KAVAKA 42: 34-36(2014)

Introduction to extremophilic fungi

T. Satyanarayana
Department of Microbiology, University of Delhi, South Campus, New Delhi-110021
Corresponding author Email: tsnarayana@gmail.com

Moderate environments are essential for sustenance of life on the Earth. Moderate means environments with pH around neutral, temperatures between 20 and 40 °C, air pressure 1 atm and adequate levels of available water, nutrients and salts. There are many extreme environments on the Earth such as acidic or hot springs, saline and/or alkaline lakes and soils, deserts and the ocean beds, which are too harsh for normal life to exist. Any environmental condition that is perceived to be beyond the normal acceptable range is considered as an extreme condition. The microbes that survive and grow in such harsh environments are known as extremophiles. These organisms not only tolerate specific extreme condition(s), but usually require them for their survival and growth. Most extremophiles are found in the microbial world. The range of environmental extremes tolerated by microbes is much broader than the other life forms. The limits of growth and reproduction of microbes include: -12 ° to more than +100 °C, pH 0 to 13, hydrostatic pressures up to 1400 atm, sugar syrups and salt concentrations of saturated brines. Besides natural extreme environments, there are also man-made extremes such as in cool houses, steam heated buildings, laundry machines, acid mine waters and industrial effluents.

Life in extreme environments is being investigated extensively and intensively focusing attention on the diversity of organisms, and molecular and regulatory mechanisms involved. The products obtainable from extremophiles such as proteins, enzymes (extremozymes) and compatible solutes are of immense interest in biotechnology. This field of research has also attracted attention because of its impact on the possible existence of life on other planets. The progress achieved in understanding research on various aspects of extremophiles and thermophiles is discussed in international conferences held every alternate year in different countries. The 9th Congress on Extremophiles was held at Sevilla (Spain) in Sept. 2012, and the next is scheduled for Sept. 2014 at St. Petersburg (Russia). While 12th Thermophiles conference was held in Sept. 2013 at Regensburg (Germany).

The abundance, long evolutionary history, and the diversity of fungi carry enormous potential to be explored. By studying more complete coverage of fungi, a more accurate census of gene diversity, regulatory elements, and genome organization will be accomplished. This increased knowledge is expected to positively affect translational science (e.g., biological engineering) in fungal biology and ultimately the successful application of fungi to solve some of the crucial energy and climate change challenges faced by human civilization (Grigoriev *et al.*, 2011). In this special segment of Kavaka, an attempt has been made to review the diversity of fungi that occur in extreme environments, their adaptations and potential biotechnological applications.

Diversity of extremophilic/extremotolerant fungi

Since the mid 1990s, fungi growing in diverse extreme environments have been discovered, and further, expanded the investigations in the domain of extremophilic microbiology, which has been traditionally focused on prokaryotic microbes. The detailed investigations on extremophilic fungi have given insight into some of the eukaryotic adaptations to extreme growth conditions. The fungal extremophiles such as halophilic and halotolerant, thermophilic and thermotolerant, osmotolerant, xerotolerant, alkalitolerant, oligotrophic, radiation tolerant, psychrotolerant, acid tolerant, heavy metal tolerant and obligate/facultative anaerobic fungi have been studied worldwide in a great variety of natural as well as man-made environments. The investigations revealed abundant and consistent occurrence of certain fungal species in the extreme environments. The advent of new and rapid sequencing technologies is helping further in elucidating the microbial diversity in the ecosystems, but more investigations are needed to document the functional role of fungi thriving in these unusual environments. The survival of cells depends on the ability of organism to sense and respond to environmental stresses.

The dominant fungi that occur in eutrophic hypersaline waters are meristematic black yeasts such as halophilic *Hortaea werneckii*, *Phaeotheca triangularis*, *Trimmatostroma salinum*, haloto-lerant *Aureobasidium pullulans* (Gunde-Cimerman *et al.*, 2000) and different species of the genus *Cladosporium* (Sterflinger, 1998). Six different species of the xerophilic genus *Eurotium* (Butinar *et al.*, 2005) and *Wallemia* (Zalar *et al.*, 2005) have been repeatedly isolated. The genus *Wallemia* is represented by *W. muriae*, *W. sebi* and *W. ichthyophaga* is known to be the most halophilic fungus to date (Zalar *et al.*, 2005). Different species of *Aspergillus* and *Penicillium* and diverse non-melanised yeasts often appear in hypersaline water of salterns (Gunde-Cimerman *et al.*, 2005).

Halophilic and halotolerant organisms thrive in environments with low a_w (water activity) because they can counterbalance the osmotic stress by accumulation of either high intracellular salt concentrations or osmotically active compatible solutes. In addition to their function of maintaining the osmotic equilibrium across the cell membrane, compatible solutes are effective stabilizers of enzyme function, providing protection against temperature extremes, freeze–thaw treatment and even dryness. Mycosporines glutaminol–glucoside and mycosporine–glutamicol–glucoside have been detected in halophilic black yeasts *Phaeotheca triangularis*, *Trimmatostroma salinum*, *Hortaea werneckii* and a halotolerant *Aureobasidium pullulans*, as well in a basidiomycetous yeast *Cryptococcus liquefacien* (Kogez *et al*., 2006). Further investigations are called for verifying whether mycosporine–glutaminol–glucoside plays an osmoprotective role in fungi living in extreme environments. The organisms in saline environments respond to osmolarity changes through multiple signaling pathways (Lenassi *et al*., 2007). The ability to survive osmotic stress requires several adaptations involving osmoregulation, ion homeostasis, accumulation of solutes, and possible modifications of the cell morphology (Hohmann *et al*., 2007).

Alkalitolerant fungi have been isolated from alkaline soils. Among fungi isolated from alkaline soils in Japan, *Acremonium alternatum*, *A. furcatum*, *Gliocladium cibotii*, *Phialophora geniculata*, *Stachylidium bicolor* and *Stilbella annulata*, *Acremonium murorum*, and *Chrysosporium murdodum* (Nagai *et al*., 1995; 1998) have been reported to be capable of growth at pH 10.0. Kladwang *et al*. (2003) isolated 490 alkalitolerant fungal isolates from soils in Thailand. Among these the species of *Acremonium* and *Stilbella* have been shown to be sources of alkaline enzymes. Zvyagilskaya and Persson (2004) isolated a yeast strain that grows vigorously at pH 9.7 and this strain is osmotolerant and halotolerant.

The Arctic and Antarctic regions have been investigated for understanding the diversity of microbes including fungi. Low temperatures induce the formation of ice crystals, and therefore, also cause low water activity (a_w). These are the dominant factors in external chemistry that influence microbial biota in cold regions. Low water activity media and low incubation temperatures have, therefore, been employed for the isolation of fungi from an Arctic environment. The dominant taxa were ascomycetous and basidiomycetous yeasts and melanized fungi mainly represented by the genera *Cladosporium* and *Aureobasidium* along with different species of the genus *Penicillium* (Gunde-Cimerman *et al*., 2003). *Cladosporium sphaerospermum*, *C. herbarum* and *Aureobasidium pullulans* had also been isolated from polar regions (Abyzov, 1993). Microfungi isolated from Arctic soils included *Acremonium*, *Geomyces*, *Mortierella*, *Phialophora*, *Phoma, Thelebolus* and sterile fungi (Bergero *et al*., 1999). *Eutypella scoparia*, *Hyphozyma variabilis* and *Ovadendron sulphureoochraceum* have been recorded for the first time.

Xerophilic fungi are yeasts and moulds that are capable of growth at or below a water activity (a_w) of 0.85. These microorganisms have developed physiological mechanisms that enable their biochemical pathways to function in environments where little water is available. External conditions of low a_w are sensed by membrane osmosensors, and xerophiles then accumulate glycerol as a compatible solute to balance the internal and external osmotic pressure. They also modify their membranes to retain this glycerol within the cell. As a group, xerophiles are extremely important in the spoilage of many processed foods and stored commodities, and in indoor environments. Moderate xerophiles include species within *Aspergillus, Penicillium* and *Eurotium*. Extreme xerophiles compete poorly at high a_w, because they require decreased a_w for growth. Some xerophiles have a preference for salt or sugar substrates, whereas other species can be isolated from both jam and salterns. Xerophiles are widely spread on the fungal tree of life. Rock-inhabiting black fungi are able to cope with different stresses that prevail in bare rocks in hot and cold extreme environments. The analysis of protein patterns in extremotolerant black rock fungi (*Coniosporium perforans, Exophiala jeanselmei* and *Friedmanniomyces endolithicus*) suggested that there is a decline in the number of proteins suggesting a down-regulation of their metabolism under extreme conditions (Tesei *et al*., 2012). *Wallemia sebi*, a xerophilic basidiomycete fungus, was found to contain 93 putative osmotic stress proteins (Padamsee *et al*., 2012).

Acid mine drainage (AMD) microbial communities contain microbial eukaryotes that confer a biofilm structure and impact the abundance of bacteria and archaea and the community composition through grazing and other mechanisms. Since prokaryotes impact iron oxidation rates and thus regulate AMD generation rates, fungal populations have been studied. Utilizing 18S rRNA and beta-tubulin gene phylogenies and fluorescent rRNA-specific probes to characterize the eukaryotic diversity and distribution in extremely acidic (pHs 0.8 to 1.38), warm (30 to 50°C), metal-rich (up to 269 mM Fe, 16.8 mM Zn, 8.5 mM As, and 4.1 mM Cu) AMD solutions from the Richmond Mine at Iron Mountain, California. The fungal 18S rRNA and tubulin gene sequences formed two distinct phylogenetic groups associated with the classes *Dothideomycetes* and *Eurotiomycetes* (Baker *et al*., 2004). *Penicillium rubrum* isolated from acidic metal sulphate-contaminated water from Birkley Pit Lake, Montana produced several polyketide metabolites (Stierle *et al*., 2012). Berkazophilones B & C and penicimplicssin exhibited capspase-1 inhibition in specific leukemia cell lines.

There are fewer than 50 species of thermophilic fungi which thrive at relatively elevated temperatures (Mouchacca, 1997; Johri *et al*., 1999). These are common in soils and in habitats wherever organic matter heats up due to decomposition. Thermophilic fungi have been isolated from manure, compost, industrial coal mine soils, beach sands, nuclear reactor effluents, Dead Sea valley soils, and desert soils of Saudi Arabia. In these habitats, thermophiles may occur either as resting propagules or as active mycelia depending on the availability of nutrients and favorable environmental conditions. Generally there is an inverse relationship between biological diversity and the adaptation required to survive in a specific habitat.

Thermophilic fungi are a small assemblage in Eukaryota, which have evolved strategies for growing at elevated temperatures up to 60 to 62 °C. During the last 50 – 60 years, many species of thermophilic fungi sporulating at 45 °C have been reported. Thermophilic fungi have been defined by Cooney and Emerson (1964), as 'those capable of growth at or above 50 °C and a minimum temperature for growth at or above 20 °C'. Several aspects of thermophilic and thermotolerant fungi are covered in the ensuing chapters.

I sincerely hope and wish that mycologists will find the contents of this Kavaka special segment informative and interesting, and encourage Indian mycologists to carry out investigations on the extremophilic fungi that are present in Indian extreme environments.

REFERENCES

Abyzov, S.S. 1993. Microorganisms in Antarctic ice. In: *Antarctic Microbiology* (Ed.: Friedmann, E.I.), Wiley Liss Inc. New York, pp. 265-295.

Baker, B.J., Lutz, M.A., Dawson, S.C., Bond, P.L. and Bandfield, J.F. 2004. Metabolically active eukaryotic communities in extremely acidic mine drainage. *Appl. Env. Microbiol.* **70**: 6264-6271.

Bergero, R., Girlanda, M., Veres, G.C., Intili, D. and Luppi, A.M. 1999. Psychrophilic fungi from Arctic soils of Franz Joseph Land. *Polar Biol.* **21**: 361-368.

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

Cooney, D.G. and Emerson, R. 1964. *Thermophilic fungi, An account of their biology, Activities and Classification*. W.H. Freeman and Co. san Fransisco, 181 pp.

Grigoriev, I.V., Cullen, D., Goodwinc, S.B., Hibbett, D., Jeffriesb, T.W., Kubicek, C.P., Kuske, C., Magnuson, J.K., Martin, F., Spataforai, J.W., Tsang, J. and Scott E. Baker, S.E. 2011. Fueling the future with fungal genomics. *Mycology*: DOI:10.1080/ 21501203. 2011.584577.

Gunde-Cimerman, N., Zalar, P., de Hoog, S. and Plemenitas, A. 2000. Hypersaline waters in salterns-natural ecological niches for halophilic black yeasts. *FEMS Microbiol. Ecol.* **32**: 235–240.

Gunde-Cimerman, N., Sonjak, S., Zalar, P., Frisvad, J.C., Diderichsen, B. and Plemenitas, A. 2003. Extremophilic fungi in Arctic ice: a relationship between adaptation to low temperature and water activity. *Physics and Chemistry of the Earth* **28**: 1273-1278.

Gunde-Cimerman N, Oren A. and Plemenitas A. 2005. *Adaptation to Life in High Salt Concentrations in Archaea, Bacteria, and Eukarya*. Springer, Dordrecht, the Netherlands.

Hohmann, S., Krantz, M. and Nordlander, B., 2007. Yeast osmoregulation. *Methods Enzymol.* **428**: 29–45.

Johri B.N., Satyanarayana, T. and Olsen J. 1999. *Thermophilc Moulds In Biotchnology*. (Eds.: Johri B.N., Satyanarayana, T. and Olsen J.). Kluwer Academic Publishers, Dordrecht. pp.354.

Kladwang, W., Bhumirattana, A. and Hywel-Jones, N. 2003. Alkaline-tolerant fungi from Thailand. *Fungal Diversity* **13**: 69 – 83.

Kogej, T., Gostincar, C., Volkmann, M., Gorbushina, A.A. and Gunde-Cimerman, N. 2006. Mycosporines in extremophilic fungi—novel complementary osmolytes? *Environ. Chem.***3**: 105–110.

Lenassi, M., Vaupotic, T., Gunde-Cimerman, N. and Plemenitas, A. 2007. The MAP kinase HwHog1 from the halophilic black yeast *Hortaea werneckii*: coping with stresses in solar salterns. *Saline Systems* **3** (3): 1-11.

Mouchacca, J. 1997. Thermophilic fungi: biodiversity and taxonomic status. *Crypt. Mycol.* **18**: 19-69.

Nagai, K., Sakai T., Rantiatmodjo R.M.,Suzuki, K., Gams, W. and Okada, G. 1995. Studies on the distribution of aikalophilic and alkali-tolerant soil fungi I. *Mycosci.* **36**: 247-256.

Nagai, K., Suzuki, K., and Okada G. 1998. Studies on the distribution of alkaliphilic and alkali-tolerant soil fungi II: Fungal flora in two limestone caves in Japan. *Mycosci.* **39**: 293-298.

Padamsee, M., Arun Kumar, T.K., Riley, R., Binder, M. et al. 2012. The genome of the xerotolerant mold *Wallemia sebi* reveals adaptations to osmotic stress and suggests cryptic sexual reproduction. *Fungal Genet. Biol.* **49**: 217-226.

Sterflinger, K. 1998. Temperature and NaCl-tolerance of rock-inhabiting meristematic fungi. *Antonie van Leeuwenhoek* **74**: 271-281.

Stierle, A.A., Stierle, D.B. and Girtsman, T. 2012. Caspase-1 inhibitors from an etremophilic fungus that target specific leukemia cell lines. *J. Nat. Prod.* **75**: 344- 350.

Tesei, D.,Marzban, G., Zakharova, K., Isola, D., Selbmann, L. and Sterflinger, K. 2012. Alteration of protein patterns in black rock inhabiting fungi as a response to different temperatures. *Fungal Biol.* **116**: 932-940.

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

Zvyagilskaya, R.A. and Persson, B.L. 2004. A new alkalitolerant *Yarrowia lipolytica* yeast strain is a promising model for dissecting properties and regulation of Na^+-dependent phosphate transporting system. *Biochemistry (Moscow)* **69**: 1607 – 1615.