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BIOTECHNOLOGICALLY POTENT HALOPHILIC FUNGAL BIODIVERSITY FROM MANGROVE ECOSYSTEM OF LASBELA, BALOCHISTAN

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Abstract

In this study, we have isolated the halophilic fungi from mangrove ecosystem and analyzed it for their biotechnological potential. Soil samples were collected from the mangrove's ecosystem of district Lasbela, Balochistan Pakistan. Temperature, pH and salt concentration of the soil were analyzed for each sample. Potato dextrose agar supplemented with 15 % salt (NaCl) was used for the initial isolation of filamentous fungal strains. The isolated fungi were initially identified with the help of growth characteristics on potato dextrose agar, structural detailed observation by microscopy and scanning electron microscopy. Total 19 different fungal species were analyzed for their halophilic and halotolerant capabilities. The fungi were then evaluated for biotechnologically important metabolites production (antibacterial, antifungal, antioxidant, polyphenolic compounds, xylanase, lipase, gelatinase, cellulases, amylase and protease). Majority of isolated species belonged to the genus *Aspergillus* (7) and genus *Penicillium* (7), 3 isolates belonged to genus *Alternaria* while one from *Fusarium* and one from the family *Pleosporaceae*. The antibacterial metabolites of the fungal isolates were found active against a range of bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Acinetobacter baumannii*. It was found that the fungal isolates from mangrove's ecosystem have a great potential for their biotechnological applications. Mangrove's ecosystems are rich in fungal biodiversity. The microbial biodiversity of such ecosystem can be a good source of biotechnologically important metabolites.

Key words: Aspergillus, Antibacterial, Enzymes, Microscopy, Mycology.

Introduction

Extremophiles, particularly eukaryotic microorganisms have been ignored in the past, they have has a great scientific research potential to be exploited for industrial applications as well as for human welfare (Ali, 2016). Fungus has long been serving the humanity in the form of antibiotics production, fermentation, similarly biodegradation of pollutants and synthesis of many catalytic metabolites (Ali, 2014a). The seas that cover nearly 71% of the surface of planet Earth contain about 35 g/L dissolved salt. Hypersaline environments are easily formed when seawater dries up in coastal lagoons and salt marshes, as well as in manmade evaporation ponds of saltern systems built to produce common salt by evaporation of seawater (Oren, 2002). Halophiles adapted to life at salt concentrations up to saturation are found in all three domains of life (Anton *et al*., 2002). Balochistan coastal area is occupying about 18,350 acres (Mirza *et al*., 1988). The coastal line consists of three major parts viz Miani Hor, Kalmat Khor and Gawadar Bay.

Fungal growth in an environment having high salt concentration was not proven till 2000. Gunde-Cimerman *et al*., (2000) and Oren, (2002) isolated fungi from solar saltern. Organisms capable of growth at 3 M salt concentration are considered as halophiles. Before this the concept of halophile was generally related to eubacteria and archaea. Halophilic organisms can withstand and exhibit their metabolic activities in the presence of high level of crystalline salt NaCl which is generally regarded as growth retarder as this creates hypertonic condition. Fungi that grow in such environment are adapted to higher concentration of salt and even to high degree of pH (Gunde-Cimerman *et al*., 2000). Fungal adaptation and their adjustment in high salt environment are different than most of prokaryotic organisms (Plemenitas *et al.*, 2008). Extreme environmental conditions have long been considered as the inhabitants of prokaryotes before 2002 (Oren, 2002).

Fungal organisms' presence in saline environment is because of their adoptive mechanisms. The biodiversity of fungi is greater than it was thought to be particularly in marine environment (Cuomo *et al*., 1988). Fungal organisms isolated from mangrove roots have been shown to produce different extracellular enzymes capable of degradation of various organic matter and pollutants (Shearer *et al.*, 2007). Various species of fungi isolated from high salt environment of mangrove ecosystem were analyzed for their biotechnological potential values. The isolated organisms were also tested for their antibacterial, antifungal and antioxidant activities. Their growth under various abiotic environmental factors was evaluated. Different enzymatic tests revealed production of versatile enzymes having good possible industrial applications. In addition to enzymes, extremophiles are a source for a large variety of metabolites that are of interest for various biotechnological applications.

The organisms inhabiting unusual environments are known as Extremophile. In simple words life existence at high temperatures, pressures, extremes of pH, salt concentration and ionizing radiation, such extreme conditions are well adopted by bacterial domain.

Extremophiles with their unusual properties signify both the abilities of these organisms to live in unusual environments and the possibilities for their economic exploitation (Dalboge, 1997).

Fungi are ubiquitous in variety of ecosystems. They may colonize on a wide range of substrates. The highest diversity of fungi is found in tropical regions, mainly in tropical forests (Hawksworth, 1991). Most of the fungi that can be found in extreme environments belong to the imperfect stage of the Ascomycota, which have been reported in mangroves, saline soils, marine sediments, sea water, salt marshes, and sand dunes (Domsch *et al*., 1993). The current study is focused on the characterization and biotechnological potential of the fungal isolates from saline environment of mangrove's ecosystem.

Materials and Methods

Sampling site description: Site selected for sample collection was mangrove ecosystem of Balochistan. The coastal belt of district Lasbela and the Miani-Hor ramsar site occupied by mangrove were selected for sampling. The Miani-Hore is about 60 km long and approximately 4 to 10 km wide. Patches of mangrove present at almost all along the lagoon. The Miani-Hore total area is about 370 km. It is occupied by three major species of mangrove. Initial screening was carried out while keeping concentration of salt (NaCl) 10% and aseptic procedures for halotolerant and halophilic fungal growth.

Sampling: Soil samples were taken aseptically in sterile containers. Wet soil below a few inches was taken after scratching the upper layers. Samples were taken from different parts from mangrove rich sites. While soil sampling, temperature of the site noted with respect to the month of season. Total hundred soil samples were taken. Each soil sample was diluted with saline water having NaCl concentration according to the salinity of the mangrove ecosystem.

Soil analysis: Soil analysis was performed by measuring pH of the soil by a pH meter. Moisture content was analyzed by calculating the difference in weight between a fresh soil sample and an overnight, oven dried soil sample. Salinity of the soil was measured with the help of salinity meter. Organic matter and the total organic carbon estimated by dichromate titration method. Total nitrogen content was assessed by Kjeldahl digestion method using a digester (Tecator DS-6) and distillation apparatus (Tecator, 1002).

Isolation of halophilic and halotolerant fungi: Halophilic and halotolerant organisms capable to grow at higher concentration of salt were isolated following the method followed by Ali *et al*., (2013) from the saline soil sample by serial dilution method and subsequent inoculation and incubation on Potato Dextrose Agar (PDA) supplemented with NaCl while keeping pH 7.4. Later on, obtained fungi (molds) were sub-cultured to get pure isolates.

Morphological identification of the isolates: Fungal identification was mainly based on physiological, morphological and molecular characteristics of the organisms. Visual and microscopic examinations were performed to know various specific characteristics features of the fungal isolates. Colony growth pattern from obverse and reverse of the culture plates of the isolates were noted. Color and texture along with other colonial characteristics of the isolated fungal species depicted their morphological features. On PDA after inoculation till maturation from initial growth along with color appearance and specific topographical appearances revealed pathways for morphological identification. Wet mount procedure was followed using lacto phenol cotton blue as a stain for light microscopy. Under 10 X, 20 X, and 80 X objective of high power field, detail of each isolate was examined and observations were recorded. Fungal hyphae, septation of hyphae, pattern of spores' formation and other structural fungal bodies helped in identification.

Scanning electron microscope (SEM) model S-3400N Hitachi having resolution of 3 nm with 30,000 times magnification was used for morphological observations. Representative specimens of the isolates grown on PDA were taken from each Petri plate for examination. Samples were loaded on adhesive stub (carbon stub) and labeled inside the safety cabin. Samples were loaded and through adjustment knobs magnification and resolution were adjusted to the required level. The isolates were identified to genus level with the help of culture growth characteristics, morphological features, compound microscopic examination and by SEM.

Characterization of the selected isolates

Growth of the isolates at different pH: All isolated fungi were subjected to grow at pH 5, pH 7 and pH 9 using PDA supplemented with 10 percent salt (NaCl). The media pH was adjusted using 2N HCl, NaOH and pH meter probe. All the isolates were incubated at 30^{o}C for 14 days. The growth characteristics as well as their dry and wet masses were determined at the end (Ali *et al*., 2013).

Growth of the isolates at different temperatures: All the fungal isolates were inoculated on PDA supplemented with 10 % salt and incubated at different temperatures (0-4^{o}C, 20^{o}C, 40^{o}C and 60^{o}C) for determining their capabilities to grow under different temperature conditions. The growth patterns along with dry and wet mass were determined after 14 days incubation (Ali *et al*., 2013).

Growth of the isolates at different salt concentrations: The PDA plates supplemented with different salt concentrations (0, 5, 10, 15 and 20%) were incubated at 30^{o}C for 14 days and the growth characteristics as well as dry and wet mass of the isolates were measured after incubation (Ali *et al*., 2013).

Screening for biotechnological potential: Fungal filtrates were prepared as suggested by (Ali *et al*., 2014b). All the Isolates were grown on enriched media for an incubation period of 7 days on Malt Extract Agar (MEA). After getting mature growth, spores from each isolate were dislodged to saline distilled water. Two ml spore suspension was added to 100 ml of Malt Extract Broth (MEB) and kept for a period of 14 days of incubation at 28-30^{o}C. Fungal mass free filtrate was obtained by filtration and the crude filtrate was then used for further tests (Ali *et al*., 2013).

Antibacterial activity determination: Agar well methods were performed for antibacterial activities following, (Akbar *et al.*, 2014). Fresh (24 h) target bacterial cultures (*Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Acinetobacter baumannii*) were adjusted at the rate of 1× 10^{8} CFU/mL in sterilized normal saline (0.9% NaCl) comparing with 0.5 McFarland. The bacteria were applied over the surface of Mueller Hinton Agar (MHA) using sterile cotton swab. Wells of 6 mm diameter were made with sterilized cork borer. The wells were filled with 100 µL of crude filtrate obtained from fungal isolates and the plates were incubated at 37^{o}C for 16-24 h. The clear zone around each well was recorded in millimeter (mm).

Antifungal activity determination: The antifungal activities of the isolates were determined using filtrate dilution and agar well methods. For filamentous fungi, the target fungal spores 1×10^{3} CFU/mL of normal saline were mixed with 1:1 dilution of Potato Dextrose Broth (PDB) and filtered sterilized filtrate of the isolates. All test tubes were kept at 30^{o}C for one week incubation. The growth inhibition was observed and recorded after incubation. For yeast species, the agar well method was applied to determine the activities of fungal isolates.

Antioxidant activity: The antioxidant activities of filtrate were evaluated by DPPH radical scavenging assay following Akbar *et al*., (2014). One milliliter filtrate were mixed with 2 mL of 0.1 mM DPPH solution and incubated for 30 min. The potential of DPPH free radical reduction was evaluated at OD 517 nm. DPPH solution without filtrate and absolute methanol was used as control. Percentage of DPPH inhibition was calculated using the following formula:

$$\% \text{ Scavenging activity} = [(Ao - A1)/Ao] \times 100$$

where Ao = Control
A1 = Test sample

Screening for potential extracellular enzymes of biotechnological importance: Five enzymes such as amylase, cellulases, lipase, protease and xylanase were screened. Preparation of liquid media and assay was carried out as per standard procedure for different enzymes literatures consulting Ali *et al*., (2014b)**.** Screening for enzymatic potential carried out systematically using the following method:

Plate screening method: Enzymes production and their extracellularly secretion were checked with slight modification in the methodology applied by (Sohail *et al*., 2009) and (Ali *et al*., 2013). To trigger the specific enzyme production a specific substrate were used in quantity of 1% (w/v) in a volume of 100 ml broth. All the isolates were tested for their ability to produce various targeted enzymes. Enzyme amylase production was checked by using soluble starch, carboxy methyl ecellulose for cellulase and Tween 80 as substrate for lipase. Similarly, casein for the production of protease and xylan for xylanase screening. To check the production of specific enzymes on solid media, the petri plates having solid media provided with substrate 1% (w/v) were used. Wells were made with help of sterilized cork borer having diameter of 6mm. Volume of 100 µl of filtrate added in each well. To settle the filtrate plates were kept at 4^{o}C for an hour. After that incubated at 30^{o}C for 24 hours. Enzymatic activity depicted from the clear zone formation around the well (in mm) and the diameter measured with scale. Detection of amylase and xylanase production were made with the help of iodine solution, while cellulase enzymes was screened with help of Congo red reagent. Similarly, for protease 1N solution of HCl was used and saturated copper sulphate solution for lipolysis analysis.

Gelatin hydrolysis: To find gelatinase production PDA was supplemented with 5% salt along with addition of 2% gelatin prepared in slants and inoculated with fungal isolates spores. Incubated at 30^{o}C and examined daily after two days for liquefaction of gelatin. The slants were observed for 20 days and results were noted.

Results

Soil analysis: While sampling for halophilic fungus isolation, the sampling site soil was analyzed for pH, salinity, temperature and organic matter in percentage. The results showed, the average temperature (24±1^{o}C), pH (8±0.5), salinity (38±2 PSU) and total organic content (8.02 ±.01 %) were recorded.

Identification and examination of halophilic halotolerant fungal isolates: Growth pattern on solid media from initial stages till to maturity along with microscopy at different stages of growth and simple and electron microscopy helped in the identification of each isolated fungal species. Electron microscopy revealed fine structural details. Initially active growth of all organisms was white and later on turned into various colors due to spore formation. It has been observed in the morphological analysis that out of the total 19 isolates 7 belonged to the Aspergillus genera, whereas 7 to the genera of *Penicillium*, 3 to the genus *Alternaria*, one *Fusarium* species and to the family *Pleosporaceae* respectively (Table 1). The macroscopic and microscopic pictures of selected fungal isolates are mentioned in (Fig. 1a,b,c,d,e,f).

Table 1. Growth characteristics on solid media (Potato Dextrose Agar).

Code	Fungal species	Color	Growth characteristics	Back color of culture plate
C1	*Aspergillus* spp.	Grey,	Velvety, granular, mound like raised colonies.	Cream color back ground.
C2	*Aspergillus* spp.	Dark grey	Granular velvety uniform growth	Cream color
C3	*Aspergillus* spp.	Grayish	Velvety	Cream color
C4	*Aspergillus* spp.	yellow	Granular appearance, dark grey spore formation	yellowish
C5	*Aspergillus* spp.	Light brown	Velvety granular with rises and ridges	Cream color
C6	Pleosporaceae Family	Dark yellow	Golden appearance smooth velvety granular	Dark brown
C7	*Penicillium* spp.	Grey	Velvety rise and fall	Off white
C8	*Aspergillus* spp.	Grayish	Velvety	Off white
C9	*Penicillium* spp.	Grey	Velvety granular	Off white
C10	*Penicillium* spp.	Grey	Velvety granular	Off white
C11	*Aspergillus* spp.	Light off white	Granular, spongy submerge	brownish
C12	*Pleospora* spp.	Grey	Velvety uniformed	Off white with brown patches
E1	*Fusarium* spp.	Grey	Velvety smooth, bluish at center	Yellow compact
E2	*Penicillium* spp.	Bluish grey	Velvety granular	Dark black
E3	*Alternaria* spp.	Light grey	Velvety granular	yellow
E4	*Penicillium* spp.	Bluish grey	Cottony granular	Off white
E5	*Alternaria* spp.	Grey	Light velvety	Yellowish
E6	*Penicillium* spp.	Grey	Velvety centrally curve	Off white
E7	*Alternaria* spp.	Grey	White cottony velvety, centrally bluish granular	Off white

Determination of dry mass of fungal mycelium on various parameters: Growth of isolates on various salt concentrations reflected that all the organisms showed their potential to grow at different salt concentrations. Organisms having code numbers C1, C3, C4, C6, C12 had shown high dry mass values on 10 % salt concentration. On 15% salt concentration organisms having code number C7, C9, C11, and E1 have shown high growth and dry mass. On 20% salt concentration organisms encoded as C2, C5, C8, C10, E2, E3, E4, E5, E6, and E7 have showed good growth with 0.5 to 0.6 grams of dry mass (Fig. 2). Growth and dry mass at provided various temperature ranges showed that all isolates have grown on all the three temperatures. On 20^{o}C temperature the encoded organisms C4, C6, C7, C8, C9, C10, C11, C12, E1, E2, E3, and E4 showed high dry mass (Fig. 3). On 40^{o}C temperature only two organisms, coded as C1 and E5 shown high growth. While at 60^{o}C temperature the organisms encoded as C2, C3, C5, E6, and E7 had shown higher growth. Isolates encoded as C1, C4, C7, E1, E2, E3, E4, E5, E6 had shown high growth at pH 5 while C2, C3, C5, C6, C8, C9, C10, C11, C12, and E7 had shown maximum growth at pH 9. The average growth and dry mass of all the isolates was higher at pH 9 compared to pH 7 and pH 5 (Fig. 4).

Antibacterial activity: The outcome of antibacterial potential by plate screening is shown in (Table 2). The antibacterial activities of the filtrate were different for different microbes (Fig. 5a). The inhibition zone ranges from zero to 20 mm. Filtrate from all isolates were found active against four target bacterial species used in this study. The action was equally noted against gram positive and negative bacterial species. The isolates with code C2, C11, C12, E6 and E7 were found active against all selected bacteria with broader zone of inhibition.

Antifungal activity: Antifungal potential of the isolates was examined by two methods. One ratio one dilution method for determining antifungal activity against molds and plate screening method was used to find out antifungal potential against yeast shown in (Table 3). Filtrate form the isolates C2, C6, C11 and E7 were found active against *A. niger*. While all the filtrates from almost all isolates were found active against *Saccharomyces boulardii* except C11. Among the isolates C1, C3, C9, C10, C12, E1, E4, E5 and E6 have shown high antifungal potential against yeast (Fig. 5b).

Screening for potential extracellular enzymes: All isolates were tested for their extracellular enzyme production. The results for each enzyme screening are mentioned in (Table 4). All the fungal isolates tested for five different extracellular enzymes production were noted positive for the production of enzyme at various degrees. The results in form of halos for different enzyme production are shown in Fig. 6 a,b,c,d. The fungal isolate with code E1, E5 and E7 was found rich for the production of different enzymes such as amylase, cellulase and proteases. Among the isolated organisms C1, C7, C8, C9, C10, C11, C12, E2, E4 and E6 were found positive for various enzymes production with the formation of moderate halo around wells.

Hydrolysis of gelatin by fungal isolates: It was found in the study that the entire fungal isolate can hydrolyze the gelatin at different degree of intensity. Results of gelatin hydrolysis are shown in (Table 5). It was found that fungal isolates with code C1, C2, C3, C4, C5, C6, C7, C9, C10, C12, E5, and E6 hydrolyzed the gelatin in the test tube in first week of incubation period. Some isolates were found as late hydrolysers as they could hydrolyze the gelatin after 2 weeks of incubation, whereas E7 was found with very low and late activity of gelatin hydrolysis.

Screening for potential antioxidant activity: The antioxidant potential of different isolates determined by DPPH method is mentioned in (Table 5). The isolates with code C4, C10 and E1 were found rich in antioxidant compounds with the ability to scavenge about 93% of the DPPH free radical.

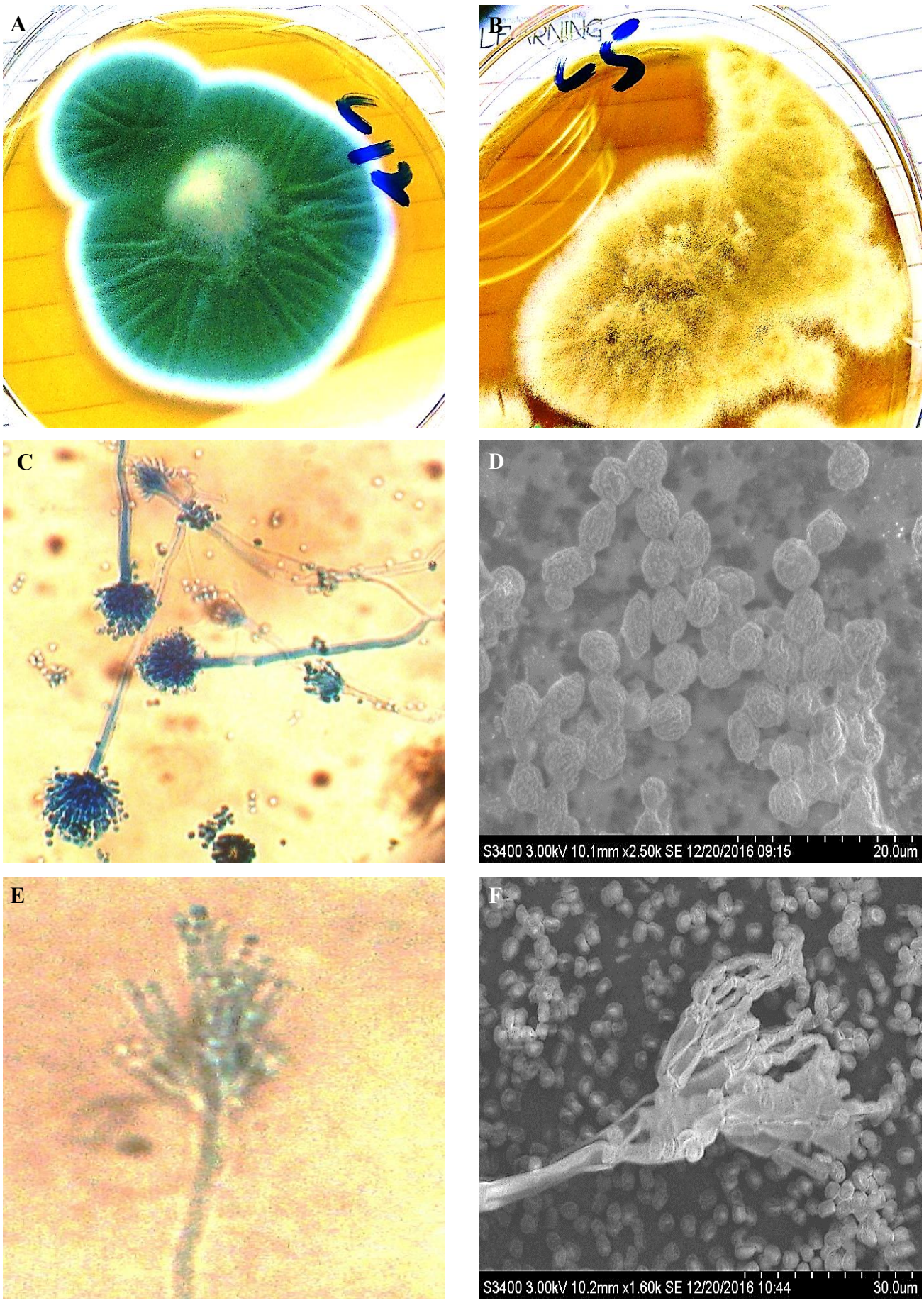

Fig. 1. (a, b) Macroscopic picture of isolates (c, d) Compound and Scanning Electron micrograph of the isolate C2 (e, f) Compound and Scanning Electron micrograph of the isolate C7.

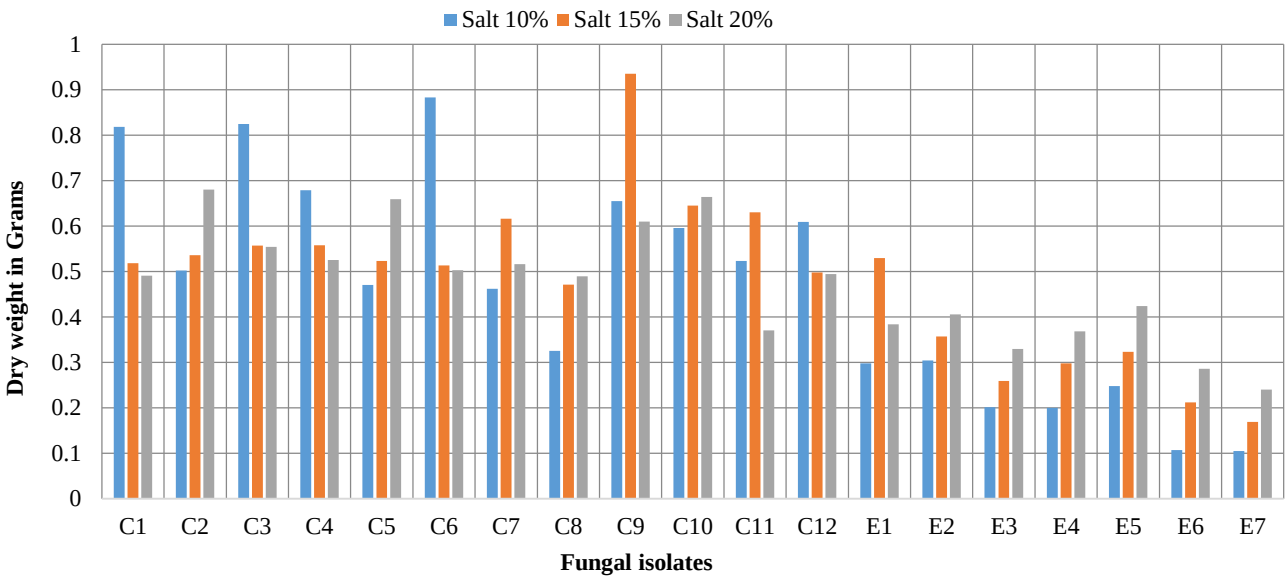

Fig. 2. Growth on potato dextrose agar with different salt concentration calculated in dry mass of organisms.

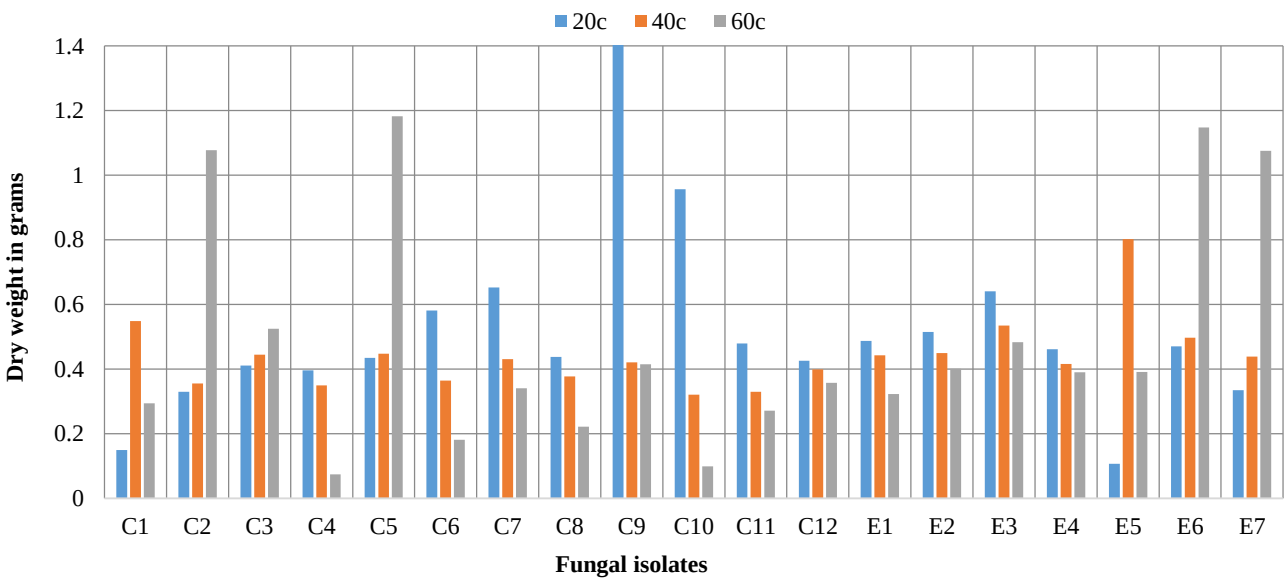

Fig. 3. Dry mass of the fungal isolates on potato dextrose agar at different temperature.

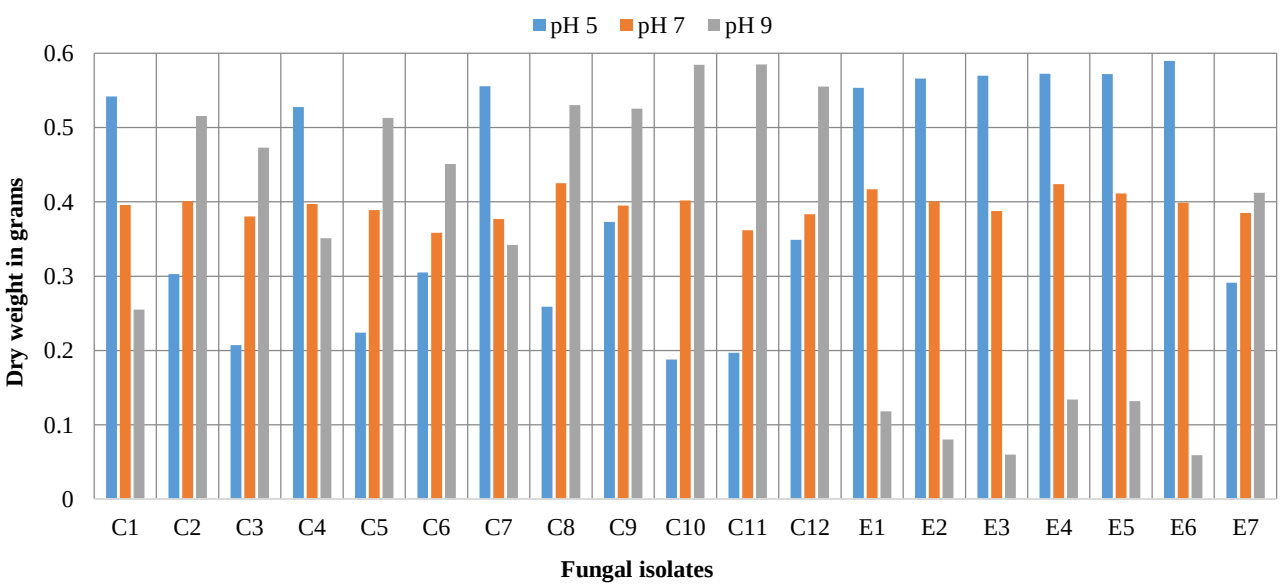

Fig. 4. Dry mass of the fungal isolates grown on potato dextrose agar with different pH.

Table 2. Antibacterial activity of the selected isolates against four different pathogenic bacteria.

Code	*P. aeruginosa*	*E. coli*	*S. aureus*	*A. baumannii*
C1	+	+	+	+
C2	++	+++	++	--
C3	+	++	+	+
C4	+	++	+	+
C5	++	++	++	--
C6	+	--	+	--
C7	--	++	++	--
C8	+	+	+	+
C9	+	++	+	--
C10	+	+	--	++
C11	++	+++	++	++
C12	+++	+++	+++	+
E1	+++	+	+	+
E2	++	+	++	++
E3	+ +	+	++	--
E4	+	+	+	++
E5	++	+	+	+
E6	++	++	+++	+++
E7	++	+++	+++	++

Note: + = 8-12 mm, ++ = 13-15 mm,+++ = more than 15 mm, =No growth *P. aeruginosa* = *Pseudomonas aeruginosa*, *E. coli* = *Escherichia coli*, *S. aureus* = *Staphylococcus aureus*, *A. baumannii* =*Acinetobactor baumannii*

Table 3. **Determination of antifungal activity of crude filtrate of the screened halophilic fungal isolates.**

Code	*Aspergillus niger*	*Saccharomyces boulardii*
C1	--	+++
C2	+	++
C3	--	+++
C4	--	+
C5	--	++
C6	+	+
C7	--	++
C8	--	++
C9	--	+++
C10	--	+++
C11	+	--
C12	--	+++
E1	--	+++
E2	--	--
E3	--	--
E4	--	+++
E5	--	+++
E6	--	+++
E7	+	++

Note: - = No activity, + = Low activity, ++ = Moderately active, +++ = Highly active

Table 4. Determination of enzymatic potential of the screened fungal isolates.

Code	Amylase	Xylanase	Protease	Lipase	Cellulase
C1	++	++	--	+	++
C2	-	-	+	-	-
C3	++	-	-	-	+
C4	-	-	-	+	+
C5	+	-	+	-	++
C6	-	-	-	+	+
C7	++	-	++	-	+
C8	+	+	++	-	++
C9	++	+	+++	-	++
C10	++	-	+	+++	+
C11	+++	+	+	-	+
C12	++	++	+++	-	+
E1	+++	+++	++	-	+
E2	+	-	+++	-	++
E3	-	-	+	+	+++
E4	+	++	++	-	++
E5	+++	-	+	-	+++
E6	++	+	++	-	++
E7	++	++	+++	-	+++

Note: + = 8-10 mm clear zone, ++ = 11-15 mm and +++ = more than 15 mm

Table 5. Gelatin hydrolysis and antioxidant activities percentage of halophilic fungal isolates.

Code	Gelatin hydrolysis	DPPH
C1	+++	30 %
C2	+++	17 %
C3	+++	25 %
C4	+++	90 %
C5	+++	53%
C6	+++	29%
C7	+++	29%
C8	++	35%
C9	+++	60%
C10	+++	80%
C11	++	69%
C12	+++	74%
E1	++	93.4%
E2	++	73.6%
E3	++	33%
E4	++	67%
E5	+++	71%
E6	+++	33.2%
E7	+	53%

Note: +++ = Hydrolysis observed after 7 days, ++ = Hydrolysis observed after 15 days, += Hydrolysis after 30 days of incubation

Discussion

Microbes are present everywhere in biosphere. They have the abilities to survive and propagate even in the harsh environment. Such environments are known as extreme environment and microbes reside there are called extremophile (Khan *et al*., 2020). These extreme conditions may be due to high or low temperature, pH, pressure, salt concentration and minerals compositions. Halophiles reside in high salt prevailed conditions (Gunde-Cimerman *et al*., 2000). Eukaryotic organisms (fungus) produce a variety of valuable secondary metabolites. Microbial origin metabolites are good source of enzymes as well as antibiotics (Fazal *et al*., 2011).

The antibiotics produced by organisms naturally inhabiting high salt environment is more effective in low available water conditions (Sepcic *et al*., 2011). All isolates have been grown in medium containing 10% NaCl salt while performing antibiotics screening tests. Activity was checked against gram positive *Bacillis subtilis* TISTR 008 and gram negative *Escherichia coli* TISTR 780. Most of the isolates shown antibacterial activity against both of the test organisms while majority shown good activity against *E. coli*. Few fungal isolates have shown antifungal activity as well. The isolates have multipotent potency by producing antibacterial as well as antifungal compounds.

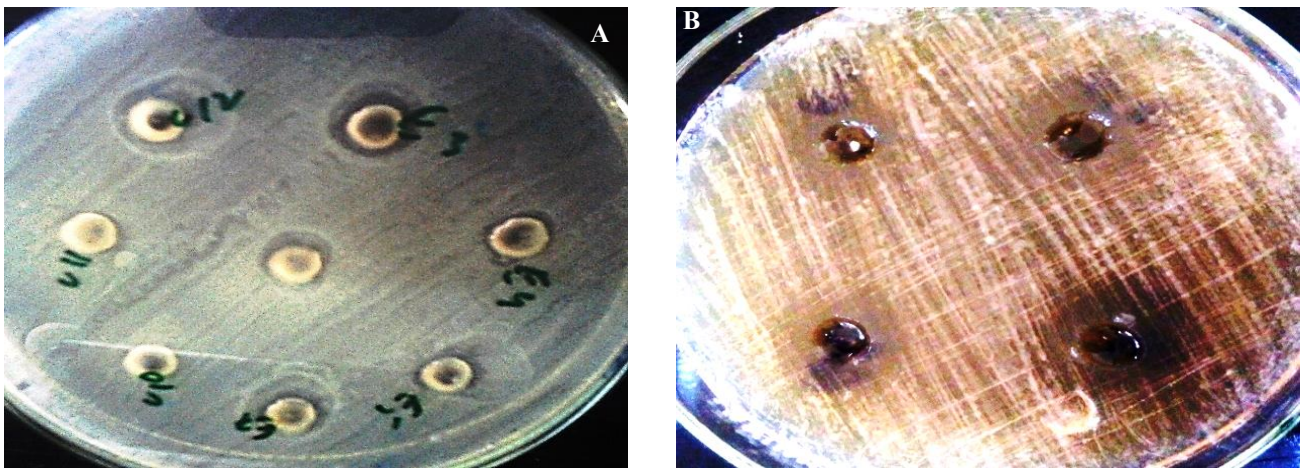

Fig. 5. Antimicrobial activity of different fungal isolates (a) activity against bacteria on Mueller Hinton Agar (b) activity against yeast spp. on Sabouraud Dextrose Agar.

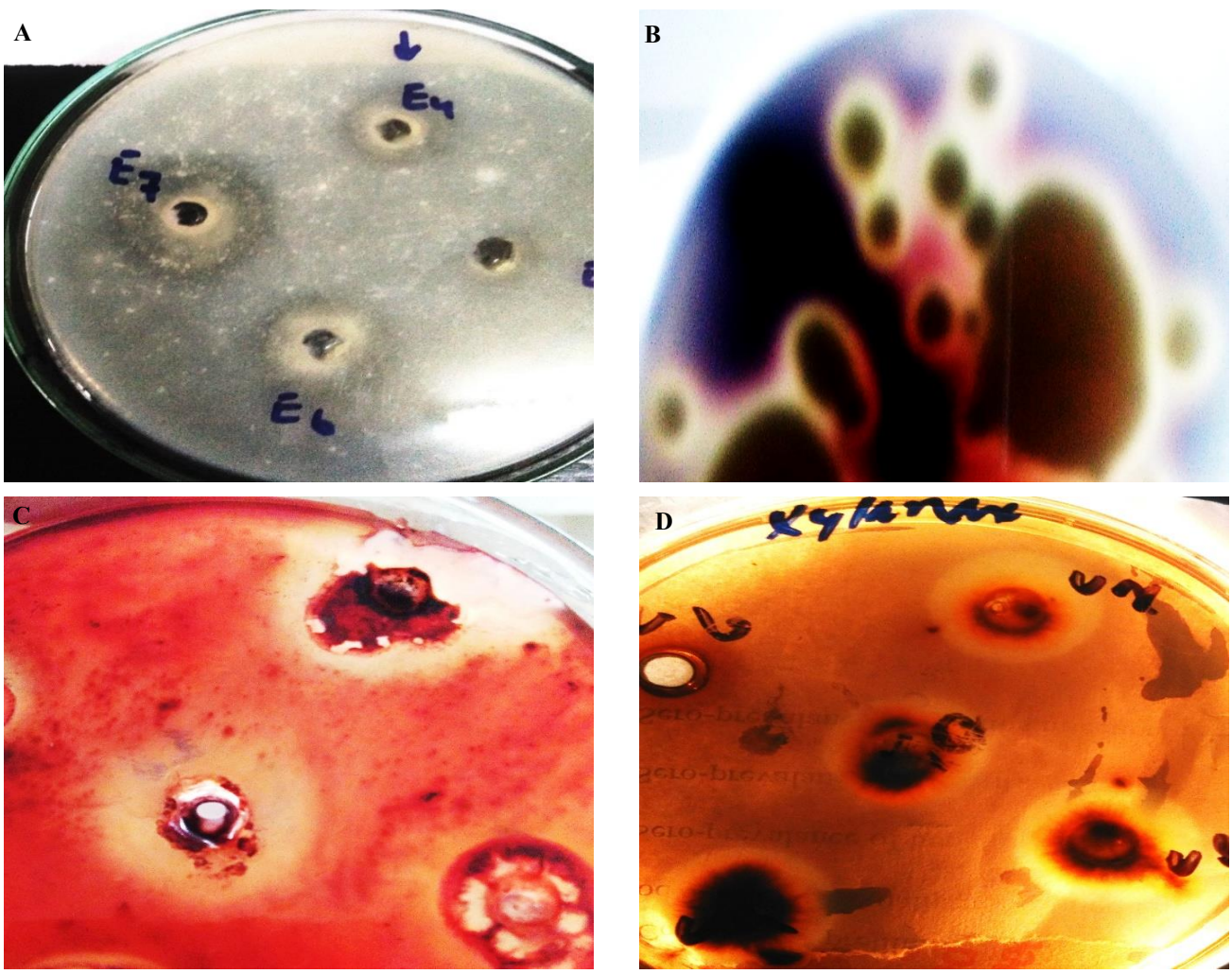

Fig. 6. Enzymatic activities of fungal isolates on different substrates supplemented media (a) Protease activity (b) Amylase activity (c) Cellulase activity (d) Xylanase activity.

All isolates were checked for their antioxidant activity. The demand for natural antioxidant is increasing day by day due to carcinogenic effect of synthetic antioxidants (Jayaprakasha & Rao, 2000). Mikaye *et al*., (2009) reported antioxidant activity of many *Aspergillus* species. Further on Ravindran *et al*., (2012) reported high antioxidant capacity by fungus present in high salt environment. Our isolates have more coincidence with that as, our isolates have been grown under high salt condition and they produced extracellular antioxidant compounds. Antioxidant compounds have great medicinal potential (Akbar *et al*., 2019; Behlil *et al*., 2019).

Industry is turning chemical to enzymatic reactions due to low cost and environment friendly nature. For different industrial reactions enzymes must cope with high pH, salt, and temperature (Asad *et al*., 2011). Enzymes from halophilic fungi were kept neglected compared to halophilic bacteria (Mukhtar & Haq, 2012). In this study the production of enzymes was screened by provided 1% substrate in PDA with 10% NaCl. This shows that enzymes can work in low water concentration as well as in high salt environment and can fulfill industrial required conditions. Various enzymes can be used for different purposes such as for saline waste water treatment amylase can be used (Ali *et al*., 2015), for biofuel production in ionic solutions cellulase and xylanase can be used (Zhang *et al*., 2011), in case of saline oil spills lipase can be used for bioremediation (Ali *et al.*, 2014c; Margesin & Schinner, 2001) and for the production of fish sauce protease can be used isolated from halophilic source (Gostinčar *et al*., 2011). Few isolates are capable of producing various types of enzymes. Halophiles are capable to be used as an efficient source for bioremediation (Bano *et al*., 2018).

Conclusion

In this study, we have isolated 19 different halophilic and halotolerant filamentous fungal species from the mangroves ecosystem of Balochistan. This is first ever study for the halophilic fungal diversity exploration of mangroves ecosystems in Balochistan and Pakistan. It was found that the fungal isolates are active for a variety of enzyme production and produce antibacterial and antifungal metabolites. Work on the isolates regarding detail profiling for different functional bioactive compounds and enzymes as well as their applications is in progress in our laboratory. These organisms can be potential bio-factories to produce different biotechnological products.

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