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ISOLATION AND IDENTIFICATION OF ANTIBIOTIC PRODUCING MICROORGANISMS FROM SOIL

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ABSTRACT: Antibiotics are most important commercially used secondary metabolites, produced by many soil microorganisms *i.e.*, bacteria and fungi and employed in wide range. Most important antibiotics used today are of microbial origin. The emergence of the antibiotic resistance and need of broad spectrum antibiotics is in focus and in demand. In present study, soil samples from different areas were collected *i.e.*, the sampling is classified based on its micro and macro environment (waste polluted soil) (normal street soil) and (agricultural soil), from a local soil and analyzed for the antibiotic production. After primary screening, bacterial isolates were identified as *Micrococcus roseus*, *Brevibacterium* sp., *Bacillus subtilis*, *Bacillus anthracis* and *Bacillus cerus*, through biochemical characterization, and fungal isolates were identified as *Tricho-cladium opacum*, *Rhizocotania* sp., *Epicoccum nipponicum*, *Aspergillus niger* and *Cladosporium cladosporides* through microscopic and macroscopic identification and checked for antibiotic activity against some common gram positive and negative bacteria namely, *Staphylococcus aureus* and *Escherichia coli*. The antibiotic test indicates that *Bacillus subtilis*, *Bacillus anthracis*, *Epicoccum nipponicum*, *Aspergillus niger* and *Cladosporium cladosporides* showed antimicrobial activity against *Saureus* whereas against *E. coli*, *Bacillus anthracis*, *Bacillus cerus*, *Bacillus subtilis*, *Trichocladium opacum* and *Cladosporium cladosporides* produces zone of inhibition. This study suggests that *Bacillus* species have the potential to produce antibiotics and can be used to control the microbial growth in future. Strains of antibiotic producing fungi could be harnessed by pharmaceutical industries and used in medicinal purposes. This work may provide potential information on the antibiotic production and further be used for the control of microbial strains.

INTRODUCTION: Antibiotics are a natural substance of biological, synthetic or semi-synthetic origin¹.

In 1928, the term antibiotic appeared as antibiosis in French microbiological literature whereas as later in 1942 the term antibiotic was introduced by Waksman.

The demand for new antibiotics growing day by day due to the emergence of multiple pathogens that are resistant to antibiotics cures for formerly life-threatening diseases. In recent years several microorganisms that are able to produce antibiotics are grown on the artificial media for the intensive

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search for antibody producing microorganisms. The most important antibiotics include Aminoglycosides, Penicillin, Macrolides, Glycopeptides, Cephalosporins and Tetracyclines². Soil is a complex and very diverse environment providing versatile source of antibiotic producing organisms³. Each year nearly 500 antibiotics were found, in which 60% of antibiotics are obtained from the soil⁴. Recent analyses have shown that screening of soil for antimicrobial activities have been carried out in many parts of the world⁵. A tea-spoon of soil contains hundred million to one billion bacteria active in each acre of the soil.

Bacillus species are gram positive, rod shaped, sporulating and aerobic or facultative anaerobic bacteria that were most abundant bacterial strains found in the soil⁶ which were capable of producing dozens of antibiotics⁷. Genus of *Bacillus* was interesting to investigate and were considered microbial factory for the production of biologically active secondary metabolites^{8,9}. Various studies confirmed *Bacillus* species to produce antimicrobial compounds having pharmaceutical and biotechnological importance^{10,11}. The main antibiotic producer of *Bacillus* sp. were *B. cereus* (Zwittermicin, Cerexin) *B. brevis* (Tyrothricin, Gramicidin), *B. circulans* (Circulin), *B. licheniformis* (Bacitracin) *B. laterosporus* (Laterosporin), *B. polymyxa* (Colistin, Polymyxin), *B. subtilis* (Bacitracin, Polymyxin, Subtilin, Difficidin, Mycobacillin,) *B. pumilus* (Pumulin) are mainly polypeptides¹² were mostly active against gram positive bacteria¹³.

An antibiotic activity of *B. pumilus* and *B. subtilis* has been reported against *Staphylococcus aureus* and *Micrococcus luteus*¹⁴. *Bacillus* species isolated from Jordanian soils shows antibacterial activity against the methicillin-resistant *Staphylococcus aureus*¹⁵. Cellulases, Subtilisins, and Amylases produced by *B. subtilis* had several industrial importances and were consumed by the laundry industries¹⁶. Fungi were extremely diversified worldwide¹⁷. On earth, there are approximately 1.5 million species of the fungi out of which 70 - 100,000 species were discovered and 95% of these have yet to be discovered¹⁸. Fungi are best source of antibiotics¹⁹ and to search antibiotics from them is very promising. Twenty best-selling medical drugs worldwide were fungal derived²⁰.

According to the International center of information on antibiotics, 338 species of fungi are able to produce antibiotics. In soil habitat, fungi compete against different other microorganisms and may induce antimicrobial production²¹. Filamentous fungi contaminated with broad spectrum antibiotics, inhibited by antibiotic resistant bacteria are able to produce the secondary metabolites. 20% of isolated antibiotics have been discovered from soil fungi²². Many species of fungi including *Penicillium*, *Aspergillus*, *Cladosporium* and Yeasts which were reported only to be the food^{23,24,25} are tremendous sources of industrially important enzymes and secondary metabolite production²⁶. Antibiotics produced by the fungal species are widely used in the chemotherapy²⁷ especially Fusidic acid, Cephalosporin and Penicillin which have both antifungal and antibacterial activity. Fungi represent an important source of potentially powerful new pharmaceutical products²⁸.

Nowadays, Pharmaceutical industries are investigating an array of fungi to increase the number of discoveries²¹. For many years ago, in many societies fungi have been recognized as palatable nutritious food and are now acceptable as a valuable source in pharmaceuticals for development of the medicines²⁸. Several thousand antibiotics have been isolated from soil microorganisms since the discovery of penicillin, but unfortunately these have been limited only to fifty, most of them being too toxic to humans²⁹. According to the food and drug administration approximately 80% of antibiotics produced from natural habitat are fed to animals and only 20% of them are used to treat infections in the humans.

The objective of this study was to find antibiotic producing microorganisms and to check their ability to inhibit the growth of *S. aureus* and *E. coli*.

MATERIAL AND METHODS:

Collection of Sample: Soil samples from different areas were collected *i.e.*, the sampling is classified based on its micro and macro environment (waste polluted soil) (normal street soil) and (agricultural soil), within Quetta city. Approximately 1 kg of soil sample was collected for further processing. Soil sample was collected in such way to get the

soil of crust and depth of at least 6 inches with the help of sterile spatula and placed in sterile plastic bags for transportation to laboratory.

Preparation of Soil Sample: Three sterile test tubes were taken, marked and labeled for each soil sample and were filled with 10 ml of distilled water. A soil suspension is made by adding 1 g of the soil sample in 10 ml of distilled water in a first test tube for each soil sample and is vortexed. 1 ml of this solution is taken and transferred to the second test tube and is vortexed and from second to third. Hence each soil sample was serially diluted in a laminar air flow.

Isolation of Microorganisms: Media was prepared for isolation of bacteria and the principle media used for this purpose was nutrient agar medium. An amount 1000 ml of distilled water in a beaker was taken and 28 g of Nutrient agar powder was dissolved in it followed by sterilization in an autoclave at 121 °C for 15 min and allowed to cool. After that media was poured in Petri plates and allowed to solidify and placed in an incubator at 37°C for 24 h in order to check its sterility. Whereas for the isolation of fungi Sabouraud dextrose agar (SDA) and Potato dextrose agar (PDA) was used.

Inoculation of Sample: The samples were inoculated on nutrient agar plates in duplicate for bacterial species isolation. An amount 0.1 ml of each soil sample from selected dilutions (usually 1:100 and 1:1000) were taken and poured using pour plate method on labelled nutrient agar plates. The Petri dishes were then inverted followed by incubation for 24 h at 37 °C to obtain the isolated colonies. For fungus growth, aliquots of 0.1 ml of the last two dilutions were inoculated on two SDA and PDA plates followed by incubation at 28 °C for 3 - 5 days.

Sub Culturing of Microorganisms: Bacterial colonies with clear margins was picked and sub-cultured on fresh nutrient agar plates using sterile loop using streak plate method in laminar air flow to purify the isolates followed by incubation for 24 h at 37 °C. For fungi, each discrete colony on SDA and PDA plates were further inoculated on fresh SDA and PDA plates followed by incubation at 28°C for 3 - 5 days.

Staining Characterization: Gram staining and spore staining was performed of the isolated and sub-cultured bacterial colonies. In gram staining a clean glass slide was taken. Smear was prepared by heat fixing and air drying. Drop of crystal violet was added on smear and allowed to stand for 60 sec and then washed with the distilled water. Then gram iodine was added and stands for 30 sec. After though decolorizer was added to title the slide followed by safranin and stand for 60 sec and was washed and dried and viewed under 100X microscope lens whereas in spore staining a clean glass slide was taken. A drop of normal saline was added on a slide then colonies of bacteria were picked using sterile loop from isolated sub-cultured colonies grown on nutrient agar plates in a laminar air flow and allowed to dry. Then Malachite green was added on smear and steam for 5 - 7 min. Safranin was added which act as counter stain and was allowed to stand for 60 sec. Slide was then washed and dried and viewed under 100X microscope lens.

Biochemical Characterization: For the identification of bacterial isolates biochemical tests were performed as described by the Bergey's manual *i.e.*, IMViC tests (indole test, citrate utilization test, catalase test, MR-VP test), triple sugar iron test (TSI), oxidase, urease, nitrate reduction and blood hemolysis.

Microscopic and Macroscopic Examination of Fungi Isolates: Microscopic examination of each fungal isolates was done by picking fungi mycelia with the help of a sterilized needle and was placed on a glass slide containing a drop of lactophenol cotton blue stain, and then covered with the cover slip and was viewed under microscope using 40 X and 100X objective lens of the microscope.

The macroscopic examination of fungi isolates was carried out by comparing the fungi isolate with the Pictorial atlas of soil and seed fungi. Some morphological features were observed that includes physical appearance, color and growth pattern of each fungus colony on SDA and PDA medium.

Test Microorganisms for Antimicrobial Activity Determination: Test organisms *i.e.*, *E. coli* and *S. aureus* were obtained from local private clinical

laboratory and tested for antimicrobial activity of antibiotic producing isolates using agar well diffusion method.

Screening of Antimicrobial Activity: For antibiotic production, Mueller Hinton agar (MHA) media was prepared by adding 17 g of MHA media in 500 ml of distilled water and autoclaved at 121°C for 20 minutes. After sterilization, the media was cooled and poured in Petri dishes and kept in incubator at 37 °C for 24 h to check its sterility. Two test tubes were taken containing 2 ml of sterilized nutrient broth. Test organisms *i.e.*, *E. coli* and *S. aureus* were inoculated in it and kept in shaker incubator for 24 h. After incubation, sterilized cotton buds were dipped in it and swabbed on MHA plates. Wells were made on MHA plates using sterile borer. Isolated and sub-cultured bacterial and fungal colonies were inoculated in test tubes containing 5 ml of NB for bacteria and PDB for fungi isolates were kept in shaker incubator at 37 °C for 24 h. After incubation, were centrifuged at 6000 rpm for 10 min and their supernatants were poured in the wells and kept in incubator for 48 hr. Zones of inhibition were observed.

RESULTS: A soil samples was collected *i.e.*, from (waste polluted soil, normal street soil and agriculture land soil). Colonies were observed in the crowded plate. Colonies showing clear margins were sub cultured on the fresh medium plates.

After incubation colonial morphology was observed. A total five culture strains of bacteria and five fungal isolates were selected from all of the soil samples *i.e.*, (waste polluted soil, normal street soil and agriculture land soil). The selected culture strains of bacteria were then subjected to gram staining, spore staining and biochemical characterization tests and their results are shown in **Table 1**. The results of the biochemical tests were checked using Bergey's manual of systematic bacteriology; as a result their morphological features were clearly observed.

The selected isolated culture strains of bacteria were identified as *Micrococcus roseus*, *Brevibacterium* sp, *Bacillus subtilis*, *Bacillus anthracis* and *Bacillus cerus* whereas the microscopic examination of fungal isolates was carried out using lactophenol cotton blue staining and its macroscopic examination was done by comparing the fungal isolate with the Pictorial atlas of soil and seed fungi as shown in **Table 2**. The fungal isolates were identified as *Trichocladium opacum*, *Rhizocotania* sp., *Epicoccum nipponicum*, *Aspergillus niger* and *Cladosporium cladosporides*. The identified cultures of bacteria and fungi were then checked for antibiotic production activity using agar well diffusion method. The zones of inhibition were observed against the test bacteria (*S. aureus* and *E. coli*) as shown in **Fig. 1** and **Fig. 2** and **Table 3** and **Table 4**.

TABLE 1: IDENTIFICATION OF BACTERIAL ISOLATES COLLECTED FROM VARIOUS SOIL

Isolates Codes	Colonial morphology					Cellular characteristic					Biochemical characterization							Identified organism	
	Whole colony	Surface texture	Edge	Pigment	Elevation	Gram stain	Spore stain	Indole	Sulphur	Motility	Citrate utilization	Catalase	MR-VP	TSI	Oxidase	Urease	Nitrate reduction		Hemolysis
N1B	circular	smooth	entire	Pink	slightly convex	+	-	-	-	-	-	+	+/[d]	A/A	[d]	-	[+]	-	<i>Micrococcus roseus</i>
N2A	irregular	smooth	entire	yellow	convex	+	-	-	-	-	[d]	+	-/-	K/A	-	+	[-]	+	<i>Brevibacterium p</i>
N2B	irregular	dry or rough	undulate (wavy)	white, dull	umbonate	+	+	-	-	+	+	+	-/+	A/NC	-	-	+	+	<i>Bacillus subtilis</i>

N3A	irregular	mat or granular	entire to undulate	whitish to cream	raised	+	+	-	-	-	+	+	[d]/+	A/A	-	-	+	-	<i>Bacillus anthracis</i>
N3B	irregular	dry	undulate	whitish to cream	flat	+	+	-	-	+	+	+	-/+	A/NC	[+]	[-]	+	+	<i>Bacillus cereus</i>

Note: + 90% or greater positive, - 90% or greater negative, [d] 26 - 75% of strains are positive, [+] 76 - 89% positive, [-] 76 - 89% negative, K/A Glucose fermentation only, Peptone catabolized, A/A Glucose, lactose or Sucrose fermentation, A/NC Ferment sugar but did not grow in anaerobic environment of butt

TABLE 2: IDENTIFICATION OF FUNGAL ISOLATES

Isolate Codes	Colonial morphology	Identified organism
S1A	Short, simple branched conidiophores, bearing single conidia apically. Dark green, aleuriosporous, ellipsoidal, clavate Conidia, usually 2 to 3 transversely septate, rarely 4-septate, warty marginally, slightly curved, with conspicuous pedicels	<i>Trichocladium opacum</i>
S1B	Pale brown hyphae, branched, septated and constricted closely near the main hyphae. Conidia not formed. Monilioid cells usually formed. Sclerotia pale brown to brown, discrete or aggregated, various in shape and size	<i>Rhizoctonia</i> sp.
S1C	Brown, porodochia discoid, composed of pseudoparenchymatous stromata and conidia. Stromata mostly brown, globose. Lacking conidiophores. Conidiogenous cells short, hyaline or pale brown, aggregated or simple on stromata, holoblastic, cylindrical. Conidia also formed on simple conidiogenous independent cells are ellipsoidal or subglobose, slightly constricted at septum and occasionally formed in chains	<i>Epicoccum nipponicum</i>
S1D	Pale brown or hyaline conidiophores usually simple, erect, thick walled, with basally foot cells, globose vesicles. Composed of catenulate conidia uniseriate or biseriaphialides on vesicles and phialides acutely tapered at apex. Conidia are single celled, phialosporous, black in mass, minutely echinulate	<i>Aspergillus niger</i>
S3A	Erect, pale brown, branched conidiophores bearing catenulate conidia in each branch. Blastosporous, pale brown or hyaline, ellipsoidal, ovate, cylindrical, subgloboseconidia, Irregular in shape, apiculate at one end, often truncate at other end	<i>Cladosporium cladosporioides</i>

TABLE 3: INHIBITION ZONE SHOWN BY DIFFERENT BACTERIAL ISOLATES AGAINST TEST ORGANISMS

Isolate Codes	Test organisms	
	<i>S. aureus</i>	<i>E. coli</i>
N1B	-	-
N2A	-	-
N2B	+	+
N3A	+	+
N3B	-	+

Note: + means active against the target bacteria, - means no activity

TABLE 4: INHIBITION ZONE SHOWN BY DIFFERENT FUNGAL ISOLATES AGAINST TEST ORGANISMS

Isolate Codes	Test organisms	
	<i>S. aureus</i>	<i>E. coli</i>
S1A	-	+
S1B	-	-
S1C	+	-
S1D	+	-
S3A	+	+

Note: + means active against the target bacteria, - means no activity



FIG. 1: CLEAR ZONES OF BACTERIAL ISOLATES

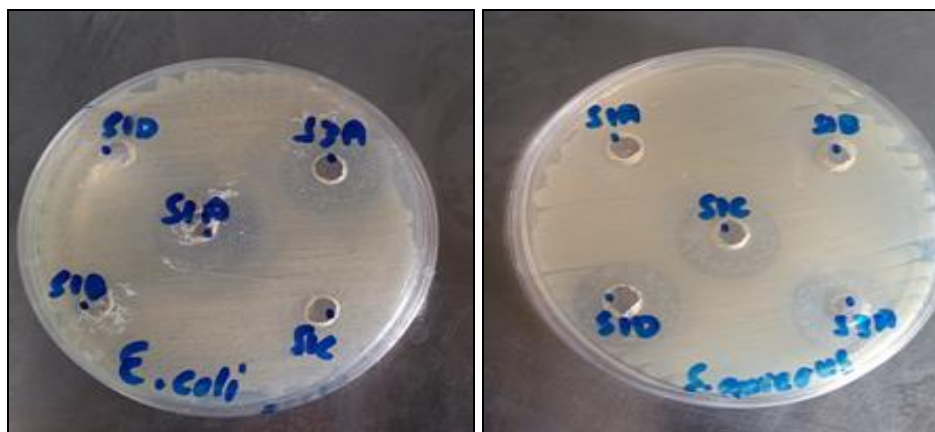


FIG. 2: CLEAR ZONES OF FUNGAL ISOLATES

DISCUSSION: In searching for new antibiotics, screening of microorganisms through relatively rapid and simple methods has been done for antibiotic production. Antibiotic production is a main feature of several kinds of soil microorganisms *i.e.*, bacteria and fungi and may thereof represent a survival mechanism. Variation in temperature may also affect the synthesis of antibiotic production. The bacteria isolated from soil shows antibiotic activity under normal growth condition and were found to inhibit some gram positive as well as some gram-negative organism. The isolates of bacteria were not organic acid producer but they are antibiotic producers^{30, 31}.

In present study bacteria and fungi were isolated from soil samples (waste polluted soil, normal street soil and agriculture land soil). The selected culture strains of bacteria were then identified by techniques, like gram staining, spore staining and biochemical characterization tests and microscopic examination of fungal isolates was carried out using lactophenol cotton blue staining and its macroscopic examination was done and both

bacterial and fungal isolates were subjected to test microorganism (*S. aureus* and *E. coli*) in order to check their ability to produce antibiotics using agar well diffusion method.

In our study results indicates that *Bacillus* species with the highest number of isolates produces clear zone of inhibition against test microorganisms. *Bacillus* species were dominant to shows antibiotic activity against *S. aureus* and *E. coli*. This finding is also corroborating the findings of Ahmed *et al.*, 2013 who screened soil microorganisms for antibiotic production and reveals that only *Bacillus* species exhibited antibacterial activity of all bacteria isolated³².

In an average of about 10^8 cells per gram rhizobacteria are found in the soil³³. Isolation of similar species from soil samples has been reported by other workers elsewhere^{34, 35}. The antimicrobial activity from a sediment habitat and resistance to antimicrobials of bacteria can easily explain the persistence and selection of such strains in this particular ecology³⁶.

On agar media cultural characteristics displayed by bacteria, were used to identify bacteria because of their specific and different growth patterns³⁷. It has been reported that *Bacillus* species and other spore forming bacteria carry genes for the production of antibiotics and breakdown of diverse carbon source³⁸. It has been reported that Bacitracin produced by *Bacillus* species inhibits both *E. coli* and *S. aureus*³⁸. Hassan et al., 2014 identified fourteen isolates of antibiotic producing *Bacillus* species from soil¹¹. For the synthesis of secondary metabolites *Bacillus* species are well known with remarkable diversity both in its function and structure³⁹. There is an argument with Aslim et al., 2002 who documented that strains of *Bacillus* had greater effects on gram positive bacteria as compared to gram negative bacteria⁴⁰.

B. subtilis has the potential to produce antibiotics and has been recognized for past 50 years. *B. subtilis* is an endospore forming rhizobacterium⁴¹. Sonenshein et al., 2002 collected several wild type *B. subtilis*, having the potential to produce more than two dozen of antibiotics⁴¹. *B. subtilis* C126 strain from sugar cane fermentation have the potential to produce polypeptide antibiotic, Bacitracin. Production of Bacitracin by *B. subtilis* is a pH dependent which gave maximum production at pH of 7.8 - 8. Strains of *B. cereus* from a soil sample have the ability to produce Bacteriocin and was active against most gram positive but not against gram negative bacteria. M15 strain of *B. cereus* possesses inhibitory effect against both gram positive and gram-negative bacteria.

Bacilli are predominant soil bacteria widely used in industrial applications, particularly antibiotics production having medically, agriculturally and veterinary importance^{42, 43}. *Bacillus* species preferred hosts for the production of many improved and new products used in genomic and proteomics⁴⁴. To enhance the yield of Bacitracin it is possible to clone and amplify the gene coding for some key enzymes in the biosynthetic pathways of Bacitracin.

In the present study, we also identified that filamentous fungi contaminated with extraordinary levels of broad spectrum antibiotics inhibited by antibiotic resistant bacteria are able to produce

antibiotics with bactericidal activity. Among identified fungal isolates *Epicoccum nipponicum*, *Aspergillus niger*, *Cladosporium cladosporides* and *Trichocladium opacum* produces clear zone of inhibition against test microorganisms.

Similar studies on distinctive fungal species were carried out by different scientists. Makut and Owolewa, 2011 screened the fungal isolates isolated from soil for antibiotic production. Results revealed that *Candida albicans* was inhibited by all fungal isolates whereas *E. coli* were inhibited by *Rhizopus stolonifera* and *Aspergillus fumigatus*. *Trichoderma viride* and *Alternaria alternata* not inhibit *Staphylococcus aureus* whereas *Pseudomonas aeruginosa* was not inhibited by *Curvularia lunata*, *Aspergillus flavus* and *Cladosporium herbarum*⁵.

Svahn et al., 2012 recognized sixty one strains of filamentous fungi predominantly various *Aspergillus* species were identified. Majority of *Aspergillus* strains shows antibiotic activity against beta lactamase producing *E. coli*, methicillin-resistant *S. aureus*, *Enterococcus faecalis* and *Candida albicans*⁴⁵. Miyake et al., 2009 reported that many *Aspergillus* species have been able to produce antioxidants⁴⁶. Gugnani 2003 reported that some *Aspergillus* species are utilized industrially for various enzymes production⁴⁷.

Ifediora 2011 conducted a study to determine the presence of fungi that has the ability to produce antibiotics in rhizosphere and sewage and check their antibiosis potency on test microorganisms (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). Six species of fungi isolated from the soil samples were examined for their antimicrobial activities which includes; *Mucor* sp., *Curvularia* sp., *Aspergillus* sp., *Penicillium* sp., *Drechslera* sp., *Rhizopus* sp. Two species; *Aurebasidium* sp. and *Cephalosporium* sp. were isolated from the sewage. All the fungal isolates were found to inhibit the growth of at least one of the test organisms. *Bacillus subtilis* was inhibited by the *Rhizopus* sp., *Mucor* sp., *Drechslera* sp., *Aurebasidium* sp. and *Curvularia* sp. *Pseudomonas aeruginosa* was inhibited by all the strains except *Cephalosporium* sp. and *Curvularia* sp. Only *Curvularia* sp. and *Aurebasidium* sp. inhibit *E. coli* whereas

Staphylococcus aureus was inhibited by the *Mucor* sp., *Cephalosporium* sp., *Aurebasidium* sp. and *Drechslera* sp. *P. aeruginosa* was inhibited by all strains except *Penicillium* sp., *Curvularia* sp., *Cephalosporium* sp. and *Rhizopus* sp.⁴⁸.

Idris et al., 2013 investigated the antimicrobial activity of endophytic fungi isolated from medicinal plant *Kigelia africana*. *Aspergillus* sp., *Aspergillus flavus*, *Cladosporium* sp. and *Curvularia lunata*, and three unknown species were screened for antibacterial test against *Staphylococcus aureus*, *E. coli* and *Bacillus subtilis*. The inhibition zones ranged from 14 - 37 mm⁴⁹.

Muhsinand Mohammad, 2012 checked antibacterial activity of fungi against *S. aureus* and *E. coli*. The inhibition zones by fungal extracts ranged from 22 - 28 mm in diameters. MIC test indicates that extract of *D. australiensis* exhibit minimal inhibition ranges from 12.5 - 6.25 ug/ml against *S. aureus* and *E. coli*⁵⁰.

Species of *Trichocadium* were frequently isolated from the soil. Pothiraj et al., 2006 isolated various species of fungi i.e., *Aspergillus niger*, *Aspergillus terreus* and *Rhizopus stolonifer* from a contaminated cassava waste soil by primary selection, serial dilution and pour plate technique and reported the production of cellulase by using solid state fermentation technique⁵¹. Sathyaprabha et al., 2011 isolated fungi namely, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus nidulans* and *Aspergillus versicolor* from crude petroleum oil contaminated soil⁵². Damisa et al., 2011 isolated strain of *Aspergillus niger* from soil samples in zaria. The samples were collected from different soil environment i.e., compost soils, rice growing field, street soil, flower beds, maize farm and fallow farm land. They were screened for cellulolytic efficacy.

However, microorganisms with higher α amylase activities facilitate the discovery of novel amylase used for industrial applications⁵³. Gautam et al., 2010 screened cellulolytic fungi isolated from municipal solid waste soil. Results clearly indicate that cellulase activity of *Trichoderma* sp. and *Aspergillus fumigatus* were relatively higher and *A. niger*, *Aspergillus flavus*, *Aspergillus nidulans*,

Alernaria species and *Penicillium* species indicates moderate activity while *Fusarium* species, *Humicolas* species and *Torula* species showed low activity⁵⁴. Mukunda et al., 2012 isolated fungi from soil of Western Ghats and screened for the production of important hydrolytic enzymes such as Cellulose, Protease, Amylase, CMCase, Lipase and pigment production. Results indicate that *Trichoderma*, *Aspergillus*, *Penicillium* and *Cladosporium* species were predominated⁵⁵.

CONCLUSION: The present study was an attempt to identify and characterize versatile strains of bacteria and fungi and to check their ability for antibiotic production. A number of different bacterial and fungal isolates were found producing clear zone of inhibition against the test microorganisms i.e., *S. aureus* and *E. coli*. The study revealed that bacterial and fungal species have potential of antibiotics production. The potential antibiotic producer bacterial species identified include *Bacillus* strains i.e., *Bacillus subtilis*, *Bacillus anthracis* and *Bacillus cerus*.

Whereas among fungal species *Trichocladium opacum*, *Epicoccum nipponicum*, *Aspergillus niger* and *Cladosporium cladosporides* were dominated. This study may contribute in providing information on the antibiotic producing microorganisms in soil. Further characterization, purification, and structural elucidation are recommended to know the novelty, quality and commercial value of these antibiotics.

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REFERENCES:

1. Nikodinovic J, Barrow KD and Chuck JA: High frequency transformation of the amphotericin-producing bacterium *Streptomyces nodosus*. Journal of microbiological methods 2003; 55(1): 273-277.
2. Demain AL: Antibiotics, natural products essential to human health. Medicinal research reviews 2009; 29(6): 821-842.

3. Baltz RH: Marcel Faber Roundtable, is our antibiotic pipeline unproductive because of starvation, constipation or lack of inspiration? *Journal of Industrial Microbiology and Biotechnology* 2006; 33(7): 507-513.
4. Molinari G: Natural products in drug discovery, present status and perspectives. *Pharmaceutical Biotechnology* 2009; 655: 13-27.
5. Makut M and Owolewa O: Antibiotic-producing fungi present in the soil environment of Keffi metropolis, Nasarawa state, Nigeria. *Eubacteria* 2011; 10(18): 19.
6. Hussein AA and AL-Janabi S: Identification of bacitracin produced by local isolate of *Bacillus licheniformis*. *African journal of Biotechnology* 2006; 5(18): 160-161.
7. Waites MJ, Morgan NL, Rockey JS and Higton M: *Industrial Microbiology an Introduction*, Blackwell Publisher London 2008.
8. Lisboa MP, Bonatto D, Bizani D, Henriques JA and Brandelli A: Characterization of a bacteriocin-like substance produced by *Bacillus amyloliquefaciens* isolated from the Brazilian Atlantic forest. *International microbiology* 2006; 9(2): 111-8.
9. Deng Y, Ju Y, Lu Z, Lu F, Yan D and Bie X: Identification of 4 -isovaleryl-spiramycin III produced by *Bacillus* sp. fmbJ *Archives of microbiology* 2014; 196(2): 87-95.
10. Awais M, Ali shah A, Abdul H and Hasan F: Isolation, identification and optimization of *Bacillus* sp. *Pak J Bot* 2007; 39: 1303-1312.
11. Hassan SA, Hanif E and Zohra RR: Isolation and screening of soil bacteria for potential antimicrobial activity. *Fuuast J Biol* 2014; 4(2): 217-219
12. Reddy PP: *Plant growth promoting rhizobacteria for horticultural crop protection*. Springer India 2014.
13. Ming LJ and Epperson JD: Metal binding and structure-activity relationship of the metallo-antibiotic peptide bacitracin. *Journal of inorganic biochemistry* 2002; 91(1): 46-58.
14. Tabbene O, Slimene IB, Djebali K, Mangoni ML, Urdaci MC and Limam F: Optimization of medium composition for the production of antimicrobial activity by *Bacillus subtilis* B38. *Biotechnology progress* 2009; 25(5): 1267-1274.
15. El-Banna NM, Quddoumi SS and Daradka H: Antimicrobial substances produced by bacteria isolated from different Jordanian sources that are active against methicillin-resistant *Staphylococcus aureus*. *African Journal of Biotechnology* 2007; 6(15): 1837-1839.
16. Morikawa M: Beneficial biofilm formation by industrial bacteria *Bacillus subtilis* and related species. *Journal of bioscience and bioengineering* 2006; 101(1): 1-8.
17. Perotto S, Angelini P, Bianciotto V, Bonfante P, Girlanda M, Kull T, Mello A, Pecoraro L, Perini C, Persiani AM and Saitta A: Interactions of fungi with other organisms. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology* 2013; 147(1): 208-218.
18. Kirk PM, Stalpers JA, Minter DW and Cannon PF: *Dictionary of the Fungi*. CABI Publishing. CAB International, Wallingford UK 2011.
19. Alii, Prasongsuk S, Akbar A, Aslam M, Lotrakul P, Punnapayak H and Rakshit SK: Hypersaline habitats and halophilic microorganisms. *Maejo International Journal of Science and Technology* 2016; 10(03): 330-345.
20. Concepcion GP, Lazaro JE and Hyde KD: Screening for bioactive novel compounds. In: *Bio-exploitation of Filamentous Fungi*. *Fungal Diversity Research Series* 6 2001.
21. Hyde KD: Increasing the likelihood of novel compound discovery from filamentous fungi. *Bio-exploitation of filamentous fungi* 2001; 77-91.
22. Berdy J: Bioactive Metabolites, a personal view. *J Antibot* 2005; 58(1): 1-26.
23. Butinar L, Zalar P, Frisvad JC and Gunde-Cimerman N: The genus *Eurotium* - members of indigenous fungal community in hypersaline waters of salterns. *FEMS Microbiology Ecology* 2005; 51(2): 155-66.
24. Cantrell SA and Baez-Félix C: Fungal molecular diversity of a *Puerto Rican* subtropical hypersaline microbial mat. *Fungal Ecology* 2010; 3(4): 402-405.
25. Zalar P, De Hoog GS, Schroers HJ, Frank JM and Gunde-Cimerman N: Taxonomy and phylogeny of the xerophilic genus *Wallemia*, Wallemiomycetes and Wallemiales, cl et ord. nov. *Antonie Van Leeuwenhoek* 2005; 87(4):311-328.
26. Carex: Roadmap for Research on Life in Extreme Environments. Carex Project Office, Strasbourg Cedex, France 2011.
27. Sethi S, Kumar R and Gupta S: Antibiotic production by microbes isolated from soil. *International Journal of Pharmaceutical Sciences and Research* 2013; 4(8): 2967.
28. Wasser SP: Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied microbiology and biotechnology* 2002; 60(3): 258-274.
29. Carlile MJ, Watkinson SC and Gooday GW: *The Fungi* Academic Press London 2000.
30. Todar K: *Journal of the Microbial World* 2009; 2: 231251.
31. Chandrashekhara S: *Tropical Journal of Pharmaceutical Research* 2010; 10: 150-200.
32. Ahmed RN, Sani AH, Ajijolakewu and Alamu FB: Soil screening for antibiotic - producing microorganisms. *Adv EnvironBiol* 2013; 7(1): 7-11.
33. Stein T: *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Molecular microbiology* 2005; 56(4): 845-857.
34. Amin M, Rakhisi Z and Ahmady AZ: Isolation and identification of *Bacillus* species from soil and evaluation of their antibacterial properties. *Avicenna Journal of Clinical Microbiology and Infection* 2015; 2(1).
35. Muhammad N, Akbar A, Shah A, Abbas G, Hussain M and Khan TA: Isolation optimization and characterization of antimicrobial peptide producing bacteria from soil. *The Journal of Animal and Plant Science* 2015; 25: 1107-1113.
36. Chelossi E, Vezzulli L, Milano A, Branzoni M, Fabiano M, Riccardi G and Banat IM: Antibiotic resistance of benthic bacteria in fish-farm and control sediments of the Western Mediterranean. *Aquaculture* 2003; 219(1): 83-97.
37. Sousa AM, Machado I, Nicolau A and Pereira MO: Improvements on colony morphology identification towards bacterial profiling. *Journal of microbiological methods* 2013; 95(3): 327-335.
38. Prescott ML, Harley PJ and Klein AD: *Microbiology*. 7th ed. Publishing Group 2008.
39. Baruzzi F, Quintieri L, Morea M and Caputo L: Antimicrobial compounds produced by *Bacillus* sp and applications in food. *Science against microbial pathogens: communicating current research and technological advances* 2011; 2: 1102-11.
40. Aslim B, Sağlam N and Beyatli Y: Determination of some properties of *Bacillus* isolated from soil. *Turkish Journal of Biology* 2002; 26: 41-48.
41. Sonenshein AL, Hoch JA and Losick R: *Bacillus subtilis* and its closest relatives. *From genes to cells*. American Society for Microbiology 2002; 3-5.

42. Akbar A, Ali I and Anal AK: Industrial perspectives of lactic acid bacteria for biopreservation and food safety. *The Journal of Animal and Plant Sciences*, 2016; 26(4): 938-948.
43. Sharga BM, Nikolaychuk VI and Maga MI: Comparative IR Spectrometry of antimicrobial substances derived antibiotic from *Bacillus*. *Mikrobiologiya* 2004; 15: 75-77.
44. Schallmey M, Singh A and Ward OP: Developments in the use of *Bacillus* species for industrial production. *Canadian journal of microbiology* 2004; 50(1): 1-7.
45. Svahn KS, Göransson U, El-Seedi H, Bohlin L, Larsson DJ, Olsen B and Chryssanthou E: Antimicrobial activity of filamentous fungi isolated from highly antibiotic-contaminated river sediment. *Infection ecology and epidemiology* 2012; 2(1): 11591.
46. Miyake Y, Ito C, Itoigawa M and Osawa T: Antioxidants produced by *Eurotium herbariorum* of filamentous fungi used for the manufacture of Karebushi, dried bonito Katsuobushi. *Bioscience, biotechnology and biochemistry* 2009; 73(6): 1323-1327.
47. Gugnani HC: Ecology and taxonomy of pathogenic *aspergilli*. *Front Biosci* 2003; 8(1-3): 346.
48. Ifediora: Studies on antibiotic production by fungi isolated from rhizosphere and sewage. *Gloria Rauchukwu* 2011.
49. Idris AM, Al-tahir I and Idris E: Antibacterial activity of endophytic fungi extracts from the medicinal plant *Kigelia africana*. *Egypt Acad J Biol Sci* 2013; 5(1): 1-9.
50. Muhsin TM and Mohammad HM: Preliminary screening of bioactive metabolites from three fungal species of *Drechslera* isolated from soil in Basrah, Iraq. *Journal of Basrah Researches Sciences* 2012; 38(2): 44-53.
51. Pothiraj C, Balaji P and Eyini M: Enhanced production of cellulases by various fungal cultures in solid state fermentation of cassava waste. *African Journal of Biotechnology* 2006; 5(20): 1882-1885.
52. Sathyaprabha G, Panneerselvam A and Muthukkumarasamy S: Production of Cellulase and Amylase from wild and mutated fungal isolates. *E-Journal of Life Sciences* 2011; 1(1): 39-45.
53. Damisa D, Ameh JB and Egbe NE: Cellulase Production by native *Aspergillus niger* obtained from soil environments. *Fermentation Technology and Bioengineering* 2011; 1: 62-70.
54. Gautam SP, Bundela PS, Pandey AK, Awasthi MK and Sarsaiya S: Screening of cellulolytic fungi for management of municipal solid waste. *Journal of Applied Science for Environmental Sanitation* 2010; 5(4): 367-371.
55. Mukunda S, Onkarappa R and Prashith KT: Isolation and screening of industrially important fungi from the soils of Western Ghats of Agumbe and Koppa, Karnataka, India. *Science, Technology and Arts Research Journal* 2012; 1(4): 27-32.

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