

# Isolation and screening of antibiotic producing fungi from solid-state waste

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## ABSTRACT

**Aim:** To evaluate the antimicrobial potential of fungal extracts isolated from solid-state waste and study their active constituents. Antibiotics are the most important secondary metabolites produced by the microorganisms that are commercially exploited for the treatment of various diseases worldwide. But with time, these microbial pathogens have become more powerful and resistant to antibiotic treatment, creating new strains that are difficult to control. To overcome the problem of multi-drug resistance, the present study aimed at exploring new sources of bioactive components.

**Methods:** A total of 22 fungal isolates were isolated from solid-state household waste dumping site at Hisar, India. The perpendicular streak plate technique was used for primary screening against three test microorganisms (*Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*). About 55% of the tested fungal isolates showed antimicrobial activity against all the three test microorganisms. The fungal isolates showing positive activity were put to secondary screening by Agar well diffusion assay against eight test microorganisms (Gram positive: *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus gordonii* and Gram negative: *Pseudomonas aeruginosa*, *Escherichia coli*, *Pseudomonas fluorescens*, *Salmonella enterica* and fungus *Candida albicans*).

**Results:** The potent fungal isolate with maximum zone of inhibition was selected for secondary metabolite extraction with dichloromethane and the crude extract was examined for antimicrobial properties. Analysis of the crude metabolites by thin layer chromatography (TLC) showed the presence of bioactive components with Rf value of 0.88. The results demonstrated that the crude extracts of fungal isolate SSR-16 exhibit great antimicrobial activity against all test microorganisms with the zone of inhibition from 2.2cm against *E.coli* and *P.aeruginosa*, 2.0cm against *C.albicans*, 1.8cm against *B.subtilis* and *P.florescens*, 1.6cm against *S.aureus* to 1.4cm against *S.entericus* and *S.gordonii*.

**Conclusion:** The selected strain SSR-16 was identified as *Aspergillus sp.* using 18 S rDNA gene sequence analysis. The study suggests that the selected strain *Aspergillus sp.* may be a source of potential antimicrobial agents.

**KEYWORDS:** Solid-state waste; Antimicrobial; TLC, aspergillus.

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## INTRODUCTION

Natural products are typically secondary metabolites that are procured from microorganisms and find many applications in the areas of industries, agriculture and medicine. The antimicrobial potential of secondary metabolites has been exploited commercially for the treatment of various infectious diseases in humans and other animals. Infections by microorganisms and development of drug resistance or even multidrug resistance among microbial pathogens are most critical problems diffusing worldwide (Bingzheng et al, 2018). In recent decades, most of the microbial pathogens are acquiring mutations and becoming more powerful and resistant to antibiotic treatment, creating new or super strains that are difficult to control (Olorunmola et al., 2013). Therefore, there is an urgent need to develop new antimicrobial agents to combat drug resistance among pathogens. From decades, plants and microbes are being exploited to produce metabolites that are considered as important sources of drugs for therapeutic applications. However, 50–60% of the therapeutic drugs are produced by plants (alkaloids, flavonoids, terpenoids, steroids and carbohydrates, etc.) and only 5% of them have a microbial origin (Srichandan and Kumananda, 2015). Among the microbes, a number of antibiotic drugs have been discovered from soil and other natural habitats, which include fungi (20% of isolated antibiotics), actinomycetes (70%) and eubacteria (10%). The streptomycetes produce a vast variety of antibiotics (Makut and Owolewa, 2011). Among the fungi, Aspergillales group ranks first in the ability to produce antibiotics (Schlegel et al., 2003).

Fungi are of great importance due to their ability to produce vast variety of bioactive metabolites for the pharmaceutical industries (Nigam and Singh, 2014). Fungi rank as the second biggest kingdom of organisms in nature and as many as 1.5–5.1 million fungal species exist (Mio et al, 2013). In the field of drug discovery, fungi played an important role by producing new bioactive compounds and

producing antibiotics such as penicillin, anti-cancer drugs such as taxol, immunosuppressants such as ciclosporin and cholesterol-lowering drugs as lovastatin (Gerke and Braus, 2014). Although the investigation of new microorganism from the uninvestigated areas will lead to many new biologically active secondary metabolites, only a small fraction (0.1%-1%) of all microorganisms have been exploited. Therefore, this study was carried out to explore uninvestigated areas, such as solid-state household waste in search of novel natural products to overcome the multi-drug resistance among pathogens.

## MATERIALS AND METHODS

### Collection of samples

Soil samples were collected from the solid-state household waste dumping site at Hisar, India. The samples were collected aseptically from four different sites with depth from 0-10cm and were brought to the laboratory in sterile polythene bags. All the samples collected were dried in oven at 45°C overnight and then stored at 4°C till further use.

### Determination of soil temperature

The temperature of the soil at four different sites was determined with the use of thermometer. The temperature was measured by inserting thermometer into the soil to a depth of 10 cm and allowed to stand for 5 minutes. Three readings were taken for each site and their average was recorded (Makut and Owolewa, 2011).

### Determination of soil pH

The soil pH of four different sites was measured using a digital pH meter by using standard methods of Watson and Brown (Makut and Owolewa, 2011). Using this method, 5g of soil sample from each site was weighed and put into a glass beaker containing 5ml of distilled water. It was then stirred with a glass rod and allowed to settle for 10 minutes. Then, the pH meter electrode was inserted into the beaker containing slurry and

swirled slowly. Thereof, three consecutive readings were taken and their average was recorded for each site.

### Isolation of fungi from soil sample

Standard serial dilution method was used to isolate the fungal strains from the soil samples. 1g of soil sample was taken into a test tube containing 9 mL sterile distilled water to make a stock solution and shaken well using vortex mixer. 1 ml of the stock solution was added to another test tube containing 9 ml sterile distilled water and so on to make further dilutions upto  $10^{-8}$ . Thereafter, 100 $\mu$ l of inoculum from  $10^{-4}$  to  $10^{-8}$  dilutions was spread onto the surface of potato dextrose agar, malt extract agar and sabouraud agar media plates in triplicates, followed by incubation at 28°C for 48-72 hrs. Distinct colonies were selected from the cultured plates and maintained on potato dextrose slants by streaking. These pure cultures were then stored at 4°C till further use.

### Test organism

Among 8 test organisms used, 5 were obtained from MTCC, IMTECH Chandigarh, India and 3 were procured from FIT laboratory, GJU, Hisar, Haryana, India. The test organisms used were gram-positive: *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus gordonii* and Gram negative: *Pseudomonas aeruginosa*, *Escherichia coli*, *Pseudomonas fluorescens*, *Salmonella enterica* and fungus *Candida albicans*. The test organisms obtained from IMTECH Chandigarh were *Bacillus subtilis*- MTCC no. 441, *Staphylococcus aureus*- MTCC no. 3160, *Streptococcus gordonii*- MTCC no. 2695, *Pseudomonas fluorescens*- MTCC no. 664 and *Candida albicans*-MTCC no. 183.

### Preliminary selection

Primary screening for antimicrobial activity was done against three test organisms i.e, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* using perpendicular cross-streak method. Fungal isolates were streaked in the middle of Mueller Hinton agar plates as a straight

line and incubated at 28°C for 48-72hrs days. The test organism was cultured for 24hrs and the density was adjusted to 0.5 McFarland. Thereafter, the test organisms were streaked perpendicular to the fungal isolate on the agar medium. Three empty plates of Mueller hinton agar streaked with the test organisms were used as control. All the plates were incubated at 37 °C and observed for the formation of the inhibition zone for 24-48 hours. The fungal isolate that showed inhibition of test microorganism by the absence of growth of the test organism near the isolates were isolated and maintained at -20°C in 60% of sterile glycerol stock.

### Secondary screening for fungal isolates via agar well diffusion technique

Fungal isolates showing positive results in the preliminary screening were cultivated in potato dextrose broth and incubated at 28°C at 100rpm for 7 days. Thereafter, the fungal cultures were extracted thrice by solvent extraction method with equal volume of ethyl acetate. (Puji Astuti and Octavian, 2014). The organic solvent extracts were evaporated to dryness, dissolved in methanol and subjected to antimicrobial studies using agar well diffusion assay (Valgas et al, 2007) (Shanmuga et al, 2002). For agar well diffusion method, 100 $\mu$ l of 24hrs old test bacteria was spread over Mueller-hinton agar plates. Then five equidistant wells of 6mm diameter were made in Mueller hinton agar with the help of sterilized core borer. Thereafter, 200  $\mu$ l of fungal extracts dissolved in methanol were poured into the wells and one well was used as the positive control. The plates were incubated at 37°C for 24 hrs and observed for the zone of inhibition around fungal extracts.

### Morphological and microscopic characterization

Fungal strain was characterized phenotypically by colonial observation of fungal isolate on five different media that were potato dextrose agar, Sabouraud's agar, Czapek-dox agar, Malt extract agar and modified media, followed by microscopic observations using lactophenol staining technique.

### Molecular characterization and phylogenetic analysis

Molecular identification of potent fungal isolate was carried out by 18S rDNA gene sequence. The strain SSR 16 was partially sequenced using commercial DNA sequencing Service, Macrogen Inc., Seoul, South Korea. Universal primer pair (forward and reverse) used were, 785F (5'-GGATTAGATACCCTGGTA-3') and 907R (5'-CCGTC AATTCMTTTRAGTTT-3'). The ITS sequences obtained were matched with related sequences available at the National Center for Biotechnology Information (NCBI) website, by using BLASTn algorithm (<http://www.ncbi.nlm.nih.gov/BLAST>). Phylogenetic relationship was established by aligning 18S rDNA sequences with other matching sequences using MEGA6 software (Dhanwal et al, 2018). The nucleotide sequence data were further deposited in the GenBank database at the NCBI.

### Selection of suitable conditions

#### Influence of different media on antibiotic production

Five modified test media were made for optimum production of antibiotic, namely media no. 1, 2, 3, 4 and 5 (Kumar et al, 2008). Composition of media were as follows:

Medium 1: Brain heart Infusion broth 10g/L, peptone 5g/L, dextrose 5g/L, NaCl 5g/L, Na<sub>2</sub>HPO<sub>4</sub> 2.5g/L, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1g/L, CaCl<sub>2</sub> 0.02g/L, KH<sub>2</sub>PO<sub>4</sub> 15g/L, yeast extract 5g/L, starch 1g/L, cysteine HCl 1g/L, MgSO<sub>4</sub> 0.2g/L.

Medium 2: Casein 4g/L, yeast extract 5g/L, dextrose 5g/L, Cysteine HCl 1g/L, starch 1g/L, NaCl 5g/L, KH<sub>2</sub>PO<sub>4</sub> 15g/L, NH<sub>2</sub>SO<sub>4</sub> 1g/L, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.2g/L, CaCl<sub>2</sub> 0.02g/L.

Medium 3: Starch 1g/L, dextrose 5g/L, casein 4g/L, C<sub>3</sub>H<sub>5</sub>NaO<sub>2</sub> 4g/L, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.5g/L, KH<sub>2</sub>PO<sub>4</sub> 15g/L, Asparagine 0.1g/L, NH<sub>2</sub>SO<sub>4</sub> 1g/L.

Medium 4: C<sub>3</sub>H<sub>5</sub>NaO<sub>2</sub> 4g/L, casein 4g/L, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.5g/L, FeSO<sub>4</sub>.7H<sub>2</sub>O 0.001g/L, Asparagine 0.1g/L, dextrose 5g/L, Starch 1g/L.

Medium 5- Minimal salt media (K<sub>2</sub>HPO<sub>4</sub> 2g/L, NaCl 1g/L, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.10 g/L, CaCl<sub>2</sub> 0.05 g/L), dextrose 5g/L, Asparagine 0.1g/L, yeast extract 5g/L, cysteine HCl 1g/L.

The pH of all the media were adjusted to pH-7.5 for maximum production of secondary metabolites. The fungal isolate SSR 16 was cultivated in media no 1 to 5 and incubated at 28°C at 100 rpm for 7 days. Thereafter, the supernatant of all the culture media were extracted with equal volume of ethyl acetate and the organic extracts were subjected to agar well diffusion assay against *Staphylococcus aureus* for determining the production of the antibiotic (Shanmuga et al, 2002).

#### Influence of different solvents on antibiotic extraction

Selected fungal isolate was cultivated in best suited medium in five flasks under same conditions of fermentation. After 7 days, the fungal culture was extracted using 5 different solvents separately. Solvents used for the extraction were ethyl acetate, chloroform, acetone/diethyl ether, dichloromethane and toluene. After the extraction, the organic extracts were subjected to agar well diffusion assay against *Staphylococcus aureus* for determining the production of the antibiotic.

#### Extraction of antibacterial metabolites

The fungal isolate exhibiting the best antimicrobial activity was cultivated in potato dextrose broth as the primary inoculum in shaking incubator at 28°C at 100rpm for 2 days. Thereafter, 5ml of primary inoculum was transferred to 3 litres of optimized medium and kept in a shaking incubator at 28°C at 100 rpm for 7days i.e .under suitable optimized conditions. After fermentation, the antibiotic was extracted by solvent extraction method. The fungal culture was centrifuged at 8000rpm for 10 minutes and 100 ml of supernatant was taken in the separating funnel. Thereafter, the supernatant was extracted with 100 ml of solvent three times and similarly the remaining supernatant was extracted. The organic solvent extracts were evaporated to

dryness, dissolved in methanol and the bioactive extract was subjected to separation and purification by thin layer chromatography.

### Separation of bioactive compounds using TLC

The concentrated ethyl acetate extract of fungal crude extract was prepared by adding 0.1mg of crude extract and 100  $\mu$ l of ethyl acetate, and subjected to primary analysis of the antibacterial substances. It was performed by thin layer chromatography (TLC) on silica gel slides by using ethyl acetate: methanol in the ratio of 4:6 in total of 25ml as a solvent system. Thereafter, 10 $\mu$ l of 1.0mg/ml crude extract was dropped on each spot on the silica plate. The TLC was run until solvent reached the top. The TLC plate was dried and chromatograms were visualized under UV light and exposed to iodine vapors.

Direct bioautography was used for the localization of antibacterial compounds in the ethyl acetate extract. The TLC slide was dried and put in sterile petri plate, in which 15ml of sterile soft nutrient agar (0.75%) seeded with 2% test organism was overlaid. Nutrient agar plate was incubated at 37°C for 24 hrs in an incubator and the formation of inhibition zone on silica gel plates was observed. The sterile zone on the media proved the presence of active antibacterial compounds (Samuel et al., 2014).

## RESULTS

### Soil characteristics

The soil samples were collected from four different locations of solid-state waste, Hisar, India. The pH and temperature of the different locations of the soil collected are shown in Table 1.

### Isolation and screening of potential antibiotic producing fungi

In the screening of potent antibiotic producing fungi, a total of 22 fungi were isolated from solid-state waste area on PDA, MEA and sabouraud agar medium. In the preliminary screening, a total

of 12 fungal isolates showed growth inhibition of all three test organisms (Fig. 1) and 7 fungal isolates showed inhibition against atleast one of these test organisms (*E.coli*, *Staphylococcus aureus* and *Candida. Albicans*) streaked perpendicular to the fungal isolate.

Table 1: Physico-Chemical Properties of soil sample of the different locations (Makut and owolewa, 2011).

Location	pH	Temperature
A	8.3 $\pm$ 0.2	26 $\pm$ 0.5
B	7.4 $\pm$ 0.2	27 $\pm$ 0.5
C	6.5 $\pm$ 0.2	27 $\pm$ 0.5
D	7.0 $\pm$ 0.2	26 $\pm$ 0.5

### Secondary screening in liquid broth

After the secondary screening, seven fungal isolates showed zone of inhibition against all eight test organisms. The potential isolates were MK-1, MK-3, MK-4, SSR-2, SSR- 12, SSR-16 and SSR- 21. During secondary screening, the crude extracts of the potential isolates were put to agar well diffusion assay to observe antimicrobial zone of inhibition (Fig. 2). Streptomycin discs of 25mg were used as positive control and methanol was used as a negative control. The results demonstrated that the crude extracts of SSR-16 and MK-3 exhibited great antibacterial activity against all test bacteria (Table 2). However, the values obtained for activity of SSR 1 show highest activity against *S. aureus* whereas the crude extract of SSR 2 and SSR 17 strains showed activity against *S. aureus* only (Table 2).

The results revealed that fungal isolates SSR 4, SSR 16 and MK 3 were active against *B. Subtilis*; fungal isolates SSR 1, SSR 4, SSR 13, SSR 16 and MK 3 were active against *E.coli*; fungal isolates MN41, SSR 16 and MK 3 against *P. aeruginosa* ; fungal



isolates SSR 1, SSR 16 and MK 3 against *S. Aureus*; fungal isolates SSR 1, SSR 16, MK 1 and MK 3 against *C. Albicans*; fungal isolates SSR 16 and MK 3 against *P. Florescens*, *S. Entericus* and *S.*

*Gordonii*. Fungal isolates SSR 16 and MK 3 showed highest zone of inhibition against *B. subtilis*, *E. coli*, *P. Aeruginosa* and *C. Albicans*.

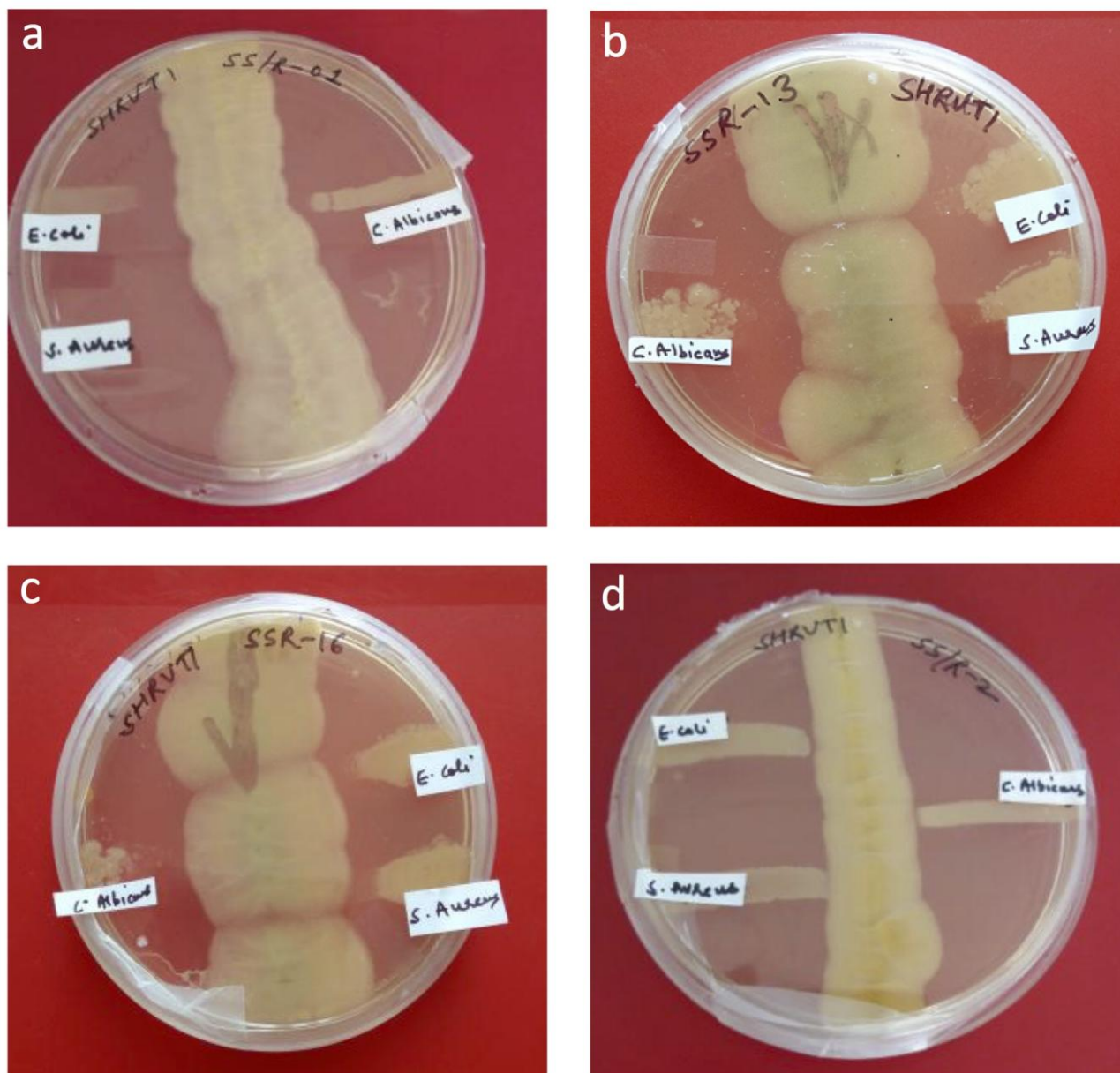


Fig. 1. Preliminary screening of different fungal isolates against *Escherichia coli*, *Staphylococcus aureus* and *candida albicans*. Isolates in fig a, b, c show inhibition against all three test organisms and fig d is shows no inhibition

#### Morphological and microscopic characterization

The pure fungal isolate SSR 16 selected from the secondary screening was cultivated on different growth media (PDA, CDA, MEA, Sabouraud's agar

and Medium 3) for characterization. The SSR 16 strain showed slower growth on CDA and medium growth on MEA and Medium 3 as compared to good growth on PDA and Sabouraud's agar (Fig.

3). Besides, SSR 16 was observed microscopically using lactophenol staining technique (Table 3)

Table 2: Diameter of zone of inhibition(in cm) by crude extracts of different fungal isolates against *B.subtilis*, *E.coli*, *P. aeruginosa*, *S. aureus*, *C. albicans*, *P.florescens*, *S. entericus* and *S. gordonii*. The -VE sign indicates no zone of inhibition and streptomycin.

<i>Fungal Isolate</i>	<i>B.Subtilis</i>	<i>E.coli</i>	<i>P.Aeruginosa</i>	<i>S.Aureus</i>	<i>C.Albicans</i>	<i>P.Florescens</i>	<i>S.Entericus</i>	<i>S.Gordonii</i>
SSR-1	1.2	2.0	1.2	2.4	2.0	1.0	1.2	1.0
SSR-2	-VE	-VE	-VE	1.0	-VE	-VE	-VE	-VE
SSR-4	2.2	1.8	1.0	1.0	1.2	1.2	1.0	-VE
SSR13	1.0	1.8	1.0	1.0	1.2	1.2	1.0	1.0
SSR16	1.8	2.2	2.2	1.6	2.0	1.8	1.4	1.4
SSR17	-VE	-Ve	-VE	1.0	-VE	-VE	-VE	-VE
MK-1	1.2	1.4	1.0	1.0	2.0	-VE	-VE	-VE
MK-3	2.0	2.0	1.8	1.6	1.8	1.4	1.4	1.0
MK-5	-VE	1.0	-VE	-VE	-VE	-VE	-VE	-VE
MK-15	1.4	1.2	-VE	-VE	-VE	-VE	1.0	-VE
Strepto- mycin disc	1.5	1.5	2.0	2.0	1.0	2.4	2.0	1.6

Table 3: Microscopic features of strain SSR 16 on staining with lactophenol blue.

Seriation	Uniseriate
Type of hyphae	Septate
Type of spores	Conidiospores
Shape of vesicles	Globose
Conidial head	Columnar

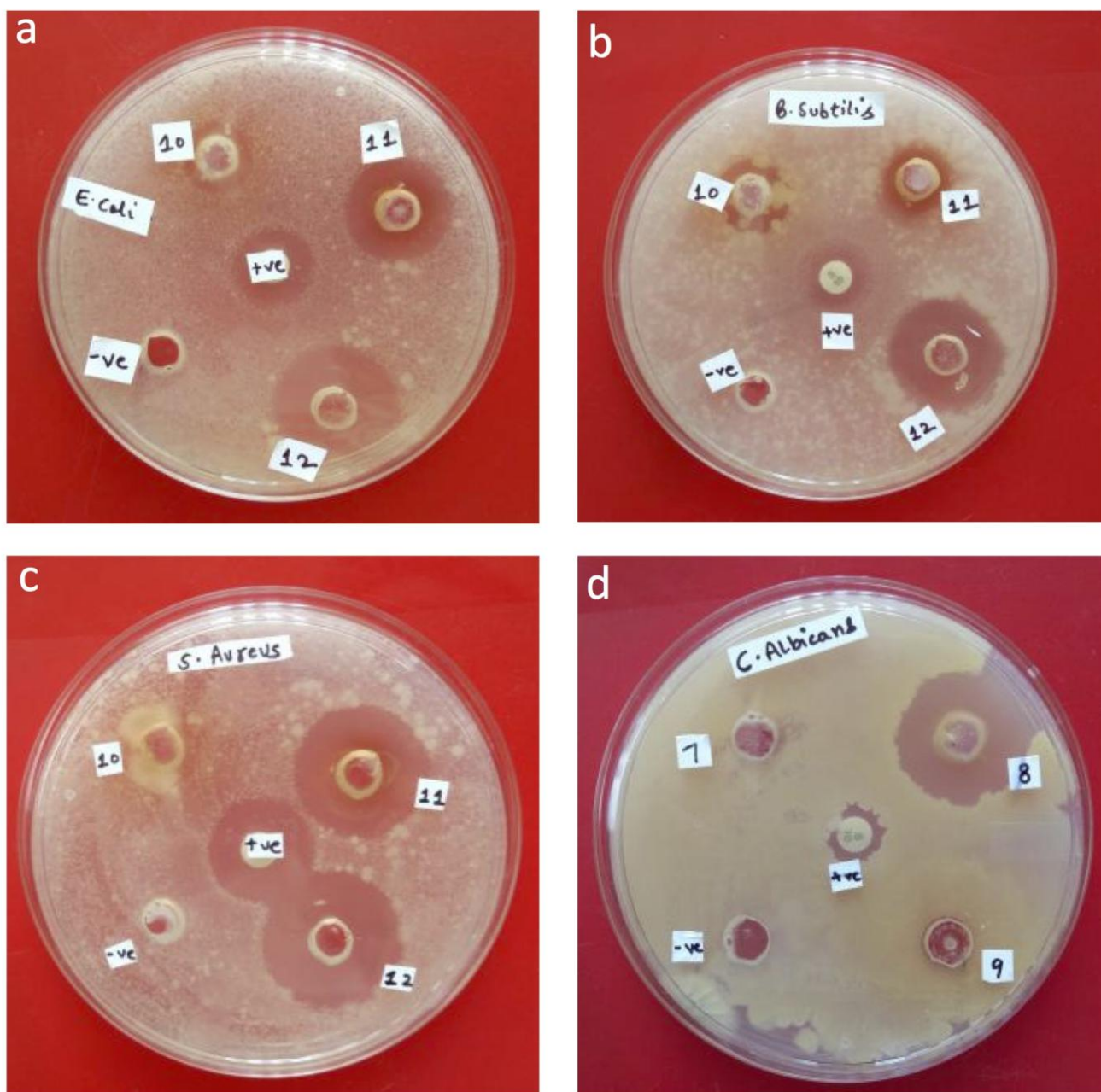


Fig. 2. Zone of inhibition by different fungal extracts against *E.coli*, *B. subtilis*, *S.aureus* and *C.albicans*. In this, +ve is streptomycin disc used (positive control) and –ve is methanol extract (negative control).

#### Molecular characterization and Phylogenetic analysis

The 18S rRNA gene of the strain SSR16 was partially sequenced to evaluate closest related

sequence. The amplified 18S rRNA sequence was compared with query sequences in the NCBI GenBank by using NCBI BLAST search. Further, the phylogenetic tree was constructed by performing multiple sequence alignment using neighbor



joining method from MEGA6 software. From the blast analysis and phylogeny analysis, SSR 16 showed the highest homology (99% identity similarity) with the *Aspergillus* sp. (Fig 3). Later, the

nucleotide sequence of the strain was submitted in the GenBank database (NCBI) with the accession number MN150488.

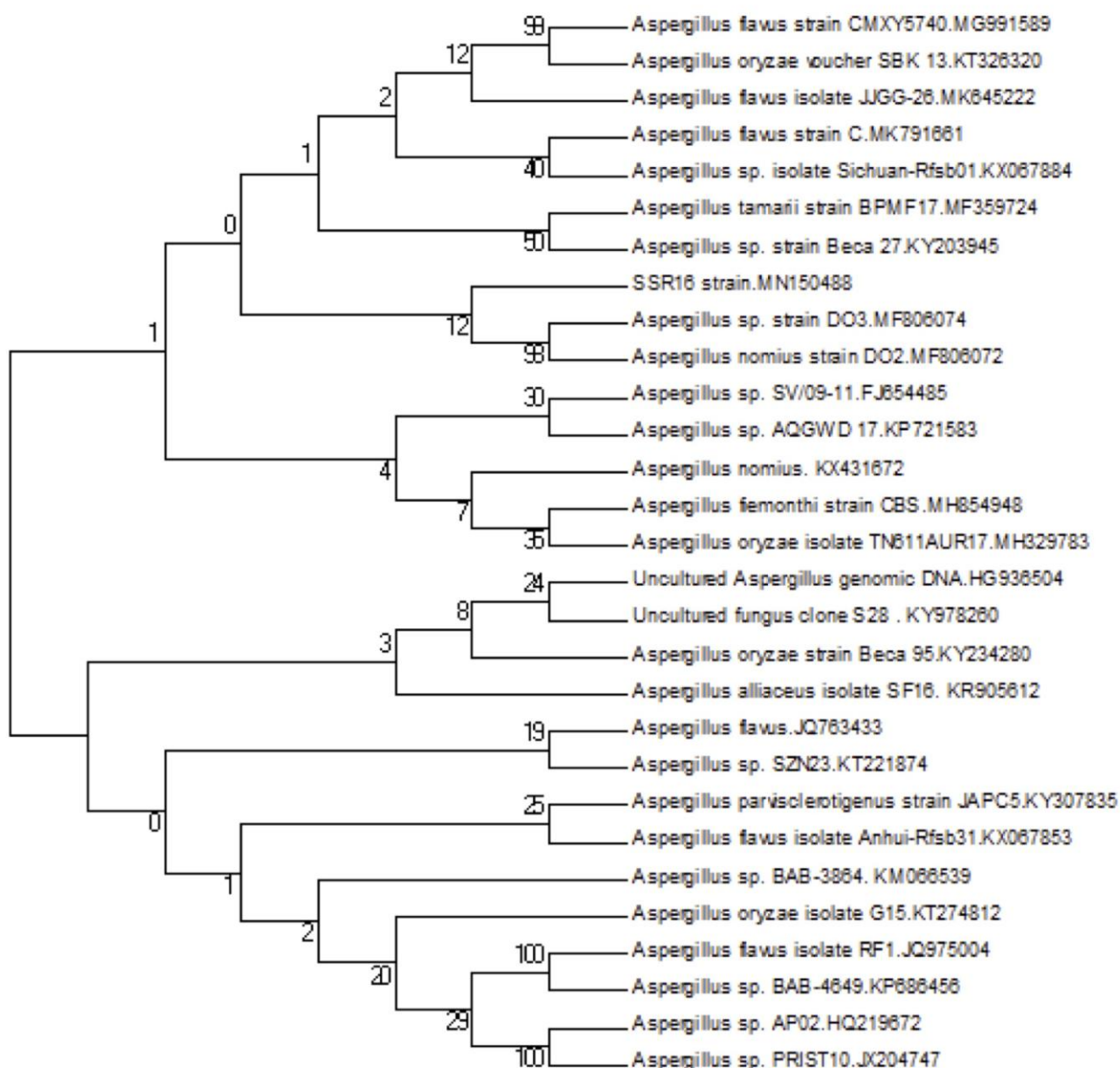


Fig. 3. Phylogenetic tree constructed to establish the evolutionary relationship of strain SSR 16 *Aspergillus* sp. (GenBank accession No. MN150488) with various related organisms using neighbor-joining algorithm from MEGA6 software.

### Influence of different media on antibiotic production

Medium no. 1, 2, 3, 4 and 5 were used for evaluating optimum production of antibiotic. The fungal isolates selected from secondary screening were grown in the above media for maximum production of secondary metabolites. The fungal isolates were cultivated in media no 1 to 5 and incubated at 30°C at 100 rpm for 7 days. Among the five media used, media no. 3 shown maximum production of antibiotic with largest zone of inhibition in agar well diffusion assay against *Staphylococcus aureus* followed by Media No. 2 > 1 > 4 > 5.

### Influence of different solvents on extraction of antibiotic

Fungal isolate was cultivated in 100ml of media no. 3 in five 250ml flasks at 30°C at 100rpm for 7 days under the same conditions of fermentation. After 7 days, the fungal cultures were extracted with 5 different solvents separately. Solvents used for the extraction were ethyl acetate, chloroform, acetone/diethyl ether, dichloromethane and toluene. Thereafter the organic extracts obtained after extraction were subjected to agar well diffusion assay. Among all the five solvents used for extraction, Dichloromethane exhibited largest zone of inhibition against *Staphylococcus aureus* followed by ethyl acetate and chloroform. The minimum or no zone of inhibition was found in Acetone and toluene.

### Extraction of antibacterial metabolites

The fungal isolate exhibiting best antimicrobial activity was cultivated in PDB as the primary inoculum in shaking incubator at 30°C at 100rpm for 2 days. Thereafter 5ml of primary inoculum was transferred to 3 litres of medium no. 3 and kept in

shaking incubator at 30°C at 100 rpm for 7days i.e. under suitable optimized conditions. After fermentation, the antibiotic was extracted by solvent extraction method using dichloromethane. The fungal culture was centrifuged at 8000rpm for 10 minutes and 100 ml of supernatant was taken in separating funnel. Thereafter, the supernatant was extracted with 100 ml of dichloromethane three times and similarly the remaining supernatant was extracted. The organic solvent extracts were evaporated to dryness, dissolved in methanol and subjected to antimicrobial studies.

### Thin layer chromatography and bioautography

Thin layer chromatography (TLC) on silica gel slides was done by using ethyl acetate:methanol in ratio of 4:6. Thereafter, 10µl of 1.0mg/ml crude extract was dropped on each spot on silica plate. The TLC was run until solvent reached the top and the results were calculated by determining the retention factor. Retention factor (Rf) value is a ratio of the distance travelled by the solute/ distance travelled by the solvent. The retention factor provides a quantitative measure of specific components and their properties in a mixture. The Rf value for *Aspergillus* was observed to be 0.88 respectively.

Direct bioautography was used for the localization of antibacterial compounds in ethyl acetate extract which provides an idea about the presence and absence of antimicrobial substances. The dried TLC slide was put in sterile petri plate, in which soft nutrient agar (0.75%) seeded with 2% of *E.Coli* as test organism was overlaid. Nutrient agar plate was incubated at 37°C for 24 hrs. The results of bioautography obtained are shown in Fig. 4. The sterile zone on the media proved the presence of active antibacterial compounds.

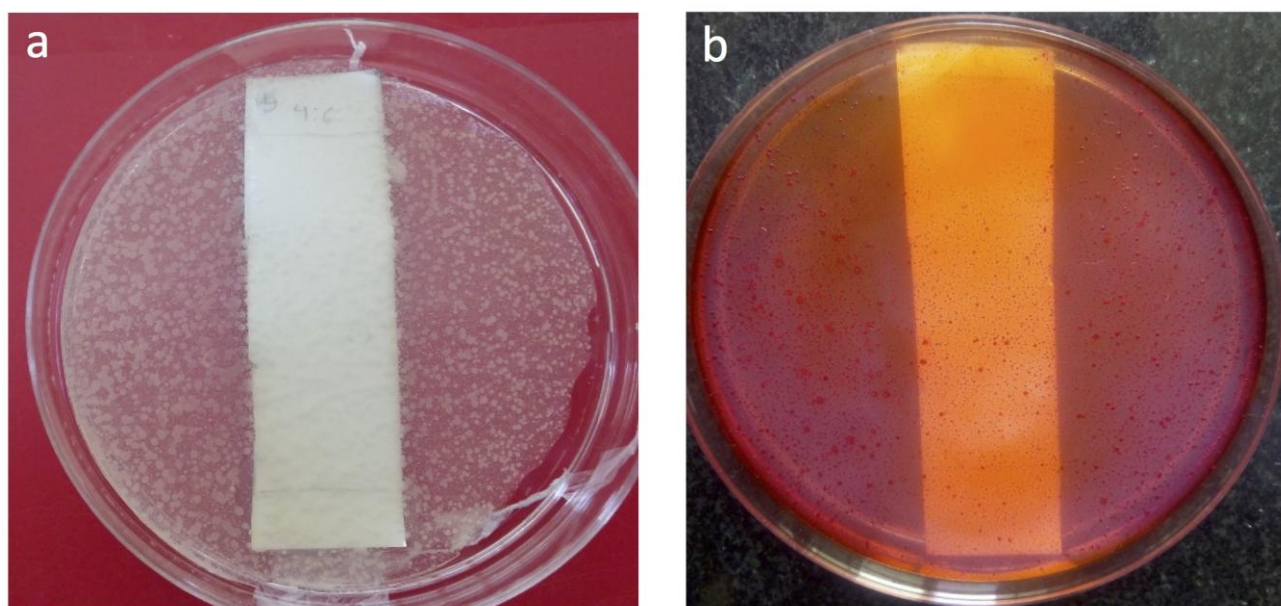


Fig.4. TLC plates showing separation of different components from SSR 16 strain with solvent system ethyl acetate: methanol (4:6). The strain SSR 16 showed inhibition against *E.coli* on direct bioautography plate.

## DISCUSSION

The discovery of new antibiotics is utmost important and challenging aspect in the 20th century to improve the quality of life and increase life expectancy. This is due to the increase in multidrug resistance among various pathogenic microbes, resulting in decreased efficacy of many antimicrobial agents against microbes causing infectious diseases (Barbosa et al., 2017). Infectious microorganisms, particularly those that are drug-resistant, cause so many deaths globally, and it has been estimated that by the year 2050, this will result in 10 million deaths per year (Shen et al., 2018). This pushes the research to the discovery of novel antimicrobial compounds. Fungi rank second in the field of secondary metabolite production after *Streptomyces*. The fungi are considered as the reservoirs for unexplored secondary metabolites; therefore, the use of fungi in search for novel antimicrobial compounds is most common due to their rich biodiversity

(Takahashi et al., 2013; Demain, 2014; Gerke and Braus, 2014).

In the present study, solid-state waste area near Hisar, India was explored and 22 fungi isolates were prepared. The screening of isolated fungi for antimicrobial properties showed significant activity against various test organisms. This led to large-scale fermentation of selected strain and isolation of bioactive compounds for further studies. The selected strain is identified as *Aspergillus*. The dichloromethane extract of *Aspergillus* strain exhibited great antimicrobial properties against Gram positive: *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus gordonii* and Gram negative: *Pseudomonas aeruginosa*, *Escherichia coli*, *Pseudomonas fluorescens*, *Salmonella enterica* and fungus *Candida albicans*. The maximum zone of inhibition obtained against test organisms was in order *E.coli* > *P.aeruginosa* and *C.albicans* > *B.subtilis* and *P.florescens* > *S.aureus* > *S.entericus* and *S.gordonii*. The culture conditions have a major impact on fungal growth and antimicrobial compound production. The production of these

bioactive compounds can be optimized by varying various media components. Also, under stress conditions, the secondary metabolite production is reported to be enhanced (Pereira et al., 2013). In this study, the potent strain was grown in six different formulated Media. Among them Medium no. 5 suited best for antimicrobial compound production. According to the molecular and phylogeny analysis, the evolutionary relationship of strain SSR 16 *Aspergillus* sp. (GenBank accession No. MN150488) with various related organisms was established using neighbor-joining algorithm from MEGA6 software. Thin Layer chromatography results suggest the presence of antimicrobial substances, which can lead to the further studies of the characterization of the compound(s). This study validates that the isolated strain *Aspergillus* could be useful for therapeutic and pharmaceutical industries that could be inexpensive and effective against multi-drug resistant microorganisms.

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## Conflict of interest statement

The authors declare no conflict of interest

## Authors' contributions

SD conceived the idea; RC collected the samples; SS, SD, HB, AK, VC conducted the experiments; VB conducted bioinformatics analysis; SD and AK wrote the manuscript. All authors have read and approved the final version of the manuscript.

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