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# Diversity and technological potential of fungi from solar salterns of southern India

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## ABSTRACT

**Twenty three species belonging to ten genera of fungi were isolated by two methods from three saltern soils of southern India. Species of *Aspergillus* were the most common fungi present in these samples. Three of these fungi, *viz*. *Aspergillus* sp. 3, *A. terreus*, and a sterile fungus (SF2) were further studied for their salt tolerance, production of some extracellular enzymes and bioactive metabolites as influenced by external salt concentration. The fungi were halotolerant and not halophilic and two of them could grow on 20% NaCl medium. Extracellular enzyme production was influenced by salt in these fungi. Some enzymes were produced through a range of salt concentration (0 to 18 %), some enzymes showed more activity in the presence of salt and a few enzymes were salt-induced. Antialgal and antifungal metabolites were elaborated only in the presence of salt.**

**KEY WORDS** :Extremozymes, halotolerant, salt tolerant fungi

## INTRODUCTION

Solar salterns are used for commercial production of salt from seawater by evaporation. Salterns have NaCl as the major salt and represent extreme hypersaline environments with the salinity at times above 300 PSU (Practical Salinity Units) [Gunde-Cimerman *et al*., 2000; Oren, 2002]. Such an extreme habitat is inimical to most life forms and only halophilic and halotolerant microbes are adapted to survive in it. Although halophilic Archaea are the common inhabitants of salterns (Ochsenreiter *et al*., 2002; Ozcan *et al*., 2007; Dillon *et al*., 2013), bacteria and the eukaryotic alga *Dunaliella salina* also occur in them (Anton *et al*., 2000; Benlloch *et al*., 2002; Baati *et al*., 2008; Dillon *et al*., 2013). The diversity of prokaryotes in salterns has been studied extensively (Benlloch *et al*., 2002). Information on filamentous fungi occurring in salterns is limited. In the year 1996, we reported the occurrence of fungi in hypersaline soils of a saltern in southern India (Suryanarayanan *et al*., 1996). Several studies by Gunde-Cimerman *et al*., (see Gunde-Cimerman and Zalar, 2014) have established that fungi are constant representatives of the eukaryotic microbial spectrum of salterns. Although salterns with their differing pH, light intensity, oxygen and nutrient levels (Pedroś-Alió, 2004; Gunde-Cimerman *et al*., 2000) are unique habitats most suitable for studying halotolerant and halophilic fungi, information on the biology of fungi inhabiting salterns and their technological potential is limited. Most of the investigations on saltern fungi are with reference to temperate zones (Kis-Papo *et al*., 2003; Gunde-Cimerman *et al*., 2004; Butinar *et al*., 2005) while limited studies have been carried out in the tropics (Suryanarayanan *et al*., 1996; Cantrell *et al*., 2006; Nayak *et al*., 2012). With the increasing need for enzymes with novel properties for effective industrial uses, it is worthwhile investigating fungi from hypersaline environments for halotolerant enzymes. In this work, we analysed the soils of the solar salterns in three different locations in southern India for their fungal diversity, screened the fungi for their salt tolerance, production of anti-fungal anti algal metabolites and extracellular enzymes as influenced by NaCl in the growth medium.

## MATERIALS AND METHODS

Hypersaline soil samples were collected from Rameswaram (Lat. 9° 17' N, Long. 79° 18' E, salinity 10.1%), Tuticorin (Lat. 8° 48' N, Long. 78° 11' E, salinity 9.9%), and Villupuram (Lat. 11° 57' N, Long. 79° 32' E, salinity 9.1%). The superficial salt encrustation was removed and the soil was collected from a depth of 2-4 cm, brought to the laboratory in sterile polythene bags and processed within 24 h of collection.

### Isolation of fungi from saltern soils by Warcup method (Warcup, 1950)

Five hundred milligram of a soil sample was evenly spread in a sterilized Petri dish (9 cm dia.) 20 ml of Czapek Dox Agar (CDA) medium ($NaNO_3$ 2.0 g, $KH_2PO_4$ 1.0 g, $MgSO_4.7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4.7H_2O$ 0.10 g, sucrose 30.0 g, Agar 20.0 g, distilled water 1L, Chloramphenicol 150 mg) was poured over it and the Petri dish was incubated at 26±1°C for 30 days. Five replicates were maintained for each sample.

### Isolation of fungi from saltern soils by dilution plating method (Waksman, 1922)

Two gram of soil was suspended in 10ml of sterile distilled water, and two-fold dilution of this was spread on 20 ml of CDA medium contained in a Petri dish (9 cm dia.) and incubated for fifteen days. Preliminary experiments confirmed that this dilution was suitable for isolation of fungi from the soil samples.

### Identification of fungi

Fungal colonies which emerged from the above treatments were isolated, mounted in water, lactophenol or cotton blue stain and observed through a Nikon Labophot (bright field/phase contrast) microscope. They were identified based on fruiting structures and spore characteristics using standard taxonomic keys (Gilman, 1967; Subramanian, 1971; Ellis, 1976; Onions *et al*., 1981; Ellis and Ellis, 1988; Barnett and Hunter 1998). Sterile forms or unidentified fungi were given code numbers to distinguish them from each other (Suryanarayanan *et al*., 1998).

For further studies, we chose *Aspergillus* sp. 3 and *Aspergillus*

*terreus* isolated from Tuticorin soil. A sterile isolate (Sterile form 2) from Tuticorin soil was also studied for comparison.

**Test for salt tolerance**

The fungi were grown on PDA (Potato Dextrose Agar) medium. After 4 days, the margin of the colony was cut with a sterile cork borer (5mm dia) and this mycelial plug was placed (mycelial surface down) at the center of 9 cm dia. Petri dish containing 20 ml of CDA amended with NaCl (0, 1%, 3.5%, 7%, 15%, 18%, or 20%) [Cantrell *et al*., 2006]. Petri dishes were incubated for 30 days at 26±1ºC and the colony diameter was measured every 24 hours. Three replicates were maintained for each treatment.

**Test for extracellular enzyme production as influenced by NaCl**

The fungi were screened for the production of amylase, cellulase, lipase, pectinase and pectate transeliminase in the presence of NaCl. A qualitative agar plate assay of Hankin and Anagnostakis, 1975; Rohrman and Molitoris, 1992, was used. The fungi were grown on agar medium containing the substrate and 0, 1, 3.5, 7, 15 or 18% of NaCl and the production of an enzyme was ascertained by measuring the zone of change in colour. Beta glucosidase production was visualized by the method of Saqib and Whitney, 2006. For detecting chitin modifying enzymes and proteases, the method of Govinda Rajulu *et al*., 2011, was used.

**Beta glucosidase:** The fungi were grown in YP media (Yeast extract 0.1 g, peptone 0.5 g, distilled water1l with different concentrations of NaCl) with 0.5% of Na-carboxymethyl cellulose as carbon source for seven days; the culture filtrate was centrifuged and the supernatant used for assay. One hundred ml of 4% agar in 0.2 M sodium acetate buffer (pH 5.0) was autoclaved and maintained at 50°C. One hundred ml of 0.2% esculin (Sigma) was mixed with 6ml of 1% $FeCl_3$ solution and heated up to 50°C in a water bath; this was mixed with the agar solution and poured in Petri dishes (20 ml per plate). After solidification, 4 wells of 0.5 cm dia. were cut and 75 µl of a supernatant was poured into each well and incubated at 37°C for 5 h. The appearance of brown colour around the well indicated enzyme activity. The zone of activity was measured with calipers.

**Chitin modifying enzymes and Proteases** (Govinda Rajulu *et al*., 2011; Thirunavukkarasu *et al*., 2017)

**Preparation of samples for detecting chitinase, chitosanases and proteases:** The fungi were grown in PD medium (Potato 200 g, Dextrose 20 g, distilled water 1L, pH 6 with different concentration of NaCl) for 5 days as static culture at 26°C. The mycelium was filtered and 100 ml of the culture filtrate was dialyzed for 15 h against distilled water. The dialyzed culture filtrate was lyophilized and used as crude enzyme source for detecting chitin modifying enzymes and different types of proteases.

**Dot blot assay for Chitin modifying enzymes:** A composite gel consisting of stacks of glycol chitin, chitosan of 38% or 1.6% degree of acetylation was layered as follows. A gel was prepared by mixing a solution of 1 ml of 30% Acrylamide/Bisacrylamide, 0.1 ml substrate (1% glycol chitin), 1.9 ml of 50 mM sodium acetate buffer (pH 5.5), 0.003 ml of 100% tetraethylmethylene-diamide (TEMED) and 0.003 ml of 40% ammonium persulphate, poured in a gel cassette and allowed to polymerize. A few drops of butanol were added to the top of the solidifying gel to help polymerization. After 20 min, the butanol was decanted and the gel was topped with a solution of 1 ml of 30 % Acrylamide/Bis acrylamide, 0.3 ml substrate (0.1% chitosan 38% Degree of Acetylation), 1.7 ml of sodium acetate buffer (pH 5.5), 0.003 ml of 100% TEMED, 0.003 ml of 40% ammonium persulphate. The surface of this gel was layered with butanol to facilitate polymerization. After 20 min, the butanol was removed and this process was repeated with 0.1% chitosan degree of acetylation 1.6%. Thus a compound gel consisting of chitin (100% degree of acetylation), and chitosans of 38% degree of acetylation or 1.6% degree of acetylation was obtained. 10 mg of the lyophilized culture filtrate of each fungus (crude enzyme source) was mixed in 1 ml of 50 mM sodium acetate buffer (pH 5.2) and centrifuged at 14000 rpm for 5 min (20˚C). 5 µl of the supernatant was loaded on the gel and incubated at 37˚C for 24 h; gels were stained with 0.1% calcofluor white for 5 min and washed with distilled water for 1 h and observed under UV transilluminator to detect zones of darkness which indicated enzyme activity.

**Dot blot assay for acidic, neutral or alkaline proteases:** A gel was prepared by mixing a solution containing 1 ml of 30 % Acrylamide/Bisacrylamide, 0.4 ml substrate (2% autoclaved gelatin), 2.3 ml of 50 mM Tris HCl buffer (pH 9.5), 0.003 ml of 100% TEMED and 0.003 ml of 40% ammonium persulphate, poured in a gel cassette and left for 20 min for polymerization. After this, the gel was topped with the above having Bis Tris buffer of pH 7.0; this process was repeated with the gel prepared using sodium acetate buffer of pH 5.0. Thus a composite gel consisting pH 5.0, pH 7.0 and pH 9.5 was obtained. For this experiment, the lyophilized culture filtrate of *A. terreus* was used. 10 mg of the lyophilized culture filtrate of *A. terreus* (crude enzyme source) was dissolved in 1 ml of the above buffers, centrifuged at 14000 rpm for 5 min (20ºC) and 5 µl of supernatant was loaded on the composite gel using a micropipette. The gel was incubated at 37°C for 24 h, stained with Coomassie brilliant blue for 3 hours, washed with distilled water and observed for the development of clear zone which indicated enzyme activity.

**Detection of antialgal and antifungal metabolites by bioautogram (Schulz *et al*., 1995)**

The fungi were grown in 100 ml PD medium with 0, 3.5, 7, 15 or 18% NaCl for 20 days; the culture filtrate was extracted with methanol and concentrated to dryness; 50 µl of this concentrated extract in methanol was spotted on a TLC aluminium sheet (pre coated Silica gel, layer thickness 0.1 mm, Merck, Germany). After drying, the chromatogram was sprayed with a suspension of an alga (*Chlorella fusca* cells) or conidia of a fungus (*Cladosporium cucumerinum*) and incubated at 26ºC in light (for the alga) or in darkness (for the fungus) for 5 days to allow for their growth. The appearance of an inhibition zone on the chromatogram indicated the

presence of bioactive secondary metabolite(s). An extract of the growth medium having different concentrations of NaCl but without the test fungus and subjected to TLC served as control.

## RESULTS

From the three soil samples, by both the isolation methods used, 23 different fungal species belonging to 10 genera were isolated (**Table 1**). These included 9 species of *Aspergillus*, 2 of *Fusarium* and one each of *Chaetomium*, *Dreschlera*, *Emericella*, *Paecilomyces*, *Sporormiella* and *Trichoderma*. An unidentified coelomycete fungus and 4 sterile forms SF1 to 4) were also isolated from the soil samples. *A. niger* and SF1 were present in all the soil samples.

**Table 1** Number of colonies of fungi isolated from soils of three different solar salterns by Warcup (W) and Dilution (D) plating methods.

| Fungus | Rameswaram (W/D) | Tuticorin (W/D) | Villupuram (W/D) |
|---|---|---|---|
| *Aspergillus flavus* | 0/0 | 0/0 | 1/0 |
| *A. niger* | >10/2 | 8/0 | 5/0 |
| *Aspergillus* sp. 1 | 0/3 | 0/0 | 2/0 |
| *Aspergillus* sp. 2 | 0/0 | 0/0 | 2/0 |
| *Aspergillus* sp. 3 | 1/0 | 1/0 | 0/0 |
| *Aspergillus* sp. 4 | 3/0 | 0/0 | 0/0 |
| *Aspergillus* sp. 5 | 0/1 | 0/0 | 0/0 |
| *Aspergillus* sp. 6 | 0/0 | 0/1 | 0/0 |
| *A. terreus* | 0/0 | 0/1 | 0/>10 |
| *Chaetomium* sp. | 0/0 | 1/0 | 0/0 |
| Coleomycete form 1 | 0/>10 | 0/0 | 0/0 |
| *Drechslera* sp. | 0/1 | 0/0 | 4/0 |
| *Emericella* sp. | 0/0 | 1/0 | 0/0 |
| *Fusarium* sp. 1 | 0/0 | 0/1 | 0/0 |
| *Fusarium* sp. 2 | 0/0 | 0/0 | 1/0 |
| *Paecilomyces* sp. | 0/>10 | 0/0 | 0/0 |
| *Penicillium* sp. | 0/0 | 2/0 | 2/0 |
| *Sporormiella* sp. | 0/0 | 0/0 | 2/0 |
| Sterile form 1 | 4/3 | 0/1 | 2/0 |
| Sterile form 2 | 2/0 | 4/0 | 0/0 |
| Sterile form 3 | 0/1 | 1/0 | 0/0 |
| Sterile form 4 | 0/1 | 0/0 | 0/0 |
| *Trichoderma* sp. | 0/0 | 3/0 | 0/0 |

The two species of *Aspergillus* grew on 20% NaCl medium, the highest concentration of salt tested (**Fig 1a, b and c**). *Aspergillus* sp. 3 grew significantly more on 3.5, 7 and 15% NaCl than on a medium without NaCl (**Fig 1a**). Its growth was also faster on NaCl-amended medium than on medium devoid of NaCl. Although *A. terreus* grew on all the NaCl concentrations tried, its growth was initially more rapid in the absence of NaCl (**Fig 1b**). With further incubation, there was no significant difference between the growth on 3.5 and 7%

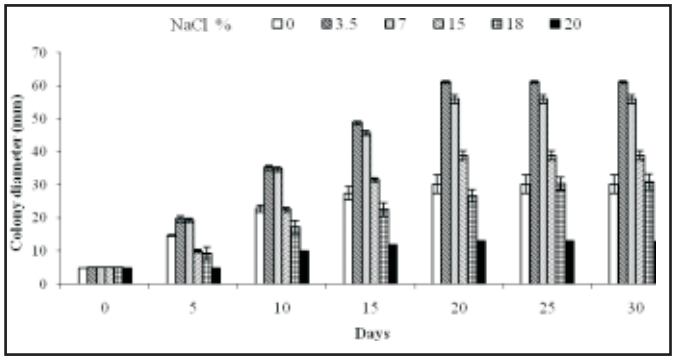


**Fig. 1a** - Effect of NaCl on growth of *Aspergillus* sp. 3**.** The mean and standard error values were calculated from six independent measurements.

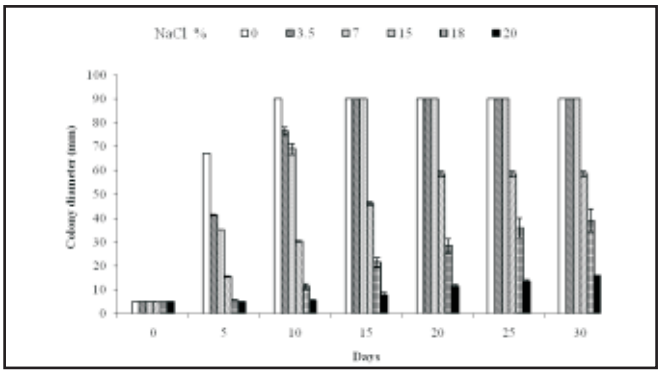


**Fig. 1b** - Effect of NaCl growth of *A. terreus*. The mean and standard error values were calculated from six independent measurements.

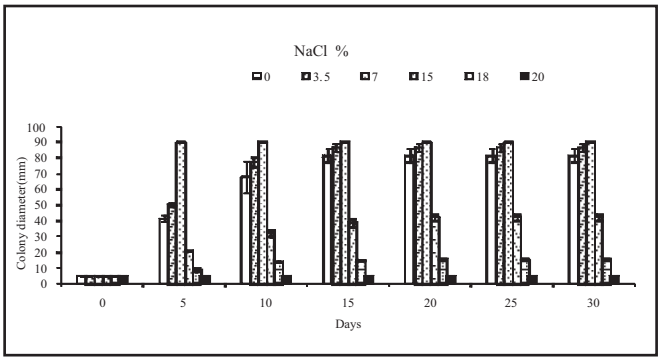


**Fig. 1c** - Effect of NaCl on growth of Sterile form 2.The mean and standard error values were calculated from six independent measurements.

NaCl. SF2 showed faster and maximum growth on 7% NaCl medium although it could grow also on higher salt concentrations (**Fig 1c**).

*Aspergillus* sp. 3 elaborated amylase, cellulase and protease in all the NaCl concentrations including the highest concentration studied (18%) (**Fig. 2a** Interestingly, this fungus showed more β-glucosidase and lipase activity in high salt medium than in medium without the salt. *A. terreus* produced all the enzymes screened in the presence of salt medium (**Fig 2b**). The activity of amylase, β-glucosidase, cellulase, lipase and protease was significantly more in the presence of salt than in the control medium (**Fig 2b and Plate 1)** Interestingly, maximum lipase activity was seen in the presence of 18% NaCl. SF2 produced β-glucosidase, pectinase and pectate transeliminase only on salt-amended media (**Fig 2c**). The activities of amylase, cellulase, lipase and protease were significantly higher on

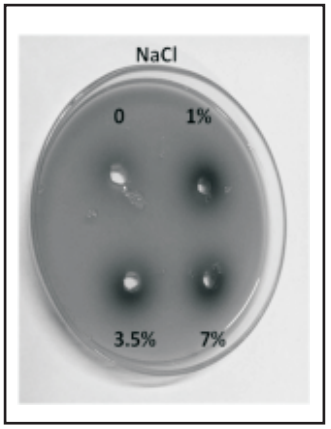


**Plate-1** Beta-glucosidase enzyme activity in the secretome of *A. terreus* grown in 0, 1, 3.5 or 7 % NaCl (agar plate diffusion assay).

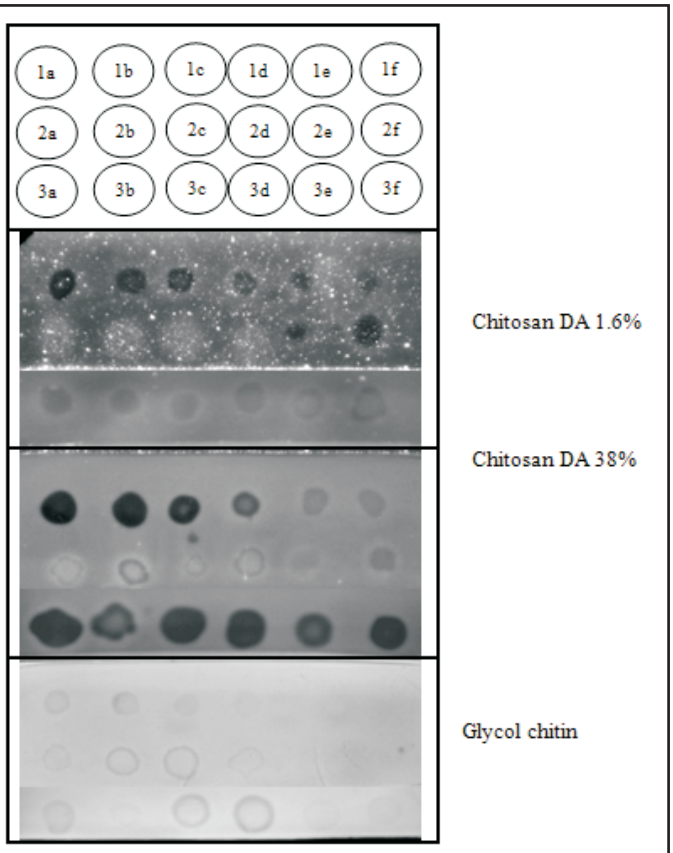


**Plate 2** - Dot blot assay for chitinase and chitosanase activity of saltern fungi as influenced by different concentrations of NaCl in the growth medium.

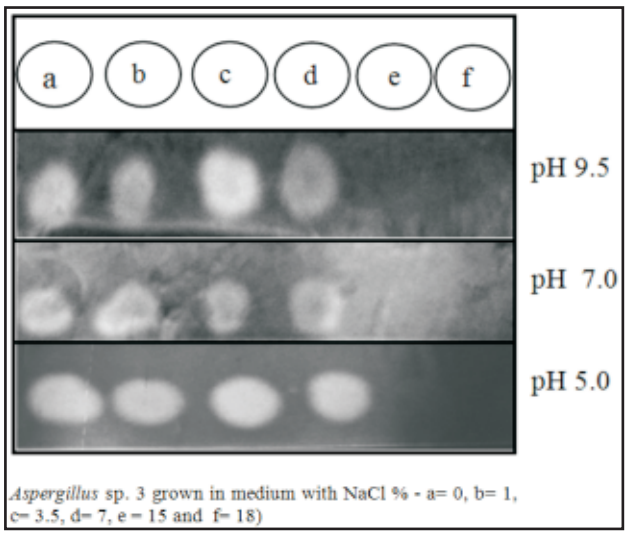


**Plate 3** - Dot blot assay for alkaline, neutral and acidic protease activity of *A. terreus* as influenced by different concentrations of NaCl in the growth medium.

salt medium than on control medium. With reference to chitin modifying enzymes, *Aspergillus* sp. 3 elaborated chitinase enzyme acting on chitosan of 1.6% degree of acetylation (DA) in all the salt concentrations (**Plate 2**). *A. terreus* did not show this enzyme activity; SF2 produced this enzyme only in the presence of 15 and 18% NaCl concentrations. *A. terreus* produced a chitosanase acting on chitosan of DA 38% in all the salt concentrations while *Aspergillus* sp. 3 produced this enzyme in a salt concentration of up to 7% (**Plate 2**). SF2 did not produce this enzyme. Chitinase enzyme activity was not discernable for the fungi under any salt concentration by the method used. Protease production by *Aspergillus* sp. 3 was also influenced by salt in the medium. This fungus showed alkaline, neutral and acidic protease activity in a salt concentration of up to 7% (**Plate 3**). At higher salt concentrations protease activity could not be detected.

**Table 2** Antialgal and antifungal activity of secretomes of fungi grown in different concentrations of NaCl.

| | Antialgal activity | | | | | Antifungal activity | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NaCl concentration in the medium | | | | | NaCl concentration in the medium | | | | |
| Fungus | 0% | 3.5% | 7% | 15% | 18 % | 0 % | 3.5% | 7 % | 15 % | 18 % |
| Extract of medium | - | - | - | - | - | - | - | - | - | - |
| *Aspergillus* sp. 3 | - | + | + | + | + | - | + | + | + | + |
| *Aspergillus terreus* | - | + | + | + | + | - | + | + | + | + |
| Sterile form 2 | - | + | + | + | + | - | - | - | - | - |

All the 3 fungi produce antialgal metabolites(s) only when grown in a medium having NaCl (**Table 2**). Antifungal metabolite(s) were produced by both the species of *Aspergillus* only in the presence of NaCl in the growth medium (**Table 2**). SF2 did not produce antifungal metabolite(s) both in the presence and absence of NaCl in the growth medium.

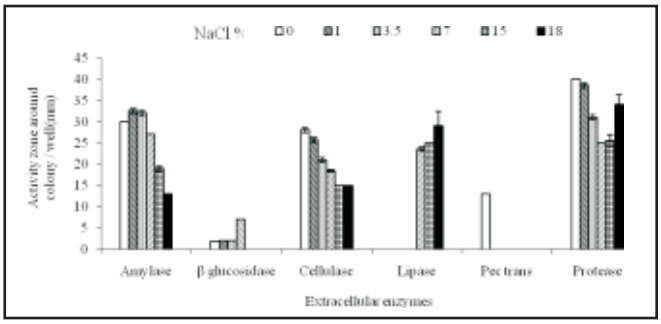


**Fig. 2a** - Extracellular enzyme production by *Aspergillus* sp. 3 in salt amended medium. The mean and standard error values were calculated from four independent measurements.

## DISCUSSION

Fungi from hyper saline environments have not been studied adequately (Suryanarayanan and Hawksworth, 2005). Extreme saline conditions such as those of salterns were thought to be inimical to filamentous fungi until it was confirmed by Gunde-Cimerman *et al*.(2004) that some species of fungi do occur in salterns and contribute to the microbial diversity of these extreme environments. In the present study, all the fungi isolated from saltern soils

(expecting the sterile forms whose identity was not confirmed) belonged to the *Ascomycota*; species of *Aspergillus* (including a species of *Emericella*, the teleomorph of *Aspergillus*) were more commonly present in the hypersaline soils. Members of *Ascomycota* and species of *Aspergillus* and *Emericella* are known to dominate the fungal community in hypersaline environments (Butinar *et al*., 2005; Gunde-Cimerman *et al*., 2004, 2009; Kis-Papo *et al*., 2001). Suryanarayanan *et al*. (1996) screened a saltern in southern India on a monthly basis for 10 months and concluded that its scanty mycoflora was represented to larger extent by species of *Aspergillus*. According to Gunde-Cimerman and Zalar (2014) of the total of 140 orders of fungi, very few, which include species of *Aspergillus*, are capable of growing in high salt environment. Cantrell *et al*. (2006) isolated many species of *Aspergillus* species including *A. flavus* and *Chaetomium globosum* from saltern environment. In the present study also, *Aspergillus flavus* and a *Chaetomium* species were isolated from saltern soils. These two fungi were isolated only by the Warcup method indicating that more than one isolation method has to be used to get a more complete picture of the species composition in hypersaline samples. Since we did not test the halotolerance of the other fungal isolates from the saltern such as *Dreschlera*, *Fusarium*, *Paecilomyces* and *Sporormiella*, it is uncertain if they constitute the natural mycobiota of this extreme environment.

The three fungi studied for salt tolerance did not show an obligate requirement of salt for their growth and hence are not halophilic. They showed a wide range of salt tolerance with *Aspergillus* sp. 3 showing optimal growth on salt-amended medium than on control medium. Fungi exhibiting such a wide range of salt tolerance are termed extremely halotolerant fungi (Gostinčar *et al*., 2010). Halotolerance rather than strict halophily as a species trait, ensures the ability to exploit wider ecological niches. Focused studies are needed to understand this ecological amplitude of fungi such as species of *Aspergillus* with reference to saline environment. The need to study the fungi of salterns more intensely is further underscored by the fact that even the limited studies on hypersaline environments have identified many new fungal species (Gunde-Cimerman and Zalar, 2014).

Little is known about the influence of salt on the production of secondary metabolites by hypersaline fungi. Butinar *et al*., (2011) indicated that production of mycotoxins by fungi of a saltern could contaminate the salt used for consumption.

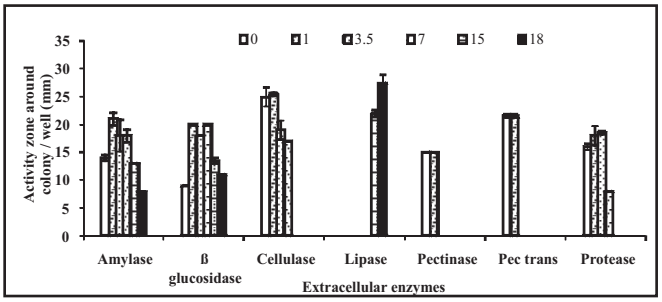


**Fig. 2b**- Extracellular enzyme production by *A. terreus* in salt amended medium. The mean and standard error values were calculated from four independent measurements.

*Wallemia ichthyophaga*, an obligate halophilic fungus, produces a haemolytic toxin under saline conditions (Gunde-Cimerman *et al*., 2009). Suryanarayanan (2012) reported that certain marine-derived endosymbiotic fungi of seaweeds produced antialgal metabolites only in the presence of NaCl in the growth medium. In the present study also, *Aspergillus* sp.3, *A. terreus* and SF2 produced antialgal metabolite(s) only in the presence of NaCl in the medium; again, salt was necessary for the two *Aspergillus* species to produce antifungal compounds. These studies indicate that the expression of certain genes in halotolerant fungi may be under the control of salt concentration in the external environment. For a halotolerant fungus, while production of antimicrobial metabolites only under saline conditions could well be a strategy to enhance its ecological fitness, it would be worthwhile studying salt-induced genes in such fungi to understand the phenomenon of halotolerance (Fang *et al*., 2014). It is pertinent to mention here that secondary metabolite profile of filamentous fungi is altered by its exposure in ionic liquids (Petkovic *et al*., 2009).

Notwithstanding the limitations of the agar plate assay (used for screening amylase, cellulase, lipase, pectinase and pectate transeliminase production) such as retarded diffusion of enzyme through agar gel, it showed that (i) some of these enzymes are produced through a range of salt concentrations, (ii) some of the enzymes show more activity in saline than non-saline environment and (iii) salt induced the production of some of the enzymes. A more sensitive dot blot test showed similar results for chitin modifying and protease enzymes. It is necessary to establish if the enhanced production of enzymes by NaCl is due to gene expression or induction of multiple forms of enzymes. Salt did not increase the activity of all the enzymes in the fungi tested; for instance while the lipase activity was pronounced in salt concentrations (**Figs. 2b & 2c** the activity β-glucosidases was less in these conditions. Thus it appears that salt does not change gene expression globally. Moreover, the apparent requirement of salt for induction of some of the enzymes in these fungi need further study. Recent studies show that the activities of chitin modifying enzymes (Venkatachalam *et al*.,

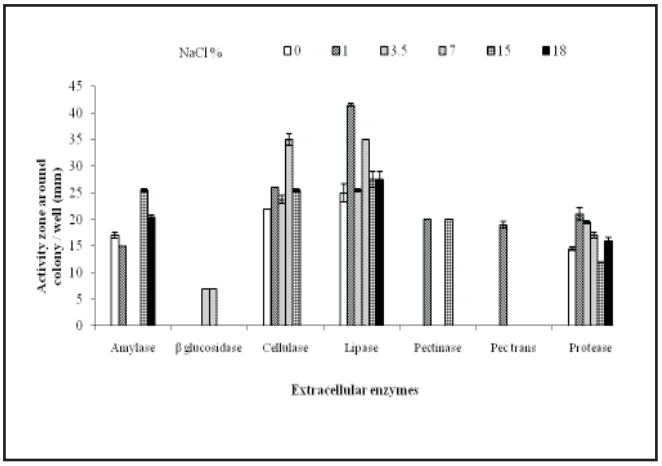


**Fig. 2c** - Extracellular enzymes production by Sterile form 2 in salt amended medium. The mean and standard error values were calculated from four independent measurements.

2015) and xylanase and xylosidase (Thirunavukkarasu *et al*., 2015) of marine-derived endophytes of seaweeds and seagrasses are induced or enhanced by NaCl. Halophilic extremozymes find use in various industrial processes including bioremediation (Gomes and Steiner, 2004; Trincone, 2011; Sengupta et al., 2017). The use of halophilic saccharification enzymes for production of biofuel from marine algae could do away with the desalination step which is critical when normal enzymes are used (Trincone, 2011). Salt tolerant proteases find use in fish processing and food industry (Trincone, 2011). However, in most cases, the source of such enzymes is Archaea (Gomes and Steiner, 2004) and bacteria (Moreno *et al*., 2013).

## CONCLUSION

Considering the importance of enzymes in food, medicine, and energy production, it would be worthwhile bioprospecting saltern fungi for novel halophilic enzymes. Saltern fungi exhibit a wide salt tolerance range and could be ideal candidates for source of novel extremozymes.

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