies were carried out to extract the pigment from strain JAR6. Furthermore, antimicrobial and antioxidant studies were carried out to underline the biological potential of extracted pigment from the isolated strain.

Materials and methods

Actinobacteria strain and its maintenance

The actinomycete strain (*Streptomyces* sp. strain JAR6) was isolated from leaf litter samples collected from VIT University campus, Vellore, Tamil Nadu, India on starch casein nitrate medium (soluble starch 10 g^{-1}, casein 0.3 g^{-1}, NaCl 2 g^{-1}, KNO_3 2 g^{-1}, K_2HPO_4 2 g^{-1}, $MgSO_4{\cdot}7H_2O$ 0.5 g^{-1}, $CaCO_3$ 0.02 g^{-1}, $FeSO_4{\cdot}7H_2O$ 0.01 g^{-1}) at pH 7.0. The pure culture was further maintained on yeast extract-malt extract agar at 4 °C.

Taxonomic studies

The cultural characteristics of strain JAR6 were monitored and examined on the basis of observations made on International *Streptomyces* Project (ISP) media described by Shirling and Gottlieb (1996). The mature sporulating aerial and substrate mycelia of strain JAR6 was investigated on ISP1-7 medium, maltose-tryptone agar and sabouraud agar at 7, 14 and 21 days. The production of melanin pigment by the isolates was observed on peptone yeast extract iron (ISP-6) agar and trypsin (ISP-7) agar. The utilization of sole carbon and nitrogen sources by strain JAR6 was investigated according to Shirling and Gottlieb (1996) as well as Tsukamura (1966). 1.0% (v/v) of the each carbon and nitrogen sources were added to make final concentration in ISP-2 medium with 2 ml (5×10^9 spores/ml) spore suspension as inocula.

Morphological analysis

Scanning electron microscopy

The morphology of strain JAR6 was analyzed by cover-slip method for 7, 14 and 21 days then further spore surface morphology and the arrangement of the spores were determined by field emission scanning electron microscope (FE-SEM). The strain JAR6 was cultured on oatmeal agar for 14 days and fresh spores were scraped from the Petri dish. The collected spores were then washed with ethanol and stored in refrigerator at 4 °C. The spores were completely dried on the stubs and then coated with the thin film of gold in vacuum evaporator. The samples were then viewed under cold field emission electron microscope (S-4700, Hitachi, Mississauga, ON, Canada) under the magnification of 22.81 K and spore morphology were determined.

Biochemical analysis

The degradation of casein (1.0% w/v) was analyzed using the protocol described by Gordon et al. (1974) at 28 °C and examined after 3, 7 and 14 days (Covan and Steel 1974). Starch (1.0% w/v) degradation was observed after 3 days on yeast malt extract medium at 28 °C by flooding plates with iodine solution. The effect of salt on growth of isolated strains was determined in TSB media supplemented with discrete doses of sodium chloride at 28 °C and examined after 3 days. The biochemical test was analyzed according to protocols mentioned by Cappuccino and Sherman (2004). The pH (4.0–10.0) was tested on ISP-2 medium and was incubated for 7–15 days on rotary shaker at 220 rpm.

Phylogenetic characterization

The genomic DNA was extracted from actinomycetes strain JAR6 according to Hopwood et al. (1985) protocol. The PCR amplification of 16S rRNA gene of strains was carried out using forward primer of 400 ng 5′-AGAGTRTGATCMTYG CTWAC-3′ and reverse primer of 400 ng 5′-CGYTAMCTT WTTACGRCT-3′, 2.5 + mM each of dNTPs, 10× Taq polymerase assay buffer and Taq DNA polymerase enzyme (1.25 units/50 µl) keeping the reaction volume up to 100 µl. The amplification reaction was further followed by initial denaturation at 94 °C for 5 min; to improve the denaturation of the DNA 5% (v/v), DMSO was added to the reaction mixture. After denaturation, annealing at 55 °C for 30 s was carried out leading to final extension at 72 °C using $MgCl_2$ with 1.5 mM final concentration. The amplified product was sequenced with the primer using ABI 3730xl genetic analyzer (Amnion Biosciences Pvt. Ltd.). The homology search

was performed using BLAST search algorithm. The nucleotide sequence of the whole gene 16S rRNA gene has been submitted in the Gen Bank (EMBL) and accession number KC842214 was obtained. The multiple sequence alignment was performed by CLUSTAL W at European Bioinformatics Institute website. The phylogenetic tree of isolated strains was constructed using neighbor joining algorithm.

Antibiogram studies

The resistance towards antibiotics of isolated strain JAR6 was examined using gentamicin, kanamycin, tigecycline, erythromycin, ciprofloxacin, clindamycin, vancomycin, ampicillin, ofloxacin, oxacillin, methicillin, penicillin, chloramphenicol, fluconazole and streptomycin using disk diffusion method on glucose yeast extract agar medium (Lechevalier and Lechevalier 1970).

Optimization of culture medium

The production of pigment was evaluated in basal medium composed of $(NH_4)_2SO_4$ 2.64 g^{-1}; KH_2PO_4 2.38 g^{-1}; $MgSO_4 \cdot 7H_2O$ 1.00 g^{-1}; $CuSO_4 \cdot 5H_2O$ 0.0064 g^{-1}; $FeSO_4 \cdot 7H_2O$ 0.0011 g^{-1}; $MnCl_2 \cdot 4H_2O$ 0.0079 g^{-1}; $ZnSO_4 \cdot 7H_2O$ 0.0015 g^{-1} with varying range of pH (4.0–10.0). The pigment production by strain JAR6 was evaluated at various pH in comparison to 3, 6, 9, 12 and 15 days and optical density was recorded at 560 nm. Carbon sources including lactose, mannitol, starch, sucrose, dextrose and nitrogen sources including peptone, sodium nitrate ($NaNO_3$), ammonium chloride (NH_4Cl), yeast extract, beef extract, casein, soybean meal, casein peptone and meat peptone were added to basal medium at 1.0% (v/v) of concentration. The spore suspension of 5 ml (5×10^9spores/ml) was added to 50 ml basal medium supplemented with various carbon and nitrogen sources, and was incubated on rotary shaker at 28 °C for 15 days. The inorganic salt medium without carbon and nitrogen sources served as control. The biomass consisting of bioactive metabolite was recorded at 600 nm (optical density) and antimicrobial activity was evaluated against clinical pathogens after 3, 6, 9, 12 and 15 days.

Production, extraction and characterization of crude pigment

The strain JAR6 was cultivated on yeast extract-malt extract medium at 28 °C for 7–10 days. After the incubation was complete, spores from freshly grown slant were scrapped and inoculated into seed medium containing yeast extract 4 g^{-1}, malt extract 10 g^{-1}, dextrose 4 g^{-1} and tyrosine 1 g^{-1} at 220 rpm for 48 h. To extract the pigment produced by strain JAR6, solid state fermentation was used and wheat bran provided commendable pigment production. 50 g of sterilized wheat bran was supplemented with 1% (w/v) of starch and peptone as carbon and nitrogen sources, respectively. To the production medium, 10.0% (w/v) of the seed medium was added and incubated at 28 °C for 10–14 days. The mature sporulating aerial mycelium producing red color pigment was observed and extracted with ethyl acetate (100 ml) twice by solid–liquid phase extraction. The obtained extract was vacuum dried under reduced pressure and was defatted with cyclohexane.

The crude pigment obtained was purified using silica gel column which was left overnight with chloroform to settle silica. 1.5 g of solid powder (red pigment) was loaded onto the top of silica column for complete adsorption on the surface of silica gel. Elutions were carried out by increasing the ratio of hexane and decreasing ratio of ethyl acetate (10:90). All the fractions were collected, concentrated and observed on silica coated thin layer chromatographic plates for single spot. The fraction exhibiting single spot was further characterized by gas chromatography–mass spectrometry and ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra of the compound in $CDCl_3$. The dried pigment was analyzed at the flow rate of 1 ml/min with the total run time of 32 min on the column. The gas chromatogram and mass spectrometry was obtained on Perkin Elmer, GC model (Clarus 680) and mass spectrometer (Clarus 600 EI).

Biological assays

Antimicrobial activity

The Gram-negative bacteria including *Escherichia coli*, *Shigella* sp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella* sp. and Gram-positive bacteria *Staphylococcus aureus, Enterococcus* sp. were acquired from Thanjavur Medical College, Thanjavur, Tamil Nadu India. These clinical isolates were further maintained in Microbial Biotechnology Lab, SBST, VIT University, Vellore, India. The test organisms were grown overnight in nutrient broth medium at 30 °C for 24 h and 100 µl of the culture was seeded onto Mueller Hinton agar plates. The sensitivity of microorganisms to the extracted pigment was tested by measuring the zone of inhibition by well diffusion method. Four different wells (4 mm in diameter) were punctured onto the agar plate and 50 mg/ml of dried ethyl acetate extract (red pigment) with different concentrations (25, 50, 75 and 100 µl) were loaded into the wells (Chauhan and Abraham 2013). The Petri plates were incubated for 24 h, and the zone of inhibition was measured around the wells. The minimum inhibitory concentration (MIC) against bacterial pathogens was determined using protocol described by Boruwa et al. (2004).

DPPH radical scavenging activity

The free radical scavenging activity of extracted pigment was analyzed by 2,2-diphenyl-1 picrylhydrazyl (DPPH). 50 mg/ml of the ethyl acetate extracted pigment (0.3 ml) was mixed with 2.7 ml of methanol solution containing DPPH radicals of 0.05 mM (Hatano et al. 1988). The reaction was shaken vigorously and allowed to stand for 30 min in dark. The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation $\%\ \mathrm{RSA} = [(A_{\mathrm{DPPH}} - A_{\mathrm{S}})/A_{\mathrm{DPPH}}]/100$, where A_{S} is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution (Barros et al. 2007).

Anticancer activity

The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37 °C, 5% CO_2, 95% air and 100% relative humidity. The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1×10^5 cells/ml. 100 µl per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to facilitate cell attachment at 37 °C, 5% CO_2, 95% air and 100% relative humidity (Mosmann 1983).

After 48 h of incubation, 15 µl of MTT (5 mg/ml) in phosphate-buffered saline (PBS) was added to each well and incubated at 37 °C for 4 h. The medium with MTT was separated and the formazan formed crystals were solubilized in 100 µl of DMSO and then the absorbance was measured at 570 nm using microplate reader. The percentage cell inhibition was determined using the following formula: % cell inhibition = 100 − Abs (sample)/Abs (control) × 100 (Monks et al. 1991). Nonlinear regression graph was plotted between % cell inhibition and log concentration and IC_{50} was determined using GraphPad Prism software.

Results and discussion

The complete sequence of strain JAR6 was determined in this study and has been deposited in GenBank database with accession number KC842214. The aligned sequence of strain JAR6 was subjected to similarity searches against public databases to infer possible phylogenetic relationships of strain JAR6. The 16S rRNA gene sequence of the strain JAR6 was compared and aligned with those sequences retrieved from NCBI Genbank database using BLAST analysis (Yellamanda et al. 2016). The phylogenetic tree (Fig. 1) from representative strains of the related species indicated that strain JAR6 should be placed in genus as *Streptomyces*. The 16S ribosomal RNA encoding gene analysis of isolated strain attested that strain was 99% related to *Streptomyces pilosus* and is depicted in Fig. 1. The morphological observations were recorded on the basis of International *Streptomyces* Project and revealed the abundant growth of aerial and vegetative mycelium on starch casein nitrate medium, yeast extract-malt extract medium (ISP-2) and oatmeal agar (ISP-3). The strain JAR6 produced red color as reverse pigment on starch casein nitrate medium as well on ISP-2.

The strain JAR6 showed an abundant growth on carbon sources including starch, inositol, lactose, dextrose, mannitol, maltose and fructose. The strain JAR6 was able to grow in the presence of 5% NaCl and tolerated up to 10% sodium chloride for its abundant growth. In addition, *Streptomyces* strain JAR6 reduced sulfur to sulfide, degraded starch and hydrolyzed lipase, however, it was not capable to degrade cellulose and gelatin. The strain JAR6 was found to be sensitive to various commercial antibiotics including gentamicin (18.5 ± 0.244), kanamycin (31.33 ± 0.249), erythromycin (11.33 ± 0.124), vancomycin (10.46 ± 0.88), ampicillin (11.53 ± 0.329), ofloxacin (21.5 ± 0.21), and streptomycin (17.43 ± 0.124), but was resistant to tigecycline, clindamycin, ampicillin, oxacillin, methicillin, penicillin, chloramphenicol and fluconazole. The antibiotic sensitivity test suggests that biological active compounds produced by strain JAR6 may be responsible for the resistance of the strain to these antibiotics (Kavitha et al. 2010). The strain JAR6 depicted spore chain morphology as represented in Fig. 2. The pigment production was found to be highest at 7.0 pH at

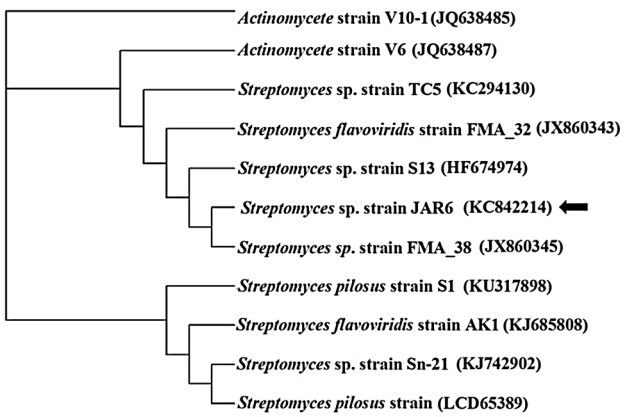

Fig. 1 Phylogenetic tree depicting *Streptomyces* sp. strain JAR6 (arrow) with its close phylogenetic neighbors. The tree was drawn using the neighbor joining method (MEGA7-Molecular Evolutionary Genetic Analysis). The accession numbers are shown in brackets

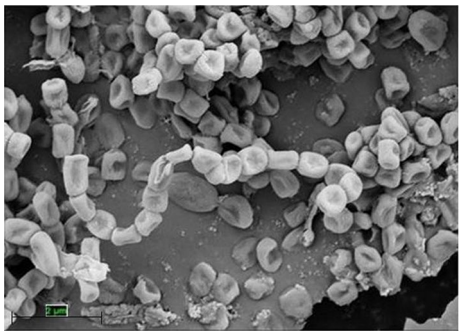

Fig. 2 Spore chain surface morphology of strain JAR6 by FE-SEM

9th day with starch and soybean meal as carbon and nitrogen sources, respectively, for JAR6 strain.

The analytical characterization of purified metabolite produced by strain JAR6 exhibited the molecular weight of compound as 389.95 which are closely similar to undecylprodigiosin (mol. wt. 392.2694). The fragmentation pattern of red color pigment has been presented in Fig. 3. Furthermore, the complete characterization was obtained by ^{1}H NMR [(D6) $CDCl_3$, 200 MHz]: $\delta = 7.50$ (br. s 1H), 6.68 (s. C=C gem 1H), 6.35 (s. heterocyclic 1H), 6.05 (s. heterocyclic 1H), 5.39 (s. C=C trans 1H), 5.37 (s. C=C trans 1H), 5.30 (s. C=C cis 1H), 5.12 (br. s 2H –NH), 3.80 (s. 3H $–OCH_3$), 2.48 (t. 2H CH_2), 1.31 (t. 16H $8CH_2$), 0.88 (s. 3H CH_3) and ^{13}C NMR [(D6) $CDCl_3$, 75.5 MHz]: $\delta = 163.7$ (C=C, imine pyrrole), 159.3 (C=C, ethylene), 142.3 (C=C, ethylene), 141.0 (C, bipyrrole), 135.4 (CH=CH, ethylene), 131.5 (C=C, attached to –NH), 126.5 (C=C, 2-pyrrole), 119.2 (CH, ethylene), 114.6 (C, N–**C**=C), 112.0 (CH, 2-pyrrole), 107.7 (CH, ethylene, 2-pyrrole), 94.6 (CH, C=**C**–H), 91.0 (CH, C=**C**–H, bipyrrole), 59.6 (CH_3, $–OCH_3$), 29.6 (8 C, chain CH_2), 22.7 (CH_2, aliphatic), 14.1 (CH_3, aliphatic). The red pigment produced by strain JAR6 manifested good antimicrobial activity against all test pathogens except *S. aureus*, *P. aeruginosa* and *K. pneumoniae*. The highest antimicrobial effect was manifested against *Salmonella* sp., *Proteus mirabilis*, *Shigella* sp. and *Enterococcus* sp., whereas the lowest zone of inhibition was manifested against *E. coli* and *Bacillus subtilis* as listed in Table 1. The minimum inhibitory concentration was determined through cfu/ml method and MIC values have been detailed in Table 2.

The free radical scavenging activity was investigated through DPPH radical and was compared with ascorbic acid. The radical scavenging activity (%) of extracted pigment was effective as it showed the color change from

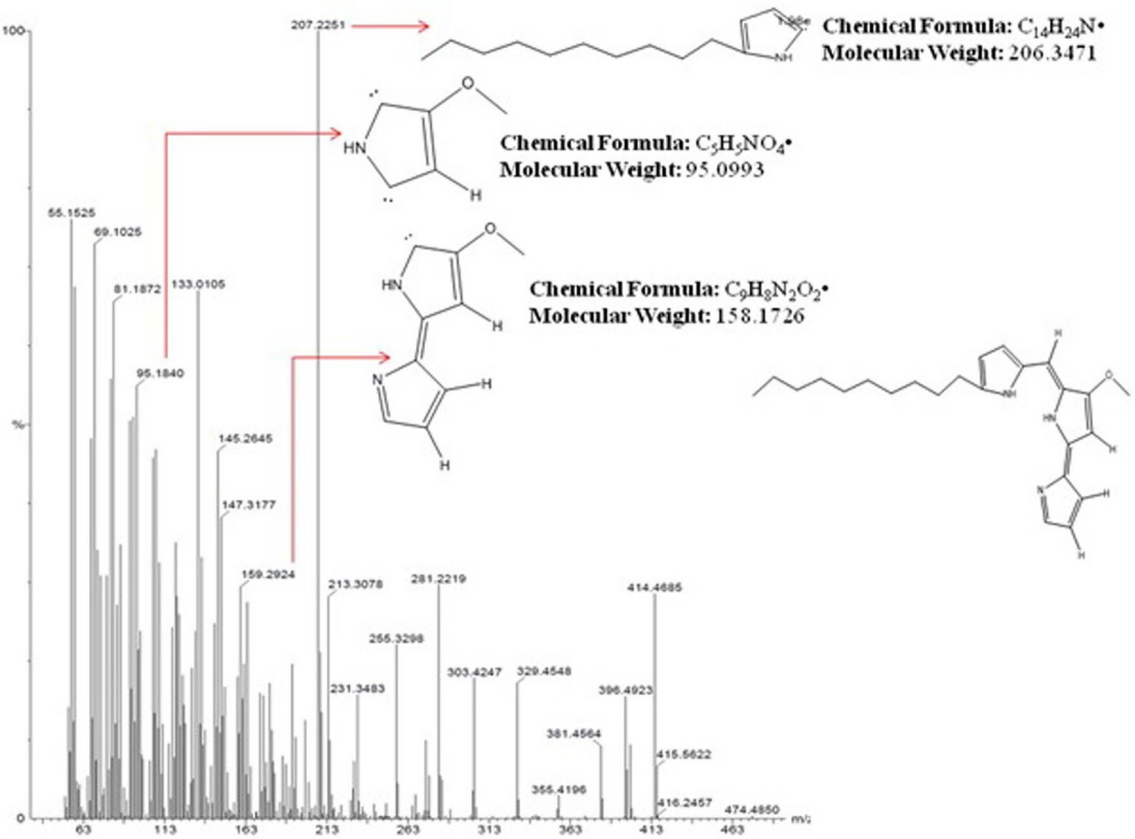

Fig. 3 GC-MS fragmentation pattern of red pigment produced by strain JAR6

Table 1 Antimicrobial activity of red pigment produced by *Streptomyces* sp. strain JAR6 against various pathogens

S. no.	Test organisms	Diameter zone of inhibition (mm)			
		25 µl	50 µl	75 µl	100 µl
1	*Staphylococcus aureus*	–	–	–	–
2	*Shigella* sp.	17.5 ± 0.294	17.3 ± 0.294	20.46 ± 0.262	22.03 ± 0.249
3	*Pseudomonas aeruginosa*	–	–	–	–
4	*Enterococcus* sp.	16.53 ± 0.205	17.3 ± 0.294	20.4 ± 0.244	22.03 ± 0.294
5	*Escherichia coli*	15.43 ± 0.2054	16.4 ± 0.374	18.56 ± 0.368	20.6 ± 0.244
6	*Bacillus subtilis*	16.23 ± 0.205	17.48 ± 0.302	18.36 ± 0.205	20.48 ± 0.139
7	*Proteus mirabilis*	27.46 ± 0.249	27.063 ± 0.129	28.53 ± 0.262	29.55 ± 0.168
8	*Klebsiella pneumoniae*	–	–	–	–
9	*Salmonella* sp.	27.5 ± 0.294	28.43 ± 0.169	28.13 ± 0.49	29.53 ± 0.286

– no inhibition zone

Table 2 Minimum inhibitory concentration of red pigment produced by strain JAR6

S. no.	Pathogenic microorganisms	MIC (µg/ml) Strain JAR6
1	*Salmonella* sp.	150
2	*Bacillus subtilis*	50
3	*Proteus mirabilis*	80
4	*Shigella* sp.	100
5	*Escherichia coli*	170
6	*Enterococcus* sp.	120
7	*Klebsiella pneumoniae*	180

Table 3 Percentage cell inhibition of HeLa cells using red pigment produced by strain JAR6

S. no.	Concentration (µg/ml)	Cell inhibition (%)
1	18.75	4.310377 ± 0.01687
2	37.5	10.717010 ± 0.0090
3	75	24.48653 ± 0.4516
4	150	43.20844 ± 0.003
5	300	90.34155 ± 0.030

purple to yellow color solution after 30 min of reaction. The strain JAR6 producing red color pigment which exhibited good antioxidant activity with 5.11% of discoloration. The strain JAR6 exhibited effective cytotoxic effect at various concentrations and IC_{50} was calculated to be 145 µg/ml. The cervical cell inhibition at various concentrations has been presented in Table 3, and Fig. 4 shows the graph with decline in cell number. Figure 5a represents the microscopic view of control of HeLa cells without any compound inoculation, whereas (b)–(d) shows the inhibition of cervical cancer cells with various concentrations 75, 150 and 300 µg/ml, respectively, of red pigment isolated from strain JAR6. Maximum of 90% cell death was observed at 30 µg/ml, whereas lower concentrations of red pigment also exhibited the cell inhibition.

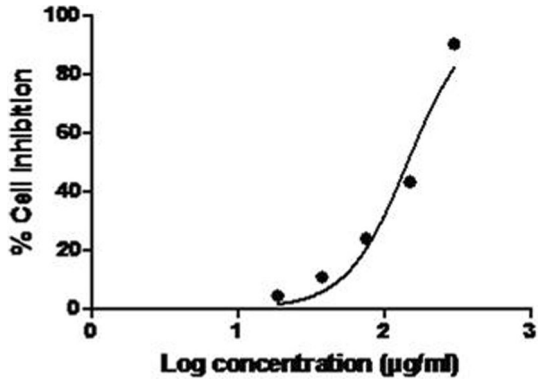

Fig. 4 Cell inhibition of HeLa cells through MTT assay by strain JAR6

With increasing demand of new natural products there is a growing interest for microbial pigments as an alternative to synthetic colorants for food, cosmetics dyes, and drugs (Dufosse 2009). *Streptomyces echinoruber* produces red pigment, rubrolone, which is already in use as natural food colorant (Gupta et al. 2004). Other natural colorants such as anthraquinone and prodigiosin have antimicrobial properties, which makes them an excellent target for the dual or multifunctional applications (Alihosseini et al. 2008). The chromogenecity of *Streptomyces* species and various color groups have become a valuable index for taxonomic diversity among *Streptomyces* as it has been well documented by Arai and Mikami (1972).

In our study, leaf litter soil samples served as a great source of pigment producing actinomycetes with effective antimicrobial potential against pathogenic microorganisms. The phylogenetic analysis through 16S rRNA gene sequencing showed 99% similarity to *Streptomyces* sp. (JAR6) confirming the strain belong to *Streptomyces* genera. The isolated strain exhibited abundant growth on ISP-medium and starch casein nitrate agar with smooth surface, suggesting that species with smooth surface fall under gray color

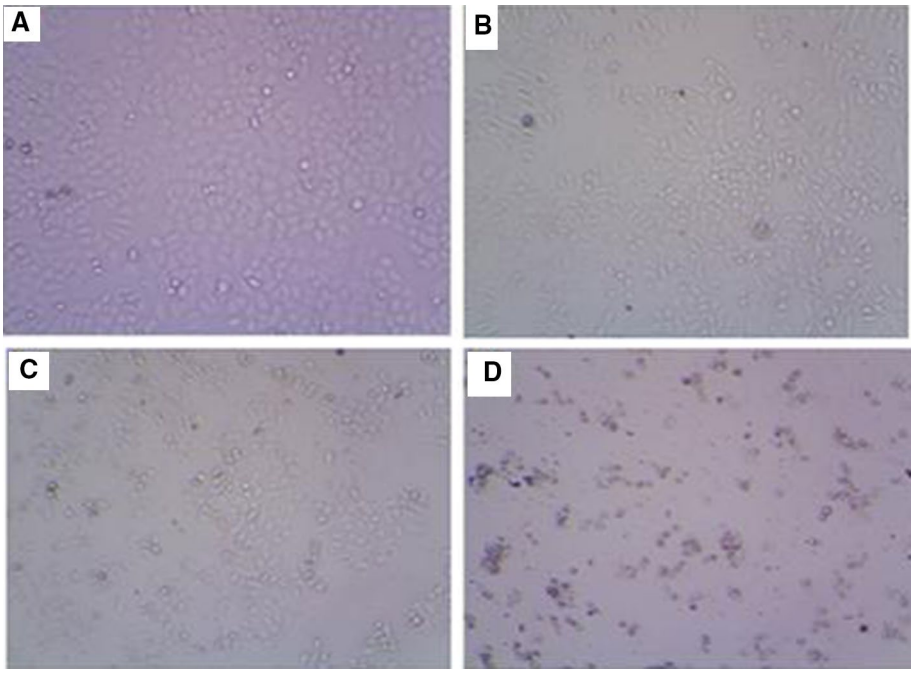

Fig. 5 Microscopic views of morphological alterations with HeLa cells. **a** Morphological characteristics of HeLa cells control without any compound. **b** Morphological alterations in HeLa cells treated with 75 µg/ml of red pigment extracted from strain JAR6. **c** Morphological alterations in HeLa cells treated with 150 µg/ml of red pigment extracted from strain JAR6. **d** Morphological alterations in HeLa cells treated with 300 µg/ml of red pigment extracted from strain JAR6

series. The strain JAR6 represented wide range of carbon assimilation and biochemical characterization revealed that it hydrolyzes starch, produces hydrogen sulfate, and exhibits lipolytic activity. According to Williams et al. (1983), biochemical identification and other test is an imperative tool for the classification of actinobacteria.

The pigment production through solid state fermentation by isolated strains was substantially affected by pH, carbon and nitrogen sources. The type, concentration of carbon and nitrogen sources and mineral composition of the culture medium had an impact on the antibiotic synthesis in *Streptomycetes* (Vijayabharathi et al. 2011). The utilization of starch, dextrose, soybean meal and peptone by the strain JAR6 for the production of bioactive metabolites suggests that these substrates act as active uptake system for other *Streptomyces* sp. which has been previously reported by Dharamraj (2009). It has also been suggested that during the complex life span, the soil-dwelling bacterial genus *Streptomyces* is challenged with diverse nutritional and environmental stresses, so the pigment production may provide competitive advantage in the environment (Chater 2001; Staric et al. 2010).

The bioactive compound produced by strain JAR6 belongs to the family of prodiginines which are designated as red, linear, tripyrrole antibiotics. The antibiotic properties of undecylprodigiosin (prodigiosin based compound) have been recognized as potential antibacterial colorant (Chater 2001; Staric et al. 2010). The strain JAR6 manifested good inhibitory activity against Gram-negative isolates; however, the inhibition was not significant against *P. aeruginosa*, *K. pneumoniae* and Gram-positive bacteria.

The antioxidant properties of undecylprodigiosin from *Streptomyces* have not been studied before, and strain JAR6 was able to reduce compounds to pale yellow hydrazine as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical which is a long-lived organic nitrogen radical with a deep purple color. The reference antioxidant used for the assay was vitamin C. In this reducing assay, the purple chromogenic radical is reduced by antioxidant or reducing compounds to pale yellow hydrazine. The free radicals produced in this reaction determine the number of DPPH molecules reduced which can be monitored by decolorization. Therefore, the reduction of free radicals could be monitored by absorbance at 517 nm and free radical scavenging activity can be determined by the physical examination, representing discoloration of the DPPH solution (Saadoun and Gharaibeh 2003).

The cytotoxicity assay of undecylprodigiosin produced by strain JAR6 exhibited effective in vitro cytotoxic activity against HeLa cell lines in dose-dependent manner and the recorded IC_{50} value was found to be 145 µg/ml. After MTT assay, it was found that 300 µg/ml of the red pigment could greatly inhibit the HeLa cell and 18.75 µg/ml of extracted pigment could inhibit the minimum of cervical cancer cells. The IC_{50} of the biological active red pigment was calculated by statistical analysis and was found to be 145 µg/ml. Similar findings have been reported from *Streptomyces* sp. CNQ766 having cytotoxicity against Mouse splenocyte T cells and macrophages with the IC_{50} value of 20 µg/ml. In other reports, actinomycetes isolated from marine sediment sources have shown anticancer property against MCF-7 and MDA-MB-231 cell lines with the IC_{50} value range from 10.13 to 18.54 (Ravikumar et al. 2012). Recently, a study

on red pigment extracted from coral reef has also exhibited good cytotoxicity effect against various cell lines (Karuppiah et al. 2013). With the discovery of actinomycin, actinomycetes have been widely studied for biological active natural pigments.

Other bacteria such as *Serratia marcescens* strains, *Vibrio* sp. DSM 14379 (Staric et al. 2010) are good producers of prodigiosins as natural colorants (Giri et al. 2004; Wei and Chen 2005) but their growth is inhibited by temperature variation. *Streptomyces* sp. JAR6 is good producer of undecylprodigiosin in solid state fermentation and it has proven to be effective antimicrobial, antioxidant and cytotoxic agent. As actinomycetes is the largest genera which produces biological active compounds, there is lot of research going on with bioactive compounds from marine sources but still terrestrial samples are the hub for new actinomycetes potent strains.

Conclusions

The present investigation reveals that the strain JAR6 isolated from leaf litter produced a biopigment which can be efficiently utilized for therapeutic applications. From the above study, it can be concluded that undecylprodigiosin is an effective antimicrobial and anticancer agent, and this biopigment has various biological and industrial applications.

Compliance with ethical standards

Conflict of interest No conflicts of interest to declare.

References

Alihosseini F, Ju KS, Lango J, Hammock BD (2008) Antibacterial colorants: characterization of prodiginines and their applications on textile materials. Biotechnol Prog 24:742–747

Arai T, Mikami Y (1972) Chromogenicity of *Streptomyces*. Appl Microbiol 23:402–406

Barros L, Ferreira MJ, Queiro B, Ferreria CFR, Baptista P (2007) Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem 103:413–419

Boruwa J, Kalita K, Barua NC, Borah JC, Mazumdar S, Thakur D, Gogoi DK, Bora TC (2004) Synthesis, absolute stereochemistry and molecular design of the new antifungal and antibacterial antibiotic produced by *Streptomyces* sp. 201. Bioorg Med Chem Lett 14:3571–3574

Cappuccino JG, Sherman N (2004) Microbiology-laboratory manual. Pearson Education Inc., New Delhi

Chater K (2001) Regulation of sporulation in *Streptomyces coelicolor* A3(2): a checkpoint multiplex? Curr Opin Microbiol 4:667–673

Chauhan R, Abraham J (2013) In vitro antimicrobial potential of the lichen *Parmotrema* sp. extracts against various pathogens. Iran J Basic Med Sci 16:882–885

Conlon JM, Kolodziejekb J, Nowotny N (2004) Antimicrobial peptides from ranid frogs: taxonomic and phylogenetic markers and a potential source of new therapeutic agents. Biochim Biophys Acta 1696:1–14

Covan ST, Steel KJ (1974) Manual for identification of medical bacteria. Cambridge University Press, Cambridge

Demain AL, Sanchez S (2009) Microbial drug discovery: 80 years of progress. J Antibiot 62:5–16

Dharamraj S (2009) Study of L-asparaginase production by *Streptomyces noursei* MTCC 10469, isolated from marine sponge *Callyspongia diffusa*. Iran J Biotechnol 9:2

Dufosse L (2009) Pigments, Microbial. In: Schaechter M (ed) Encyclopedia of microbiology, 3rd edn. Academic, Oxford, pp 457–471

Giri A, Anandkumar N, Muthukumaran G, Pennathur G (2004) A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia marcescens* isolated from soil. BMC Microbiol 4:1–10

Gordon RE, Barnett DA, Handarhan JE, Hor-Nay Pang C (1974) *Nocardia coeliaca*, *Nocardia autotrophica* and the nocardin strains. Int J Syst Evol Microbiol 24:54–63

Gupta D, Khareb SK, Laha A (2004) Antimicrobial properties of natural dyes against Gram-negative bacteria. Color Technol 120:167–171

Harvey AL (2008) Natural products in drug discovery. Drug Discov Today 13:19–20

Hatano T, Kagawa H, Yasuhara T, Okuda T (1988) Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem Pharm Bull 36:2090–2097

Hopwood DA, Bibb MJ, Chater KF, Kjester T, Bruton CJ (1985) Genetic manipulation of *Streptomyces*: a laboratory manual. John Innes Foundation, Norwich

Jose PA, Jebakumar SRD (2013) Non-streptomycete actinomycetes nourish the current microbial antibiotic drug discovery. Front Microbiol 4:240

Karuppiah V, Aarthi C, Sivakumar K (2013) Statistical optimization and anticancer activity of red pigment isolated from *Streptomyces* sp. PM4. Asian Pac J Trop Biomed 8:650–656

Kavitha A, Prabhakar P, Vijayalakshmi M, Venkatshwarlu Y (2010) Purification and biological evaluation of the metabolites produced by *Streptomyces* sp. TK-VL_333. Res Microbiol 161:335–345

Lechevalier MP, Lechevalier HA (1970) Composition of whole-cell hydrolysates as a criterion in the classification of aerobic actinomycetes. In: Prauser H (ed) The Actinomycetales. G. Fisher Verlag, Jena, pp 311–316

Locci R (1989) *Streptomycetes* and related genera. In: Williams ST, Sharpe ME, Holt JG (eds) Bergey's manual of systematic bacteriology. Williams and Wilkins, London, pp 2451–2493

Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-wolff A (1991) Feasibility of high flux anticancer drug screen using a diverse panel of cultured human tumour cell lines. J Natl Cancer Inst 83:757–766

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Newsman DJ, Cragg GM, Snader KM (2003) Natural product as sources of new drugs over the period 1981–2002. J Nat Prod 66:1022–1037

Ravikumar S, Fredimoses M, Gnanadesigan M (2012) Anticancer property of sediment actinomycetes against MCF-7 and MDA-MB-231 cell lines. Asian Pac J Trop Biomed 2:92–96

Saadoun I, Gharaibeh R (2003) The *Streptomyces* flora of Badia region of Jordan and its potential as a source of antibiotics active against antibiotic-resistant bacteria. J Arid Environ 53:365–371

Shirling EB, Gottlieb D (1996) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:313–340

Smaoui S, Mathieu F, Elleuch L, Coppel Y, Merlina G, Karray-Rebai I, Mellouli L (2012) Taxonomy, purification and chemical characterization of four bioactive compounds from new *Streptomyces* sp. TN256 strain. World J Microbiol Biotechnol 28:793–804

Solanki R, Khanna M, Lal R (2008) Bioactive compounds from marine actinomycetes. Indian J Microbiol 48:410–431

Staric N, Danevcic T, Stopar D (2010) *Vibrio* sp. DSM 14379 pigment production-a competitive advantage in the environment. Micro Ecol 60:592–598

Tsukamura M (1966) Adansonian classification of mycobacteria. J Gen Microbiol 45:253–273

Vijayabharathi R, Bruheim P, Andreassen T, Raja DS, Devi PB, Satyabhama S, Priyadarshini VB (2011) Assessment of resistomycin, as an anticancer compound isolated and characterized from *Streptomyces aurantiacus* AAA5. J Microbiol 49:920–926

Wei YH, Chen WC (2005) Enhanced production of prodigiosin-like pigment from *Serratia marcescens* SMDR by medium improvement and oil-supplementation strategies. J Biosci Bioeng 99:616–622

Williams ST, Goodfellow M, Alderson G, Wellington EM, Sneath PH, Sackin MJ (1983) Numerical classification of *Streptomyces* and related genera. J Gen Microbiol 129:1743–1813

Yellamanda B, Vijayalakshmi M, Kavitha A, Reddy DK, Venkateswarlu Y (2016) Extarction and bioactive profile of compound produced by *Rhodococcus* sp. VLD-10. 3 Biotech 6:261