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Biopotential of Endophytic *Streptomyces* sp. AZRA 34 for Recovery of Enzymes and Antimicrobial Compounds Isolated from the Root of *Azadirachta indica*

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ABSTRACT

Actinomycetes are a prolific producer of biotechnologically important compounds of various interests. In recent past, researchers showed the interest to isolate actinomycetes from medicinal plants and have assessed their potential use for human welfare. In this study, a *Streptomyces* sp. AZRA 34 has been isolated from the root of *Azadirachta indica* and was screened for extracellular production of amylase, cellulase, pectinase and chitinase. Further, these enzymes were quantified in solid-state fermentation and submerged media. Compared to submerged medium, the production of all enzymes in solid-state fermentation was approximately double. Ethyl acetate extracted crude metabolites of isolate (AZRA 34) was also evaluated for antibacterial and antioxidant activities. Out of five human pathogenic bacteria selected for antibacterial activity, three, *Staphylococcus aureus* ATCC 25923, *Salmonella Typhi* MTCC 3216, and *Aeromonas hydrophila* IMS/GN11 were found susceptible to crude metabolites while *Shigella flexneri* ATCC 12022 and *Enterococcus faecalis* IMS/GN7 were resistant. The crude extract was separated by column chromatography and thin-layer chromatography, and each purified fraction was screened for the antibacterial activity against all three susceptible bacterial species. Two fractions showed positive antibacterial activity against all tested bacteria. GC-MS analyses of these two fractions confirmed them as 2, 4-diphenyl-2, 3-dihydro-1, 5-benzothiazepine, and 17-(1, 5-dimethylhexyl)-10, 13-dimethyl-3 styryl hexadecahydrocyclopenta [a] phenanthren-2-one. Effective concentration (EC50) for antioxidant activity of the crude metabolite was recorded as 77.61 µg/ml.

Keywords: Endophytes, *Streptomyces*, Antibacterial activity, Bioactive compounds

INTRODUCTION

Ever since the evolution of plants and humans, both of them have faced a constant interaction with a blend of a large number of beneficial and deleterious microbes. These microbes play a crucial role in the survival of human and plant on the Earth by supporting their growth and health. Plant tissues are a specific niche for different types of microbes which has been less explored. Some microbes grow on the exterior of the plant surface while some live inside the plant tissues as endosymbiont called endophytic microbes. Endophytic microbes include bacteria, actinobacteria and fungi which rest inside the plant tissues without any discernible disease symptoms. Endophytes play a salient role in nurturing the host fitness by furnishing resistance to biotic and abiotic stresses, enhancing resistance to insects and pests, producing phytohormones and other bioactive compounds (Joseph and Priya, 2011; Parthasarathi *et al*., 2012; Verma *et al.*, 2014; Kharwar, 2024). Endophytes are well known producer of large number of bioactive metabolites like antibacterial, antifungal, anticancer and antioxidants. These microbial endophytes represent a lesser-investigated stockpile for unique species of budding interest in the quest of novel compounds for utilization in agriculture, pharmaceutical, and other industries (Qin *et al*., 2011; Rao *et al*., 2015). Among 60000-80000 of reported microbial metabolites, 47 % exhibit at least one kind of biological activity, a ratio far higher in comparison to plant (7%) and animal (3 %) derived compounds (Berdy, 2012). Among all microbial group, more than two-third of bioactive metabolites available for therapeutic use, are produced by actinomycetes (Berdy, 2005; Dasí-Delgado *et al.,* 2025; Wongso *et al*., 2023). Bioactive secondary metabolites that have been reported from endophytic actinomycetes belong to different classes of compounds like non-ribosomal peptides (Ezra *et al*., 2004), macrolides (Zhao *et al*., 2004), alkaloids (Igarashi *et al*., 2004) etc. The species of genus *Streptomyces* represent over 80 % of known actinobacteria compounds and are well recognized for their ability to secrete a different class of metabolites having antibiotic

potential. Two most studied *Streptomyces* spp. are *S. griseus* utilized for industrial production of an antibiotic (streptomycin) and *S. coelicolor* used in genetic studies (Procópio *et al*., 2012). Various biotechnologically and industrially important enzymes have been reported from several actinomycetes which is an important step towards revolutionizing green technology that can reduce the cost of enzymes production compared to other sources (Nawani *et al*., 2013). Actinomycetes are also known to produce antioxidant molecules. Antioxidants are contemporary needs which play a decisive role in scavenging and restricting free radicals that ultimately protect against various degenerative and infectious diseases to human beings (Sharma and Gupta, 2008). In a study, Verma *et al.* (2011) isolated 29 filamentous fungal taxa from *Azadirachta indica* (Neem plant), but the present work exploits the potential of actinobacteria from neem. Recently, Kumar *et al.* (2016) have reported activation of antimicrobial property of endophytic *Streptomyces coelicolor* using the epigenetic modulators and have identified a protein responsible in escalating the antimicrobial properties belonging to porin family. We hypothesize that endophytic actinobacteria from *A. indica* may produce industrially important enzymes and bioactive compounds. We screened and selected the most active endophytic actinobacterium *Streptomyces* sp. AZRA 34 isolate, associated with the root of *A. indica*, for further assessment of its potential for enzymes production, antioxidants and antibacterial activities. Further the metabolites were purified through column and thin layer chromatography and most active fractions of the metabolites were identified through GC-MS.

MATERIALS AND METHODS

Isolation of endophytic actinomycetes

For endophytic actinomycetes isolation, healthy root tissues of neem were collected from Botanical garden of Banaras Hindu University, Varanasi, Uttar Pradesh, India (25°16'03.2"N 82°59'25.3"E). Root samples were washed in running tap water to eliminate soil particles and other debris. For surface sterilization, approximately 05 g of air-dried root were dipped in 70 % ethanol for 1 min and thereafter transferred to the solution of 4% sodium hypochlorite for 2 min, and again kept in 70% ethanol for 10 seconds. Subsequently, the samples were washed thrice in sterile distilled water. Tissue samples were crushed with 10 ml sterilized distilled water using mortar and pestle. About 0.2 ml of crushed sample spread over SCA plates supplemented with 150 µg/ml nystatin to arrest the mycelial growth. Plates were incubated at 28 ± 2°C for 21 days. After the emergence colonies, they were transferred to fresh SCA plates to procure pure culture.

Identification of endophytic actinomycetes

The isolated culture was identified by the molecular method using 16S rDNA sequencing. Briefly, the isolation of total genomic DNA was done utilizing endophytic actinomycete following the method described by Kumar *et al*. (2016) and Neumann *et al*., (1992). Approximately, 100 ng of pure genomic DNA was taken for amplification of 16S rDNA gene using a thermal cycler (Mycycler, BioRad). The almost entire gene was amplified by using the forward primer PA (AGA GTT TGA TCC TGG CTC AG) and the reverse primer PH (AAG GAG GTG ATC CAG CCG CA), were used. A final volume of 50 µl was prepared by mixing PCR buffer (10X) 5 µl, 0.66 µl of (3u/µl) Taq polymerase (Genei, India), 2 µl of each primer, 1 µl dNTPs (Genei, India), 2 µl of template DNA and 37.34 µl MQ water for each amplification reaction mixture. PCR amplified DNA was purified by HiYield PCR DNA mini kit from Real Biotech Corporation (RBC, India) through gel excision method. Purified DNA was sequenced at Centre for Genetic Disorder, Institute of Life Science, Banaras Hindu University, Varanasi. The obtained 16S rDNA sequence was compared with the NCBI databases using the nBLAST search program. The 16S rDNA sequence comparisons were made with their closest neighbours found in NCBI.

Extracellular enzyme assay

Enzymatic activity including amylase, cellulase, pectinase, and chitinase were performed for purified isolate *Streptomyces* sp. AZRA 34. The cellulolytic activity was observed by Reese's mineral media containing cellulose (1% w/v) as a carbon source (Rautela *et al*., 1966). The amylase and pectinase activities were observed on Reese's mineral media containing 1% (w/v) starch and pectin as a carbon source for respective enzymes. The chitinase activity was observed on Reese's mineral media containing 1% (w/v) colloidal chitin as a carbon source.

Quantification of enzymes produced in submerged (SmF) and solid-state fermentation (SSF)

For the quantification of enzymes produced in submerged fermentation, Basal salt media (broth) amended with 1% substrate was autoclaved and inoculated with culture and incubated at 37° C in the shaking condition. One ml of spent medium (crude enzyme extract) was drawn aseptically after every 24 hours for quantification of enzymes. The activity of enzymes was determined by the dinitrosalicylic acid (DNS) method (Miller, 1959). Two ml reaction mixture contained 1 ml of substrate for respective enzymes in phosphate buffer saline (pH 7.4), and 1 ml crude enzyme extract. The well vortexed mixture was incubated in a water bath shaker at 37° C for 30 min. The reaction was arrested by the addition of 2 ml DNS reagent and 6 ml distilled water followed by heating at 100° C for 10 min. The coloured solution was centrifuged at 10,000 rotations per min (rpm) for 10 min, and the absorption of the test sample was measured at 540 nm using UV-Vis spectrophotometer. For the quantification of enzyme produced in solid-state fermentation, 5g of wheat bran was taken and washed thrice with distilled water. One gram of each carbon source was amended with washed wheat bran and autoclaved. Culture was inoculated on it and kept at 28 ± 2°C for enzyme production. Enzyme quantification was done by previously described DNS methods.

Extraction of secondary metabolites and screening of antibacterial activity

For the extraction of crude compounds, the endophytic *Streptomyces* sp. AZRA 34 isolate was grown in starch casein potassium nitrate broth (SCB) and incubated at 37 ± 2°C in BOD incubator (NSW Calton, India) for 21 days. After the completion of the incubation period, the broth was centrifuged for 15 min at 10,000 rpm and bioactive compounds were extracted thrice by mixing supernatant with the same amount of ethyl acetate. The extracts were combined and evaporated to dryness in vacuum by rotary evaporator (Ika, Germany). Concentrated crude compounds were collected in a fresh tube and kept in a fume hood for complete drying. Collected crude compounds were weighed and dissolved in methanol to the final concentration of 100 µg/µl. Antibacterial activity of crude compounds was evaluated by the method described by Kumar *et al*., (2016) and Bauer *et al*., (1966). Each sterile paper disc was impregnated with 10 µl methanolic extract and kept at room temperature to complete dryness. The antibacterial activity was tested against selected human pathogenic bacteria (*Salmonella typhi* MTCC 3216, *Enterococcus faecalis* IMS/GN7, *Staphylococcus aureus* ATCC 25923, *Aeromonas hydrophila* IMS/GN11 and *Shigella flexneri* ATCC 12022). The bacterial lawn of the test pathogen was prepared on the Mueller- Hinton agar (MHA) plates using a sterile cotton swab. The paper discs loaded with 1 mg crude compounds were placed over the bacterial lawn made on the MHA plates. Experimental control was also included, which was only a paper disc impregnated with pure methanol. All these plates were incubated at 37° C for 24 hr. The inhibition zones developed around each paper disc were measured. Each test was done in three replicates.

Antioxidant assay by DPPH scavenging activity

The antioxidant test was done by the method given by Blois (1958) with minor changes. Microtitre plates were used for screening of antioxidant activity. In brief, 10 µl of 100 µg/µl DMSO solution of the compound was mixed with 290 µl of 0.2 mM DPPH in a microtiter well and kept in dark for half an hour. A mixture of DPPH+DMSO was also set simultaneously as a negative control. The results were noted based on the colour change of DPPH from purple (free radical form) to yellow (reduced form).

For quantitative estimation of antioxidant activity, a series of different concentrations of compound dissolved in DMSO. Aliquots of 100 µl of the three-fold test dilutions were taken into the plastic tubes and mixed with 2900 µl of 0.2 mM methanolic DPPH solution. A negative control of DPPH and DMSO mixture (2900µl + 100µl) was also put in place. Tubes were wrapped with black paper and incubated in dark to avoid light contact for an hour at room temperature. The change in absorbance due to reduction by antioxidant (sample) was recorded at 517 nm against a methanol-DMSO (2900µl + 100µl) blank on double-beam UV/Vis spectrometer, (SPECORD ®210 Plus-analytikjena, Germany) in triplicate. Strength of DPPH scavenging activity of compound was calculated as percent inhibition using equation mentioned in antibacterial test section. EC_{50} value is the effective concentration of

sample at which it scavenges 50% of initial DPPH radicals and this value was calculated by linear regression analysis of dose-response curve plotting between percent inhibition and concentrations of test compounds

Separation of bioactive fractions from crude compounds

Ethyl acetate extracted crude compounds were separated by column chromatography having silica of 100- 200 mesh size packed in glass column. Crude compounds were impregnated in little amount of silica and loaded above packed silica column. Pure hexane was used for separation of non-polar compounds. Fractions of 100 ml hexane eluent were collected, concentrated and checked for presence of compounds on thin layer chromatography. If there was no additional compound present in last fraction of pure hexane then 95:5 ratio of hexane and ethyl acetate was applied in column. This process was repeated just like pure hexane process. Different ratio of hexane: ethyl acetate (90:10, 80:20, 70:30 etc.) were used for fractionation of crude compounds by column chromatography. Each fraction procured from column chromatography was checked by thin-layer chromatography for the presence of single or multiple compounds. Each band present on TLC plate was carefully scraped and collected in a separate vial. Different fractions obtained from column chromatography were run simultaneously on the same plate to check for the presence of similar compounds in different fractions. Bands of same RF value in different fractions were designated as the same compounds and collected in a single vial. Scraped silica gel that contains compounds was mixed with ethyl acetate for recovery of compounds. For dissolving all compounds in ethyl acetate, it was mixed in it and left for half an hour for settling down silica particles on the bottom. Ethyl acetate was carefully removed from vials and kept into fresh vials. For complete removal of the silica particles from the compounds dissolved in ethyl acetate, it was filtered through a membrane filter.

Inhibitory concentration 50 of separated compounds by MTT assay

For calculation of inhibitory concentration 50 (IC_{50}), spectroscopically maintained bacterial inoculum (2 µl) was suspended in 2.9 ml MH broth and mixed with a series of sample dilutions prepared in 98 µl DMSO and incubated at 37 ± 2°C for overnight in BOD incubator. The incubated bacterial tubes were treated with 200 µl MTT ((3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; 5 mg/ml in fresh PBS buffer) dye and further incubated for half an hour at 37°C. After incubation, samples were treated with acidified isopropanol and 3% SDS to lyse bacterial cell and dissolve insoluble formazan dye formed from MTT due to presence of viable cells. Absorbance was recorded at 517 nm on double-beam UV/Vis spectrophotometer, (SPECORD® 210 Plus-analytikjena, Germany) against MH broth and DMSO blank. Percentage inhibition of bacterial growth by sample was calculated as per given formula below:

% inhibition = [(Absorbance of control - Absorbance of sample)/ Absorbance of control] ×100

IC_{50} value was calculated by linear regression analysis of dose-response curve plotted between % inhibition and dilutions of test compounds.

Characterization of antibacterial compounds by gas chromatography- mass spectrometry (GC-MS)

Two purified compounds that were active against three human pathogenic bacteria were characterized by GC-MS analysis by National Institute of Pharmaceutical Education and Research (NIPER), Mohali. The gas chromatography coupled with mass spectrometry was performed on Shimadzu QP-5000. The GC oven method was as followed: initial temperature: 50°c, initial time: 2 min., number of ramps: 1, Rate #1 (degree/min.): 8.0, final temperature #1: 280, hold time: 8.0 min., maximum temperature: 350°c, prep run timeout: 10.0 min., equilibration time: 0.50 min. the mass spectra was recorded on EI mode from MW 50-70.

RESULTS

Isolation and identification of endophytic actinomycetes

After emergence of actinobacterial colonies on SCA plates, each isolate was transferred to separate SCA plate for obtaining pure isolates. These isolates were preliminary screened for antibacterial activity and one isolate (AZRA 34) that showed very promising antibacterial property against selected human bacterial pathogens, was selected for mass culture to obtain crude compound. Based on 16S rRNA gene sequencing analysis, the AZRA

34 isolate was identified as a member of the genus *Streptomyces*. The obtained 16S rRNA gene sequence was submitted to the NCBI GenBank database and assigned the accession number KY858967. This molecular identification and phylogenetic tree analysis confirms the taxonomic placement of the isolate within the *Streptomyces* genus, which is well known for its prolific production of bioactive secondary metabolites (**Figure 1**).

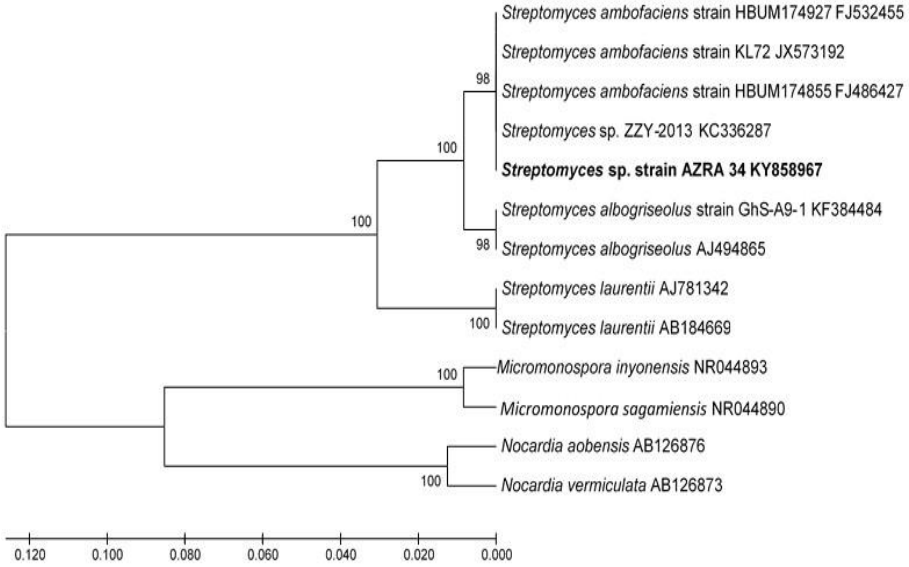

Figure 1: Phylogenetic tree constructed from the comparison of 16S rDNA gene sequences of *Streptomyces* sp. and selected most close reference strains of Actinobacteria retrieved from the NCBI database

Extracellular enzymes assay

The isolate AZRA 34 was screened for extracellular production of amylase, chitinase, cellulase and pectinase. Amylase and pectinase activities were found positive on plate assay and based on the result, quantification of these two enzymes was done in solid-state and submerged fermentation medium. Production of pectinase in solid-state fermentation (SSF) was far higher than that of submerged medium (SmF) on fifth days of incubation. In SSF medium, pectinase production was 1.627 µM/ml/min in comparison with SmF of 0.201 µM/ml/min. Amylase production was less in comparison with pectinase but the trend was found similar as in SSF (0.167 µM/ml/min) and SmF (0.130 µM/ml/min) (**Figure 2).**

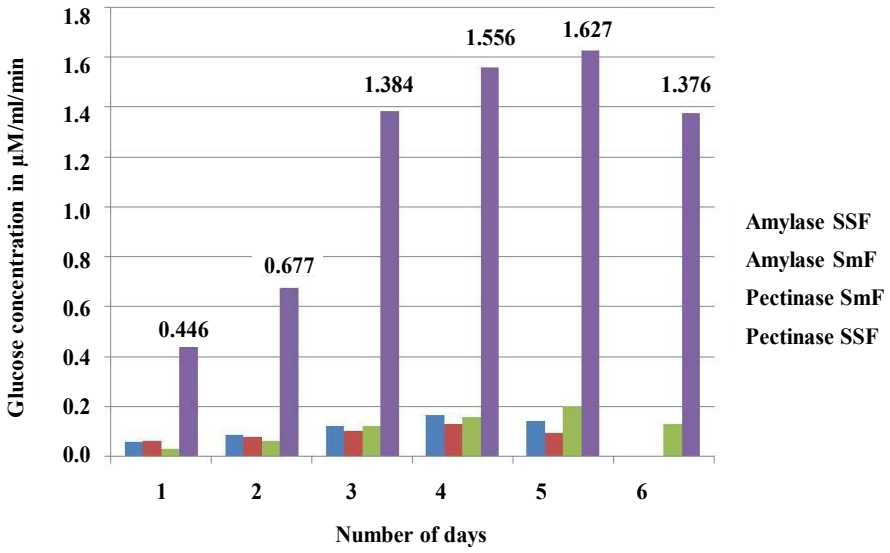

Figure 2: Graph illustrating the production of amylase and pectinase in submerged fermentation (SmF) and solid-state fermentation (SSF) media at various time intervals

Antibacterial activity

The crude compound obtained from the isolate showed strong antibacterial activity against three human pathogenic bacteria including *Aeromonas hydrophila* IMS/GN11, *Salmonella typhi* MTCC 3216, and *Staphylococcus aureus* ATCC 25923. However, it was found inactive against *Enterococcus faecalis* IMS/GN7 and *Shigella flexneri* ATCC 12022.

Antioxidant activity

DPPH free radical scavenging activity of crude compound was performed to demonstrate antioxidant activity. Ascorbic acid was taken as positive control. At 50 µg/ml concentration, crude compound showed 39 % of free radical scavenging activity in comparison of 55% activity of standard ascorbic acid. It was found that antioxidant activity was increased up to 85 % at 300 µg/ml concentration (**Figure 3**). Effective concentration 50 (EC_{50}) of crude compound was recorded as 77.61 µg/ml.

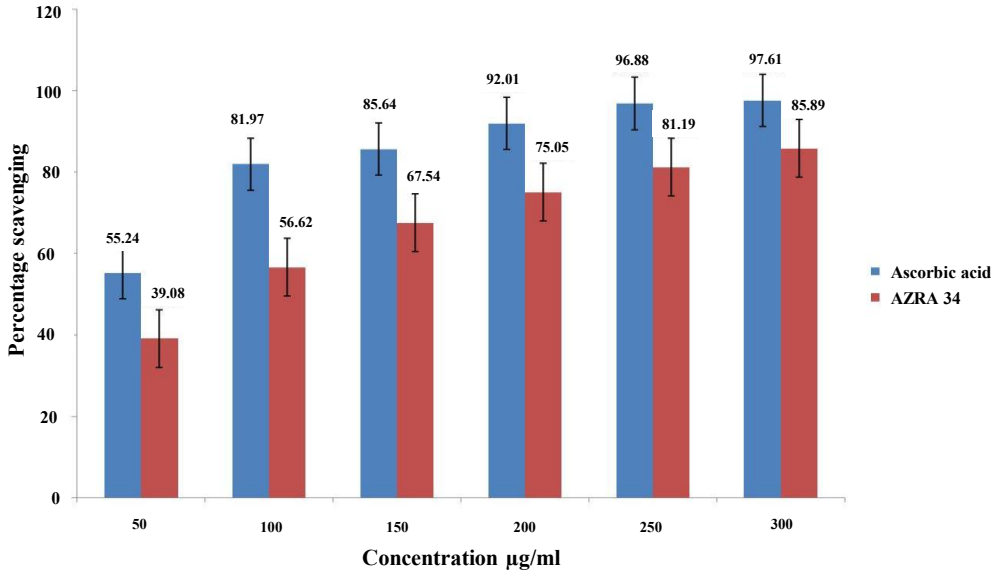

Figure 3: Graph showing the percentage of free radical scavenging activity of the crude compound at different concentrations, with ascorbic acid as a positive control

Separation of bioactive fractions and their antibacterial activity

Crude compounds were separated by column chromatography and thin layer chromatography. More than 25 fractions were collected and compound from each fraction was evaluated for antibacterial properties. Out of all recovered fractions, two (F1 and F3) were found active against three bacteria namely *Salmonella typhi* MTCC 3216, *Aeromonas hydrophila* IMS/GN11, *Staphylococcus aureus* ATCC 25923. IC_{50} of these compounds were calculated by MTT method. IC_{50} of compounds in F1 was found 9.77 µg/ml for *Aeromonas hydrophila*, 12.07 µg/ml for *Salmonella typhi* and 10.5 µg/ml for *Staphylococcus aureus*. In the case of compound F3 IC_{50} value was recorded as 28.14 µg/ml, 22.77 µg/ml and 30.42 µg/ml for *Aeromonas hydrophila*, *Salmonella typhi* and *Staphylococcus aureus*, respectively (Table 1).

Table 1: IC_{50} values (in µg/mL) of isolated compounds from *Streptomyces* sp. AZRA 34 against five human pathogenic bacteria. Where NA= No activity

Compounds	*Aeromonas hydrophila*	*Salmonella typhi*	*Staphylococcus aureus*	*Shigella flexneri*	*Enterococcus faecalis*
F1	9.77	12.07	10.5	NA	NA
F3	28.14	22.77	30.42	NA	NA

Characterization of antimicrobial compounds by Gas chromatography-mass spectrometry (GC-MS)

Two fractions compounds that were active against three human pathogenic bacteria were characterized by GC-MS analysis. Main compounds in F1 and F3 were characterized as 2, 4-diphenyl-2, 3-dihydro-1, 5 benzothiazepine and 17-(1, 5-dimethylhexyl)-10, 13-dimethyl-3-styrylhexadecahydrocyclopenta[a] phenanthren-2-one respectively (**Figure 4a** and **4b**).

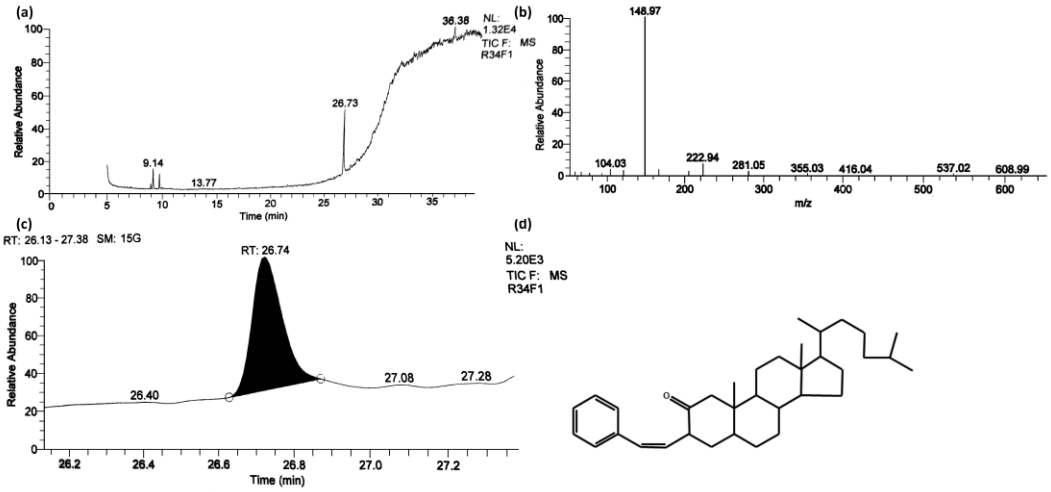

Figure 4a: GC-MS analysis of compound F1: (a) chromatogram, (b) mass spectrum, (c) retention time, and (d) molecular structure of 2,4-diphenyl-2,3-dihydro-1,5-benzothiazepine

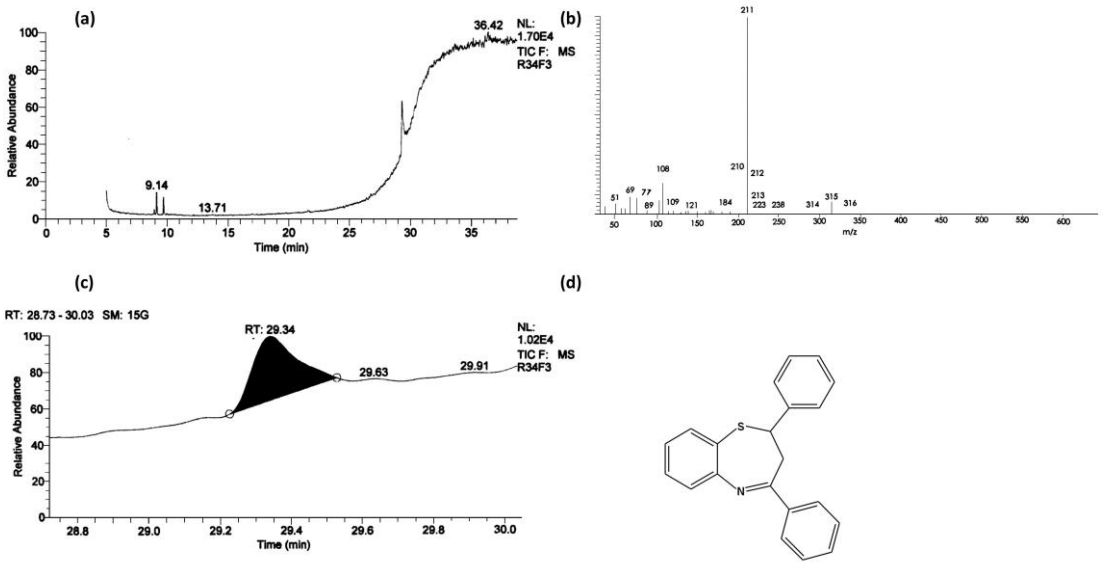

Figure 4b: GC-MS analysis of compound F3: (a) chromatogram, (b) mass spectrum, (c) retention time, and (d) molecular structure of 17-(1,5-dimethylhexyl)-10,13-dimethyl-3-styrylhexadecahydrocyclopenta[a]phenanthren-2-one

DISCUSSION

Endophytic actinobacteria have been very potential source of bioactive molecules. Many research groups are involved in exploring potential of endophytic actinobacteria for their bioactivities and their industrial applications (Golinska *et al*., 2015). Endophytes may also mimic the host compound, as many of them have capability to produce the same or similar bioactive compounds for which host plant is known (Zhao *et al*., 2010). Endophytic actinomycetes are regarded as one of the relatively untapped potential sources of the antibacterial

compounds against antibiotic-resistant pathogens (Ranjan and Jadeja, 2017). *Azadirachta indica* is a well-known plant in Indian subcontinent and almost every part of this plant is being used as folk medicine since time immemorial. In this experiment an endophytic actinobacterium was isolated from root of *Azadirachta indica* and identified as *Streptomyces* sp. AZRA 34 using 16S rDNA sequencing followed by phylogenetic analysis. Phylogenetic analysis confirms its taxonomic position and we have submitted it as *Streptomyces* sp. AZRA 34KY858967 to NCBI database (**Figure 1**). Further this isolate was evaluated for antibacterial, antioxidant activity and the production of enzymes. Amylase and pectinase were produced by this isolate on screening plates, and the production of both the enzymes was further quantified on submerged and solid-state fermentation media. SSF medium was found better in both the enzymes production and it was approximately double to SmF medium. Moreover, in SSF, cheap and easily available substrates were used that has reduced the cost of enzyme production. Gonzalez (1998) clearly described the advantage of SSF process where enzyme titer is higher than SmF (Viniegra-González, 1998).

Natural antioxidants are known to be more effective with minimum side effects and therefore, microbial metabolites can be good alternative for it. Antioxidant activity and EC_{50} of crude compounds from AZRA 34 was found quite close and comparable to some of earlier reported antioxidant compounds (Arumugam *et al*., 2010). Antioxidant compounds such as 2-allyloxyphenol Saccharomonopyrone A have been reported from different actinomycetes (Arumugam *et al*., 2010, Yim *et al*., 2017). In present study we report antioxidant activity in crude, but are unable to specifically identify the pure compound responsible for the antioxidant activity.

The crude compounds showed strong antibacterial activity against *Salmonella typhi* MTCC 3216, *Aeromonas hydrophila* IMS/GN11 and *Staphylococcus aureus* ATCC 25923. However, growth of *Enterococcus faecalis* IMS/GN7 and *Shigella flexneri* ATCC 12022 were not inhibited. There are several mechanism including enzymatic inactivation of drug, target site modification, efflux of drug outside the cell, biofilm formation etc that may be used by bacterial pathogens to survive against antibacterial compounds (Belay *et al.*, 2024). However, the exact reasons are not exactly known for the resistance displayed by these pathogens against the crude compounds of *Streptomyces* sp. AZRA 34. However, Verma *et al.* (2009) isolated 55 endophytic actinomycetes from *A. indica* and screened the antimicrobial activity against *Bacillus subtilis*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pythium oligandrum* (FC011), *P. ultimum* (FC036), *P. cactorum* (FC514), *P. aphanidermatum* (FC001), *Phytophthora cypogea* (FC509), and *P. infestans* and about 60% isolates displayed a broad-spectrum antimicrobial activity against both fungi and bacteria (Verma *et al*., 2009). After separation of crude compounds, 25 pure fractions were recovered. Two fractions (F1 and F3) showed similar antimicrobial activity as of shown by its crude. GC-MS analysis of fraction F1 and F3 confirmed them as 2, 4-diphenyl-2, 3-dihydro-1, 5 benzothiazepine and 17-(1, 5-dimethylhexyl)-10, 13-dimethyl-3-styrylhexadecahydrocyclopenta[a] phenanthren-2-one respectively (**Figure 4a** and **4b**). Previously reported two compounds of 4-hydroxy coumarin, derivatives of 1,5-benzothiazepine, were evaluated for antibacterial activity and found to be most active against *Escherichia coli* and *Staphylococcus aureus* (Sahoo *et al*., 2012). Moderate to good antibacterial activity was exhibited by 2, 3-dihydro-1, 5-benzothiazepines against *Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and *Staphylococcus epidermidis* (Sawant and Nirwan, 2013). In a recent study, an endophytic actinomycete, *Aeromicrobium ponti* was found to produce several antibacterial compounds including 1-acetyl-β-carboline, indole-3-carbaldehyde, 3-(hydroxyacetyl)-indole, brevianamide F, and cyclo-(L-Pro-L-Phe) (Gos *et al*., 2017). Naphthalene derivative isolated from an endophytic fungus of *Aegle marmelos* exhibited the positive antibacterial activity against clinical isolates of human pathogenic bacteria such as *Salmonella paratyphi* IMS/GN4, *Shigella flexnii* IMS/GN1, *Salmonella enteritidis* IMS/GN3, *Shigella boydii* IMS/GN2, *Pseudomonas aeruginosa* ATCC 27853 and *Morganella morganii* IMS/GN6 (Gond *et al*., 2013). A phenanthren alkaloid was reported from the leaf of *Bryophyllum pinnatum* which inhibited the growth of several test bacteria and yeast including *Staphylococcus aureus, Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans* (Ebere Okwu and Uchenna, 2011). In this study, we reported the production of two compounds

from F1 and F3 by the endophytic *Streptomyces* sp. AZRA 34 with significant antibacterial activity against important human pathogens including *Aeromonas hydrophila*, *Salmonella typhi* and *Staphylococcus aureus*. There are few reports available about the production of derivatives of 1, 5-benzothiazepine and phenanthren by other endophytic and non-endophytic microbes. Benzothiazepine containing compounds are generally chemically synthesized and display the broad-spectrum antibacterial and anti-cancer activity (Wang *et al*., 2009; Cherkupally *et al*., 2008; Prabhakar *et al*., 2017). Phenanthren was reported to produce by some plants and microbes (Cresp *et al*., 1974; Ebere Okwu and Uchenna, 2011) with a broad range of antimicrobial activity. Extracellular enzyme production, antioxidant activity and production of antibacterial metabolites by endophytic isolate AZRA 34 examined in the present study suggesting its high biotechnological potential.

This strain of *Streptomyces* can be used for large scale production of extracellular enzymes and antibacterial compounds. We are looking further to improve the quantity and quality of antibacterial compound production. Recently, genome editing tools are gaining popularity to produce microbial metabolites and CRISPR (clustered regularly interspaced short palindromic repeats) is one of them. Tong *et al.* (2018) have developed a CRISPR-Cas9 toolkit with high efficiency for actinomycetes genome editing and it was successfully demonstrated in *Streptomyces coelicolor* A3(2) and *S. collinus* Tü 365 species.

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CONFLICT OF INTEREST

Authors do not have any conflict of interest.

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