Pak. J. Bot., 46(3): 983-988, 2014.

SCREENING OF POTENTIAL BIOTECHNOLOGICAL APPLICATIONS FROM OBLIGATE HALOPHILIC FUNGI, ISOLATED FROM A MAN-MADE SOLAR SALTERN LOCATED IN PHETCHABURI PROVINCE, THAILAND

IMRAN ALI[1,2,4*], NAPA SIWARUNGSON[3], HUNSA PUNNAPAYAK[4*], PONGTHARIN LOTRAKUL[4], SEHANAT PRASONGSUK[4], WICHANEE BANKEEREE[4] AND SUDIP K. RAKSHIT[1]

[1]Food Engineering and Bioprocess Technology, School of Environment, Resources and Development, Asian Institute of Technology, Klong Luang, Pathumthani 12120, Thailand,
[2]Institute of Biochemistry, University of Balochistan, Quetta, 87300, Pakistan, [3]Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand, [4]Plant Biomass Utilization Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand
**Corresponding author's email: imranalisheik@gmail.com*; *phunsa@chula.ac.th*

Abstract

This is the continuation study of obligate halophilic fungi isolated from a man-made solar saltern located in Phetchaburi province, Thailand. The isolation site was hypersaline extreme habitat. Six halophilic fungi which were screened for applications belonged to 4 species and 5 strains of *Aspergillus* genus namely *A. flavus*, *A. gracilis*, *A. penicillioides* (2 strains) and *A. restrictus*. A yeast namely, *Sterigmatomyces halophilus* was also one of the isolate screened for biotechnological potentials. Antibacterial, antioxidant and enzymatic activity were determined for each of the strains. All of the isolates in this study were found to have at least one of the applications screened. *A. flavus*, *A. gracilis* and *A. penicillioides* (1) were found to have high antibacterial potential. *A. flavus* and *A. penicillioides* (2) showed the most antioxidant activity while *A. gracilis* and *A. restrictus* were found to be most promising candidates for enzymes (of the five enzymes tested) having activity under saline conditions.

Introduction

Extreme environments were long considered to be free of life. However with intensive research recently a wide diversity of extremophilic microorganisms have been discovered (Turk *et al*., 2011). Halophilic fungi are defined as those fungi which can grow *In vitro* at salt concentration of 3 M and can be isolated from global environments at salinities above 1.7 M (Gunde-Cimerman *et al*., 2005).

Solar salterns are systems where salt is produced by the evaporation of sea water (Oren, 2002). Sodium chloride (NaCl) is the most abundant ion and the pH is from neutral to slightly alkaline in such systems (Gostinčar *et al*., 2011; Zehra *et al*., 2013). Salinity affects the physiology of plants (Muhammad & Hussain, 2012). Fungi were not considered to be the part of hypersaline environments until they were first reported to be the active inhabitants of solar salterns a decade ago (Gunde-Cimerman *et al*., 2000). Many species including *Aspergillus, Cladosporium, Pencillium* and some yeasts including those which were previously reported only to be the food contaminants have been isolated from hypersaline habitats (Butinar *et al*., 2005; Zalar *et al*., 2005; Cantrell & Baez-Félix, 2010). The function of fungi in hypersaline environments is still not fully understood (Gunde-Cimerman *et al*., 2004).

The potential biotechnological applications of extremophiles and their metabolites, capable of activity under extreme conditions, are one of the main reasons for studying them (Antranikian, 2005; Amils *et al*., 2007). Hypersaline habitats are tremendous sources of microorganisms that can provide industrially important enzymes (CAREX, 2011). Halophiles have been used in several common fermentation processes (Gostinčar *et al*., 2011). They have also been utilized in some new industrial processes like the production of bioactive compounds such as beta-carotene and ectoine (Lentzen & Schwarz, 2006; Oren, 2010). Similarly they have been reported for the production of biorhodopsin for optical computing, biosurfactants for enhanced biodegradability, exopolysaccharides for efficient oil recovery, food additives and compatible solutes working as stress protectants (Margesin & Schinner, 2001b; DasSarma & DasSarma, 2002). The problem of water scarcity and increasing salinity in soil makes halophilic fungi as excellent candidates to acquire the genes for increasing the halophilic and xerophilic tolerant characteristics in food producing crops (Gostinčar *et al*., 2011). Increasing demand of bioremediation of hypersaline environments together with the unavoidable need to treat saline oil marshes and wastewater from industries is stimulating the search for industrial halophilic organisms (Margesin & Schinner, 2001a; DasSarma & DasSarma, 2002; Chung *et al*., 2009).

However, the research on halostable/halophilic metabolites has mostly focused on prokaryotic sources. This is specially monopolized by the extensive study on halophilic bacteria, although the halophilic fungi can be a better source of extracellular metabolites (Dalboge, 1997). The aim of the present study was to determine the antimicrobial, antioxidant and enzyme production (five different enzymes) potential of the obligate halophilic fungi, isolated from a man-made solar saltern located at Phetchaburi province of Thailand. To the best of our knowledge, this is the first study of such a wide range of applications from obligate halophilic fungi.

Methodology: All screening studies were carried out using aseptic techniques and NaCl concentration of 10% (w/v) was supplemented in all medium used for the growth of obligate halophilic fungi. Three replicates were made in all experiments along with sterile distilled water

media which served as negative controls in plate screening methods. Appropriate blanks were used in spectrophotometric analysis.

Site description and sampling: Necessary summarized information is provided here. For further details of area, identification and characteristics of obligate halophilic fungi in this study please consult our recent report (Ali *et al*., 2013). The man-made solar saltern from which the sample was collected is located in Ban Laem district, Phetchaburi province, Thailand. The area is near the sea, is surrounded by mangrove forests and has a large number of man-made solar salterns used for the production of salt. Temperature throughout the year is not much variable with an average of minimum about 24^{o} C and maximum of about 32^{o}C (Ali *et al*., 2013). Salt concentration (NaCl) in the soil sample obtained from a depth of 10-15 cm in the saltern was found to be 13.11% (w/v), pH was recorded to be 7.4 and moisture content was 27.16%. Very low levels of nitrogen, organic carbon and total organic carbon were found in the soil samples. All fungi were isolated using a serial dilution method on potato dextrose agar (PDA) supplemented with 15% NaCl (w/v). Obligate halophilic fungi were selected on the basis of being unable to grow on NaCl free medium. Obligate halophilic fungi were molecularly and morphologically identified (Ali *et al*., 2013).

Screening for potential antibacterial activity: Fungal filtrates were prepared as suggested by Elaasser *et al*., (2011). Isolates were grown in malt extract agar (MEA) for seven days. Spores were scrapped in saline distilled water and 2 ml of spore suspension was added in 100 ml of malt extract broth (MEB). The broth flasks were incubated at 30^{o} C for 14 days and mycelium was then harvested by filtration. Crude filtrate was used further. Activity was checked against gram positive *Bacillis subtilis* TISTR 008 and gram negative *Escherichia coli* TISTR 780 by following two methods.

Plate screening method: Plate screening method for antibacterial activities was carried out using the method suggested by National Committee for Clinical Laboratory Standards (NCCLS, 2003). Bacteria were applied on nutrient agar (NA) by cotton swab. Holes of 1cm diameter were made in the NA using a cock borer. Holes were filled by 100 µl of crude filtrate obtained from fungal isolates. Plates were incubated at 4° C for one hour for proper diffusion and then incubated for 37° C for 24 hour. Diameter of inhibition zones were recorded in millimeter (mm).

Spectrophotometric analysis: Antibacterial screening by spectrophotometric analysis at 600 nm was carried out using the method described by Mahmood *et al*., (2011) with some modifications. Activated bacteria of 100 µl with optical density value of 1.0-1.5 was inoculated in 10 ml of nutrient broth (NB) into which was added 100 µl of fungal filtrates. After 24 hour of incubation at 37^{o} C, the optical density of the NB was determined and compared with control having no fungal filtrate.

Screening for potential antioxidant activity: Methodology for the preparation of fungal extracts used for determining antioxidant activity was adapted from the method followed by Arora & Chandra, (2010). Fungal isolates were grown on yeast extract glucose agar (YGA) for seven days followed by incubation in 100 ml Czapek dox's broth having a composition (w/v) of 3% sucrose, 0.2% sodium nitrate, 0.1% di-potassium hydrogen phosphate, 0.05% magnesium sulphate, 0.05% potassium chloride and 0.001% of ferrous sulphate. After 10 days of incubation at 30^{o} C the filtrates obtained were used for determining antioxidant activity following two methods given below:

Thin layer chromatography (TLC): The radical scavenging method described by Cavin *et al*., (1998) was one of the methods followed. TLC plates were spotted with fungal filtrates and developed. Dried TLC plates were sprayed with 0.2% (w/v) 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution. Plates were immediately stored in dark and observed after 30 min.

Total phenolic content (TPC): Methodology adopted by Arora & Chandra, (2010) for finding TPC was used as a second measure of antioxidant activity. All test tubes during this study were covered by aluminum foil to prevent light penetration. Test samples of 0.5 ml were treated with 0.2 ml of Folin–Ciocalteu (FC) reagent. After 10 min, 0.6 ml of 20% (w/v) sodium carbonate was added. The reaction mixture was incubated at 40^{o} C for 30 min. Absorbance for activity was determined at 765 nm. Gallic acid was taken as the standard.

Screening for potential extracellular enzymes: Five enzymes: amylase, cellulase, lipase, protease and xylanase were screened. Screening for enzymatic potential was carried systematically using the following two methods.

Plate screening method: Plate screening method was used for checking the presence of enzymes. The methodology described by Sohail *et al*., (2009) was followed with modifications. Isolates were inoculated at 30^{o} C for 7 days in potato dextrose broth (PDB) 100 ml supplemented with 1% (w/v) of soluble starch for amylase, cellulose for cellulase, Tween 80 for lipase, casein for protease and xylan for xylanase screening. Similarly PDA plates supplemented with 1% (w/v) of specific substrates were prepared and wells with diameter of 1 cm were made using a cock borer. The holes were filled with 100 µl of fungal filtrates. Plates were incubated at 4^{o}C for 1 hour and then at 30^{o}C for 24 hour. Diameters of resultant clear zones indicating activity of enzymes were recorded in millimeters (mm). Iodine solution was used for aiding amylase and xylanase screening, while congo red reagent helped in measuring the halos made by cellulase enzymes.

Crude enzyme assay: Spectrophotometric assay for crude enzymes was carried out based on the results of the plate screening method described above. Only those enzymes which showed clear zones in plate screening

were assayed. Preparation of liquid media and assay were carried out as per standard procedure for different enzymes in literatures consulted (Ghose, 1984; Abe *et al*., 1988; Gwande & Kamat, 1998; Mahadik *et al*., 2002; Khucharoenphaisan *et al*., 2008; Siala *et al*., 2009). One unit of enzyme activity (U) is defined as the amount of enzyme that produces 1 µmol of product in 1 min.

Results

Screening for potential antibacterial activity

Plate screening method: The results of the plate screening method for antibacterial activity are shown in table (Table 1). Positive (+) activity in the table indicates clear zones ranging from 1-10 mm, moderate activity (++) means clear zones ranging from 10-15 mm and high (+++) activity means clear zone above 15 mm. All the fungal isolates have been found to express some extent of antibacterial activity. Their extracts were more active against *E. coli* than *B. subtilis*, showing more potential against gram negative bacteria. The supernatant from *A. flavus* was the only one showing high activity against *B. subtilis*. Supernatants from *A. gracilis* and *A. penicillioides* (1) have shown high activity against *E. coli*. Moderate activity was exhibited by the supernatant from *S. halophilus* against *E. coli*.

Spectrophotometric analysis: The results obtained on spectrophotometric analysis of the culture broth with fungal filtrates are shown in Table 1. Positive (+) activity in the table means inhibition of bacterial growth ranging from 0-0.1 nm, moderate activity (++) means inhibition of bacterial growth ranging from 0.1-0.2 nm and high (+++) activity means inhibition of bacterial growth above 0.2 nm. All fungal isolates were found to have active antibacterial potential in this study. Spectrophotometric analysis confirmed the results of plate screening method and provided some more detailed analysis of bacterial growth inhibition in liquid media. *A. penicillioides* (1 and 2) and *A.restrictus* have shown a moderate activity against *B. subtilis*. Other than that all the results of plate screening method remains unchanged in spectrophotometric analysis.

Table 1. Determination of antibacterial potential of the screened obligate halophilic fungi with + indicating positive activity, ++ indicating moderate activity and +++ indicating high activity.

Isolates	Plate screening method		Spectrophotometric method	
	B. subtilis	***E. coli***	***B. subtilis***	***E. coli***
A. flavus	+++	+	+++	+
A. gracilis	+	+++	+	+++
A. penicillioides (1)	+	+++	++	+++
A. penicillioides (2)	+	+	++	+
A. restrictus	+	+	++	+
S. halophilus	+	++	+	++

Screening for potential antioxidant activity

Thin layer chromatography (TLC): The results of the antioxidant potential of the filtrates from screened cultures of obligate halophilic fungi using TLC are shown in Table 2. Positive results were inferred for those having yellow spots on a purple background. *A. flavus* and *A. penicillioides* (2) were found to have antioxidant potential in this method. There was some difficulty ascertaining if some of the strains were really negative or some had some antioxidant activity. Hence a second method of testing for this activity was carried out.

Total phenolic content (TPC): The results of antioxidant activity using the TPC tests are shown in Table 2. The positive (+) activity in the table means TPC ranging from 0-5 mg/ml, moderate activity (++) means TPC ranging from 5-10 mg/ml and high (+++) activity means TPC above 10 mg/ml. The results confirmed the TLC method and analyzed the strength of antioxidant potential by fungal filtrates. *A. flavus* was found to have moderate while *A. penicillioides* (2) was found to have high antioxidant potential.

Screening for potential extracellular enzymes

Plate screening method: The results of the tests to determine the extracellular enzyme production activity of the screened fungi are shown in Table 3. Positive (+) activity in the table means clear zones ranging from 1-10 mm, moderate activity (++) means clear zones ranging from 10-15 mm and high (+++) activity means clear zone above 15 mm. Low protease and lipase presence were observed in plate screening method. *A. gracilis* and *A. restrictus* were found to be the most promising species showing the presence of maximum number of extracellular enzymes screened in this study, followed by *A. penicillioides* (1). Xylanase found in this study from *A. gracilis* and *A. penicillioides* (1) holds the potential of being cellulase free xylanase.

Table 2. Determination of antioxidant potential of the screened obligate halophilic fungi with + indicating positive activity, ++ indicating moderate activity and +++ indicating high activity

Isolates	TLC method	TPC method
A. flavus	+	++
A. gracilis	-	+
A. penicillioides (1)	-	+
A. penicillioides (2)	+	+++
A. restrictus	-	+
S. halophilus	-	+

Table 3. Determination of enzymatic potential of the screened obligate halophilic fungi with + indicating positive activity, ++ indicating moderate activity and +++ indicating high activity.

Isolates	Plate screening method					Crude enzyme assay				
	Amylase	Cellulase	Lipase	Protease	Xylanase	Amylase	Cellulase	Lipase	Protease	Xylanase
A. flavus	-	+++	-	-	-	-	+	-	-	-
A. gracilis	+++	-	+	-	++	+	-	+	-	+
A. penicillioides (1)	+++	-	-	-	+++	+	-	-	-	+
A. penicillioides (2)	-	-	-	-	-	-	-	-	-	-
A. restrictus	-	++	+	+	-	-	+	+	+	-
S. halophilus	-	-	+	-	-	-	-	+	-	-

Crude enzyme assay: The results of enzyme assay are shown in Table 3. Positive (+) symbol in the table express the positive enzyme activity. Enzyme assay results confirmed the presence of enzymes found in plate screening method.

Discussion

Living organisms produce wide range of bioactive molecules, making them rich source of different types of medicines (Fazal *et al*., 2011). Constant need of new antibiotics due to antibiotic resistance make our isolates as good choice of natural sources due to their active antibacterial activity. Sepcic *et al*., (2011) reported that antibiotic activity from the halophilic/halotolerant fungi has been more effective in low water activity or increase in salt concentration. Their isolates were more active against *B. subtilis* than *E. coli*. In our study all of our isolates have grown at 10% NaCl (w/v). Most of our isolates have shown high activity against *E. coli* and moderate activity against *B. subtilis*. *A. flavus* has been found most effective against *B. subtilis* but *A. penicillioides* (1) stands out as most impressive strain amongst our isolates in antibacterial activity because it has shown highest activity against *E. coli* and has also exhibited moderate activity against *B. subtilis*, proving a wide range of antibacterial potential. It has been reported that halophilic/halotolerant bacteria are resistant to most antibiotics (Ghosh *et al*., 2010), in this case our antibacterial results provide an opportunity for proper antibiotic extraction from halophilic fungi which may act best against halophilic/halotolerant bacteria at high salt concentrations.

Antioxidants delay the process of oxidation by blocking the oxidizing chain reactions. Restriction on the use of synthetic antioxidants due to their carcinogenicity (Ito *et al*., 1983) increased the demand of natural antioxidants (Jayaprakasha & Rao, 2000). Filamentous fungi are considered as a source of food preservation. Many *Aspergillus* have been reported to produce antioxidants (Mikaye *et al*., 2009). Recently Ravindran *et al*., (2012) have reported an increased in antioxidant capacity of fungi exposed to high salt conditions and more extracellular antioxidant productivity than intracellular production. Our findings are similar to these results as our isolates were grown in saline conditions and high extracellular antioxidants were released than intracellular antioxidants (results of intracellular antioxidants not shown). Crude filtrates of *A. flavus* and *A. penicillioides* (2) have shown high TPC near 10 mg/ml which certainly can be increased by concentrating the filtrates. Phenolic compounds are found to be related with the life span of fungi by their antioxidant defense against the stress of environmental factors (Cook & Samman, 1996). Therefore, isolates from our study can be good candidates for antioxidant production processes, requiring high salt concentration.

For different industrial applications, enzymes must be stable under extreme process conditions (Asad *et al*., 2011). Enzymes obtained from halophilic organisms display polyextremophilic properties. Generally they are haloalkalophilic and thermotolerant which makes them applicable in wide range of industrial processes carried out at extreme pH and temperature (Setati, 2010). Halophilic fungal enzymes have been neglected unlike the extensive study on halophilic bacterial enzymes (Mukhtar & Haq, 2012). Except *A. penicillioides* (2), all isolates in our study have shown the presence of at least one enzyme screened. In plate screening method the isolates were provided only PDA supplemented with 1% (w/v) of specific substrates which can be a cheap raw material for enzyme production. As all enzymes were produced in 10% NaCl (w/v) so they are expected to be halotolerant. They can work at low water activity and high saline conditions and can be applicable for industrial processes requiring high salt concentrations such as: amylase for saline waste water treatment (Margesin & Schinner, 2001a), cellulase and xylanase for biofuel production in ionic solutions (Zhang *et al*., 2011), lipase for bioremediation of saline oil spills (Margesin & Schinner, 2001a) and protease for fish sauce production (Gostinčar *et al*., 2011). Any other extreme behavior of our enzymes under study will be an additional value.

Conclusion

This study was conducted with an aim to reveal the potential of biotechnological applications of obligate halophilic fungi. All of the obligate halophilic fungi in this study were found to have at least one of the applications screened. High levels of screened potentials can be excellent candidates for the application in appropriate industries. Further characterization of these applications is under study to explore their extremophilic behavior and relationship with salt concentrations. The possibility of using these natural substances in food, pharmaceutical and detergent industry will help to reduce the use of harmful chemicals which are commonly used in many products. We believe that this research will pioneer and open a new era of using halophilic fungi for their applications rather than just their diversity and characteristic studies.

Acknowledgements

The research grant funds have been provided by agreement on Post-Doctoral Research Grant Allocation from the Ratchadaphisek Somphot Fund.

References

Abe, J., F.W. Bergman, K. Obata and S. Hikuri. 1988. Production of raw starch digesting amylase by *Aspergillus* K-27. *Appl. Microbio. Biotechnol.*, 27: 447-450.

Ali, I., L. Kanhayuwa, S. Rachdawong and S.K. Rakshit. 2013. Identification, phylogenetic analysis and characterization of obligate halophilic fungi isolated from a man-made solar saltern in Phetchaburi province, Thailand. *Ann. Microbiol.*, 63: 887-895. doi: 10.1007/s13213-012-0540-6.

Amils, R., C. Ellis-Evans and H.G. Hinghofer-Szalkay. 2007. *Investigating life in extreme environments—A European perspective*. (Ed.): N. Walter, ESF, Cedex Strasbourg, France.

Anonymous. 2003. National Committee for Clinical Laboratory Standards: *Performance standards for antimicrobial disc susceptibility tests*. 8th edition. Approved Standard, document M2-A8, NCCLS, Wayne, Pennsylvania.

Antranikian, G. 2005. Extreme environments as a resource for microorganisms and novel biocatalysts. *Mar. Biotechnol*., 96: 219-262.

Arora, D.S. and P. Chandra. 2010. Assay of antioxidant potential of two *Aspergillus* isolates by different methods under various physio-chemical conditions. *Braz. J. Microbiol.*, 41: 765-777.

Asad, W., M. Asif and S.A. Rasool. 2011. Extracellular enzyme production by indigenous thermophilic bacteria: partial purification and characterization of α-amylase by *Bacillus* sp. wa21. *Pak. J. Bot*., 43: 1045-1052.

Butinar, L., P. Zalar, J.C. Frisvad and N. Gunde-Cimerman. 2005. The genus *Eurotium*-members of indigenous fungal community in hypersaline waters of salterns. *FEMS Microbiol. Ecol.*, 51: 155-166.

Cantrell, S.A. and C. Baez-Félix. 2010. Fungal molecular diversity of a Puerto Rican subtropical hypersaline microbial mat. *Fungal Ecol.*, 3: 402-405.

Carex. 2011. *Roadmap for Research on Life in Extreme Environments*. Carex Project Office, Strasbourg Cedex, France.

Cavin, A., K. Hostettmann, W. Dyatmyko and O. Potterat. 1998. Antioxidant and lipophilic constituents of *Tinospora crispa*. *Planta Med*, 64: 393-396.

Chung, J., S. Shin and J. Oh. 2009. Characterization of a microbial community capable of reducing perchlorate and nitrate in high salinity. *Biotechnol. Lett.*, 31: 959-966.

Cook, N.C. and S. Samman. 1996. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. *J. Nutr. Biochem*., 7: 66-76.

Dalboge, H. 1997. Expression cloning of fungal enzyme genes: A novel approach for efficient isolation of enzyme genes of industrial relevance. *FEMS Microbiol. Rev.*, 21: 29-42.

DasSarma, S. and P. DasSarma. 2002. *Halophiles, Encyclopedia of Life Sciences*. Wiley, London.

Elaasser, M.M., M.M. Abdel-Aziz and R.A. El-Kassas. 2011. Antioxidant, antimicrobial, antiviral and antitumor activities of pyranone derivative obtained from *Aspergillus candidus*. *J. Microbiol. Biotech. Res.*, 4: 5-17.

Fazal, H., N. Ahmed, Ikramullah, H. Inayat, L. Khan and B.H. Abbasi. 2011. Antibacterial potential in *Parthenium hysterophorus, Stevia rebaudiana* and *Ginkgo biloba. Pak. J. Bot*., 43: 1307-1313.

Ghose, T.K. 1984. Measurement of Cellulase activities. *Pure & Appl. Chem*., 59: 257-268.

Ghosh, R., P.K. Chattopadhyay, B. Chattopadhyay and D. Pal. 2010. Antibiotic resistance profile of halophilic microorganisms isolated from tannery effluent. *Indian J. Biotechnol.*, 9: 80-86.

Gostinčar, C., M. Lenassi, N. Gunde-Cimerman and A. Plemenitaš. 2011. Fungal adaptation to extremely high salt concentrations. *Adv. in Appl. Microbiol.*, 77: 71-96.

Gunde-Cimerman, N., J.C. Frisvad, P. Zalar and Ana. Plemenitaš. 2005. Halotolerant and halophilic fungi. In: *Biodiversity of Fungi-Their Role in Human Life*. (Eds.): S.K. Deshmukh and M.K. Rai. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 69-128.

Gunde-Cimerman, N., P. Zalar, S. De Hoog and A. Plemenitaš. 2000. Hypersaline waters in salterns: natural ecological niches for halophilic black yeasts. *FEMS Microbiol. Ecol.*, 3: 235-240.

Gunde-Cimerman, N., P. Zalar, U. Petrovič, M. Turk, Kogej. T. S. De Hoog and A. Plemenitaš. 2004. Fungi in the salterns. In: *Halophilic microorganisms*. (Ed.): A. Ventosa. Springer-Verlag, Heidelberg. pp 103-113.

Gwande, P.V. and M.Y. Kamat. 1998. Production, characterization and application of *Aspergillus sp*. xylanase immobilized on Eudragit S-100. *J. Biotechnol.*, 66: 165-75.

Ito, N., S. Fukushima, A. Hasegawa, M. Shibata and T. Ogiso. 1983. Carcinogenecity of buthylated hydroxyanisole in F344 rats. *J. Natl. Cancer Inst.*, 70: 343-347.

Jayaprakasha, G.K. and L.J. Rao. 2000. Phenolic constituents from lichen *Parmotrema stuppeum* (Nyl.) Hale and their antioxidant activity. *Z. Naturforsch.*, 55: 1018-1022.

Khucharoenphaisan, K., S. Tokuyama and V. Kitpreechavanich. 2008. Characterization of the thermostability of xylanase produced by new isolates of *Thermomyces lanuginosus*. *Sci. Asia*., 34: 187-192.

Lentzen, G. and T. Schwarz. 2006. Extremolytes: Natural compounds from Extremophiles for versatile applications. *Appl. Microbiol. Biotechnol.*, 72: 623-634.

Mahadik, N.D., U.S. Puntambekar, K.B. Bastawde, J.M. Khire and D.V. Gokhale. 2002. Production of acidic lipase by *Aspergillus niger* in solid state fermentation. *Process Biochem.*, 38: 715-721.

Mahmood, N., S.A. Ditta and M. Zameer. 2011. Biochemical screening of different local fungi for their comparative antibacterial potential. *IJAVMS*., 5: 322-326.

Margesin, R. and F. Schinner. 2001a. Biodegradation and bioremediation of hydrocarbons in extreme environments. *Appl. Microbiol. Biotechnol.*, 56: 650-663.

Margesin, R. and F. Schinner. 2001b. Potential of halotolerant and halophilic microorganisms for biotechnology. *Extremophiles.*, 5: 73-83.

Mikaye, Y. C. Ito, M. Itoigawa and T. Osawa. 2009. Antioxidants produced by *Eurotium herbariorum* of filamentous fungi used for the manufacture of karebushi, dried bonito (katsuobushi). *Biosci. Biotechnol. Biochem.*, 73: 1323-1327.

Muhammad, Z. and F. Hussain. 2012. Effect of NaCl salinity on the germination and seedling growth of seven wheat genotypes. *Pak. J. Bot*., 44: 1845-1850.

Mukhtar, H. and I. Haq. 2012. Purification and characterization of alkaline protease produced by a mutant strain of *Bacillus subtilis. Pak. J. Bot*., 44: 1697-1704.

NCCLS. 2003. *Performance standards for antimicrobial disk susceptibility tests; approved standard*. 8th Ed, Wayne, Pennsylvania, USA.

Oren, A. 2002. Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. *J. of Industrial Microbiol. and Biotech.*, 28: 56-63.

Oren, A. 2010. Industrial and environmental applications of halophilic microorganisms. *Environ. Technol.*, 31: 825-834.

Ravindran, C., G.R. Varatharajan, R. Rajasabapathy, S. Vijayakanth, A.H. Kumar and R.M. Meena. 2012. A role for antioxidants in acclimation of marine derived pathogenic fungus (NIOCC 1) to salt stress. *Microb. Pathog.*, 53: 168-179.

Sepcic, K., P. Zalar and N. Gunde-Cimerman. 2011. Low water activity induces the production of bioactive metabolites in halophilic and halotolerant fungi. *Mar. Drugs.*, 9: 43-58.

Setati, M.E. 2010. Diversity and industrial potential of hydrolase-producing halophilic/halotolerant eubacteria. *Afr. J. Biotechnol.*, 9: 1555-1560.

Siala, R., A. Sellami-kamoun, M. Hajji, I. Abid, N. Gharsallah and M. Nasri. 2009. Extra cellular acid protease from *Aspergillus niger* I1: purification and characterization. *Afr. J. Biotechnol.*, 8: 4582-4589.

Sohail, M., S. Naseeb, S.K. Sherwani, S. Sultana, S. Shehzad, A. Ahmed, Saleem. S and S.A. Khan. 2009. Distribution of hydrolytic enzymes in native fungi: *Aspergillus* the predominant genus of hydrolase producers. *Pak. J. Bot.*, 41: 2567-2582.

Turk, M., A. Plemenitaš and N. Gunde-Cimerman. 2011. Extremophilic yeasts: plasma-membrane fluidity as determinant of stress tolerance. *Fungal. Biol*., 115: 950-958.

Zalar, P., S. De Hoog, H. Schroers, J.M. Frank and N. Gunde-Cimerman. 2005. Taxonomy and phylogeny of the xerophilic genus *Wallemia* (Wallemiomycetes and Wallemiales, cl. et ord. nov.). *Antonie Van Leeuwenhoek*., 87: 311-328.

Zehra, A., B. Gul, R. Ansari, A.A. Altar, A.K. Hegazy and M.A. Khan. 2013. Interactive effect of salt, light and temperature on seed germination and recovery of a halophytic grass – *Phramites kara*. *Pak. J. Bot*., 45: 725-736.

Zhang, T., S. Datta, J. Eichler, N. Ivanova, S.D. Axen, C.A. Kerfeld, F. Chen, N. Krypidis, P. Hugenholtz, J. Cheng, K.L. Sale, B. Simmons and E. Rubbin. 2011. Identification of a haloalkaliphilic and thermostable cellulase with improved ionic liquid tolerance. *Green Chem.*, 13: 2083-2090.

(Received for publication 12 November 2012)