

Biodata of **Sybren de Hoog**, author of “*Relation of Halotolerance to Human-Pathogenicity in the Fungal Tree of Life: An Overview of Ecology and Evolution under Stress*”

**Dr. Sybren de Hoog** is a senior researcher at the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands and associate professor at the Institute of Biodiversity and Ecosystem Dynamics at the University of Amsterdam. His research focuses on fungal taxonomy and molecular phylogeny, particularly of black yeasts and relatives. He wrote more than 275 refereed papers, mostly concerning clinically relevant moulds, and including the diagnostic handbook “Atlas of Clinical Fungi” (2nd ed., 2000, 1126 pp.). Currently he is President-elect of the International Society for Human and Animal Mycology (ISHAM).

E-mail: [de.hoog@cbs.knaw.nl](mailto:de.hoog@cbs.knaw.nl)





## RELATION OF HALOTOLERANCE TO HUMAN-PATHOGENICITY IN THE FUNGAL TREE OF LIFE: AN OVERVIEW OF ECOLOGY AND EVOLUTION UNDER STRESS

SYBREN DE HOOG<sup>1,2</sup>, POLONA ZALAR<sup>3</sup>, BERT GERRITS VAN DEN ENDE<sup>1</sup> and NINA GUNDE-CIMERMAN<sup>3</sup>

<sup>1</sup>Centraalbureau voor Schimmelcultures, P.O. Box 85167, NL-3508 AD Utrecht, The Netherlands; <sup>2</sup>Institute of Biodiversity and Ecosystem Dynamics, Kruislaan 318, NL-1098 SM Amsterdam, The Netherlands; <sup>3</sup>Biotechnical Faculty, Biology Department, Večna pot 111, SI-1000 Ljubljana, Slovenia

### 1. Introduction

It is a general belief in mycology that fungi growing on substrates with low water activity display a general xerophylic phenotype (Northolt et al., 1995), determined primarily by the water potential of the medium rather than by the chemical nature of the solute (Hocking, 1993; Pitt and Hocking, 1997). Therefore fungi are considered xerophilic if they grow well at water activity ( $a_w$ ) of 0.85 or less, corresponding to 17% NaCl or 50% glucose added to the growth medium. In contrast to obligate halophilic bacteria, only a few species of food-borne fungi (*Basipetospora halophila*, *Polypaecilum pisce* and *Hortaea werneckii*) were known to be stimulated by NaCl but without any obligate requirement (Andrews and Pitt, 1987; Pitt and Hocking, 1997). Therefore Pitt and Hocking (1997) concluded that there was no evidence of halophily in fungi. This conviction remained unchallenged, until fungi as well as bacteria, able to adapt to a wide range of salinities, were isolated from natural hypersaline environments (Gunde-Cimerman et al., 2000; Oren, 2002) and the question how to define a halophile was reopened.

Over the years, several definitions of halophily have been proposed. Kushner (1978) divided microorganisms into four groups, based on the salt concentrations optimal for their growth. Lanyi (1978) suggested the requirement for salt as the basis of halophily, while Brock (1994) accommodated also marine microorganisms among halophiles, as long as they required sodium ions. In his recent monograph, Oren (2002) included in his list of halophilic microorganisms those which are able to grow above 100 g l<sup>-1</sup> salts, even if their salinity optimum is lower.

Fungi display a halophilic behaviour different from that of the majority of halophilic prokaryotes. Although halophilic fungi do not require salt for viability, they are able to grow and adjust to the whole salinity range, from freshwater to almost saturated NaCl solutions. This flexibility enables halophilic fungi to survive periods of extreme environmental stress in a resting state. When conditions improve, they immediately respond with increased metabolic activity, growth and propagation. Therefore Gunde-

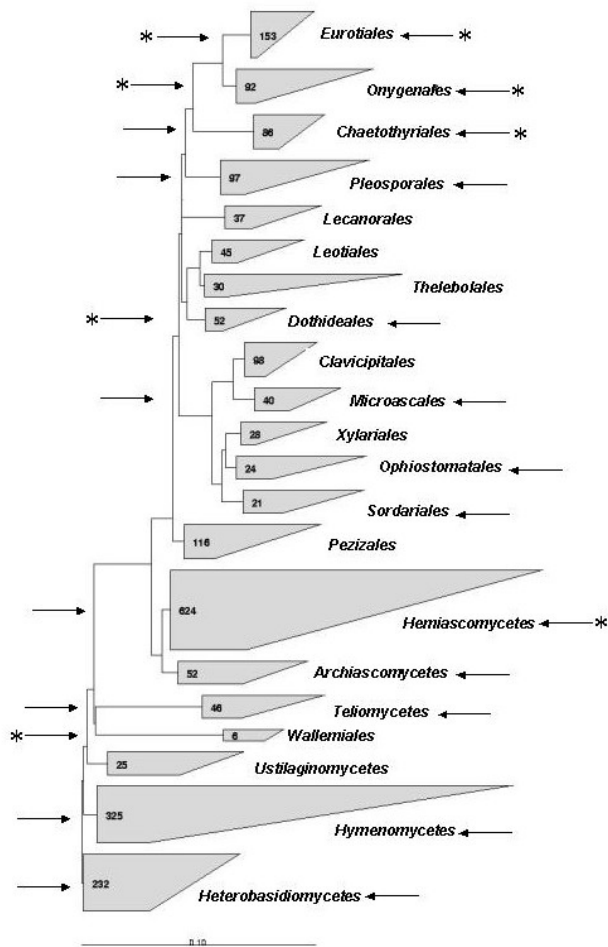
Cimerman et al. (2000, 2005) considered fungi isolated from hypersaline environments as halophilic if they were isolated with high frequency on selective saline media from environments at salinities above 10% and able to grow *in vitro* with 17% NaCl. They were regarded as halotolerant if they were isolated from water with lower salinities but nevertheless able to grow *in vitro* with 17% NaCl.

Although halotolerance and halophily according to these definitions are uncommon amongst fungi, truly halophilic species do exist. On the other hand, xerotolerance is observed more frequently. Fungi able to grow with little available water are rare in lower fungi, in the divisions *Chytridiomycota* and *Zygomycota*. For example, thraustochytrids and labyrinthulid non-filamentous fungi (*Labirinthuloides minuta*, *Schizochytrium* sp. and *Thraustocytrium* sp.) have been isolated from a sandy beach of Great Salt Lake, Utah (Amon, 1978). In the *Zygomycota*, *Mucor hiemalis*, *M. racemosus*, *M. circinelloides*, *Rhizopus nigricans*, *R. oryzae* and *R. stolonifer* have occasionally been isolated from saline soil and *Mucor circinelloides* and *M. hiemalis* from salterns (Abdel-Fattah et al., 1977; Abdel-Hafez, 1982; Guiraud et al., 1995; Grishkan et al., 2003; Mahdy et al., 1996; Steiman et al., 1995, 1997). In addition, *Mucor circinelloides*, *M. racemosus*, *M. spinulosus* and *Rhizopus stolonifer* are known as food-borne fungi.

Halophily is scattered amongst the higher fungi and occurs in several orders of the *Basidiomycota* and *Ascomycota* (Fig. 1, Table 1). In any particular order, growth at decreased water activity is in most cases limited to a few species or a single genus of an order. In the orders *Wallemiales*, *Eurotiales* and *Dothideales*, however, halophily is expressed in several groups of the same order that are not each others nearest phylogenetic neighbours. This suggests that this otherwise rare property either is an evolutionary ancient trait (plesiomorph) within these orders or that different species of genera independently have adapted to halidic conditions. If its origin is evolutionary old, there may be a general constitutional and physiological cellular mechanism underlying this behaviour. In the following we will review how xerotolerance, halotolerance and halophily is expressed in individual species.

Despite the known phylogenetic diversity of the xerotolerant fungi, we have made a remarkable observation when we compared the distribution of xerotolerance in the fungal kingdom with that of fungi able to invade warm-blooded animals. Table 1 compares the occurrence of xerotolerance with opportunism in humans and animals in orders of *Asco-* and *Basidiomycota*. At present, a total of 106 orders of fungi are known (Kirk et al., 2001). Tolerance of low water-activity is apparent in only ten of these (Table 2). Pathogenicity and consistent opportunism (BioSafety Levels 2 or 3; de Hoog et al., 2000) are also found in ten orders. A further twelve orders include species with low or insignificant pathogenicity, shown in occasional infections and therefore regarded as BSL-1 (de Hoog et al., 2000). Table 2 lists the orders with species proven to belong to BSL-2 or 3. Both properties, i.e. consistent xerotolerance as well as consistent invasive ability, are uncommon in the fungal kingdom. Nonetheless, the two lists show total overlap: eight orders with xerotolerance also contain opportunistic fungi of BSL 2-3, while the remaining three contain occasional opportunists (BSL-1). This strongly suggests that the genetic backbone of each of these eight orders encodes properties that are useful for both life strategies. Focusing on individual species, we notice, however, a dual tendency. With only a few exceptions discussed below, species exhibiting xerotolerance have no BSL attribution at all or belong to BSL-1 (Table 1). BSL-1 species have either never been encountered in medical mycology, or morbidity was

insignificant, coincidental or extremely rare). Thus, the eight orders of fungi including xerotolerant and opportunistic species respectively strongly coincide. However, the individual species within these orders nearly always have only one of the two properties. Thus, at the species level, xerotolerance and pathogenicity seem to be mutually exclusive.



**Figure 1.** Phylogenetic tree of the higher fungi (*Basidiomycota* and *Ascomycota*) based on 2229 near-complete SSU sequences, aligned with reference to *Saccharomyces cerevisiae*. The tree was constructed with 1514 positions using the Neighbour Joining algorithm. Each flag displays the number of sequences it contains. Arrows at the left indicate the presence of X<sub>T</sub> or H<sub>T</sub> species, additional asterisks indicate the presence of X<sub>P</sub> or H<sub>P</sub> species. Arrows at the right indicate the presence of species with BioSafety Levels 1-2, additional asterisks indicate the presence of species with BSL-3. *Saccharomycetales* are under *Hemiascomycetes*, *Filobasidiales* are under *Heterobasidiomycetes*, *Polyporales* are under *Hymenomycetes*, and *Sporidiales* are under *Teliomycetes*.

TABLE 2. Approximate overview of xerotolerant, xerophilic, halotolerant and halophilic species in the fungal kingdom and their BSL.

	Xero- /halo- tolerance	BSL		Xero- /halo- tolerance	BSL
<b>Wallemiales</b>			<b>Eurotyales (continued)</b>		
<i>Wallemia ichthyophaga</i>	H <sub>P</sub>	-	<i>Penicillium corylophilum</i>	X <sub>T</sub>	1
<i>Wallemia muriae</i>	X <sub>P</sub>	-	<i>Penicillium chrysogenum</i>	X <sub>T</sub>	1
<i>Wallemia sebi</i>	X <sub>T</sub>	1	<i>Penicillium citrinum</i>	X <sub>T</sub>	1
			<i>Penicillium crustosum</i>	X <sub>T</sub>	-
<b>Filobasidiales</b>			<i>Penicillium cyclopium</i>	X <sub>T</sub>	-
<i>Trichosporon mucoides</i>	X <sub>T</sub>	2	<i>Penicillium digitatum</i>	X <sub>T</sub>	-
<i>Trichosporonoides nigrescens</i>	X <sub>T</sub>	-	<i>Penicillium duclauxii</i>	X <sub>T</sub>	-
<i>Trichosporonoides oedocephalis</i>	X <sub>T</sub>	-	<i>Penicillium expansum</i>	X <sub>T</sub>	-
			<i>Penicillium fagi</i>	X <sub>T</sub>	-
<b>Sporidiales</b>			<i>Penicillium fellutanum</i>	X <sub>T</sub>	-
<i>Rhodosporidium sphaerocarpum</i>	X <sub>T</sub>	1	<i>Penicillium flavigenum</i>	X <sub>T</sub>	-
<i>Rhodotorula babjevae</i>	X <sub>T</sub>	-	<i>Penicillium freii</i>	X <sub>T</sub>	-
<i>Rhodotorula laryngis</i>	X <sub>T</sub>	-	<i>Penicillium frequentans</i>	X <sub>T</sub>	-
			<i>Penicillium funiculosum</i>	X <sub>T</sub>	-
<b>Polyporales</b>			<i>Penicillium glabrum</i>	X <sub>T</sub>	-
<i>Phanerochaete chrysosporium</i>	X <sub>T</sub>	1	<i>Penicillium herquei</i>	X <sub>T</sub>	-
			<i>Penicillium implicatum</i>	X <sub>T</sub>	-
<b>Dothideales</b>			<i>Penicillium islandicum</i>	X <sub>T</sub>	-
<i>Aureobasidium pullulans</i>	X <sub>T</sub>	1	<i>Penicillium janczewskii</i>	X <sub>T</sub>	-
<i>Cladosporium sphaerospermum</i>	X <sub>T</sub>	1	<i>Penicillium janthinellum</i>	X <sub>T</sub>	-
<i>Cladosporium herbarum</i>	X <sub>T</sub>	1	<i>Penicillium lanosum</i>	X <sub>T</sub>	-
<i>Cladosporium cladosporioides</i>	X <sub>T</sub>	1	<i>Penicillium manginii</i>	X <sub>T</sub>	-
<i>Hortaea werneckii</i>	H <sub>P</sub>	1	<i>Penicillium miczynskii</i>	X <sub>T</sub>	-
<i>Phaeoheca triangularis</i>	H <sub>P</sub>	-	<i>Penicillium melinii</i>	X <sub>T</sub>	-
<i>Trimmatostroma salinum</i>	H <sub>P</sub>	-	<i>Penicillium montanense</i>	X <sub>T</sub>	-
			<i>Penicillium nordium</i>	X <sub>T</sub>	-
<b>Eurotiales</b>			<i>Penicillium oxalicum</i>	X <sub>T</sub>	-
<i>Aspergillus caespitosus</i>	X <sub>T</sub>	-	<i>Penicillium polonicum</i>	X <sub>T</sub>	-
<i>Aspergillus candidus</i>	X <sub>T</sub>	1	<i>Penicillium purpurogenum</i>	X <sub>T</sub>	1
<i>Aspergillus carneus</i>	X <sub>T</sub>	1	<i>Penicillium restrictum</i>	X <sub>T</sub>	-
<i>Aspergillus caesiellus</i>	X <sub>T</sub>	1	<i>Penicillium rugulosum</i>	X <sub>T</sub>	1
<i>Aspergillus clavatus</i>	X <sub>T</sub>	1	<i>Penicillium roquefortii</i>	X <sub>T</sub>	-
<i>Aspergillus conicus</i>	X <sub>T</sub>	1	<i>Penicillium simplicissimum</i>	X <sub>T</sub>	-
<i>Aspergillus elegans</i>	X <sub>T</sub>	-	<i>Penicillium sizovae</i>	X <sub>T</sub>	-
<i>Aspergillus egyptiacus</i>	X <sub>T</sub>	-	<i>Penicillium steckii</i>	X <sub>T</sub>	-
<i>Aspergillus flavipes</i>	X <sub>T</sub>	1	<i>Penicillium solitum</i>	X <sub>T</sub>	-
<i>Aspergillus flavus</i>	X <sub>T</sub>	2	<i>Penicillium spinulosum</i>	X <sub>T</sub>	-
<i>Aspergillus fumigatus</i>	X <sub>T</sub>	2	<i>Penicillium sumatrense</i>	X <sub>T</sub>	-
<i>Aspergillus heteromorphus</i>	X <sub>T</sub>	-	<i>Penicillium variabile</i>	X <sub>T</sub>	-
<i>Aspergillus homomorphus</i>	X <sub>T</sub>	-	<i>Penicillium verrucosum</i>	X <sub>T</sub>	-
<i>Aspergillus melleus</i>	X <sub>T</sub>	-	<i>Penicillium viridicatum</i>	X <sub>T</sub>	-
<i>Aspergillus niger</i>	X <sub>T</sub>	1	<i>Penicillium waksmanii</i>	X <sub>T</sub>	-
<i>Aspergillus niveus</i>	X <sub>T</sub>	1	<i>Penicillium westlingii</i>	X <sub>T</sub>	-
<i>Aspergillus ochraceus</i>	X <sub>T</sub>	1	<i>Polypaecilium pisce</i>	H <sub>P</sub>	-
<i>Aspergillus oryzae</i>	X <sub>T</sub>	1	<i>Talaromyces flavus</i>	X <sub>T</sub>	-
<i>Aspergillus parasiticus</i>	X <sub>T</sub>	-	<i>Talaromyces dupontii</i>	X <sub>T</sub>	-

<i>Aspergillus penicillioides</i>	X <sub>T</sub>	-	<i>Talaromyces stipitatus</i>	X <sub>T</sub>	-
<i>Aspergillus proliferans</i>	X <sub>T</sub>	-	<i>Talaromyces wortmanii</i>	X <sub>T</sub>	-
<i>Aspergillus puniceus</i>	X <sub>T</sub>	-	<i>Xeromyces bisporus</i>	X <sub>P</sub>	-
<i>Aspergillus restrictus</i>	X <sub>T</sub>	1			
<i>Aspergillus sclerotiorum</i>	X <sub>T</sub>	1	<b>Chaetothyriales</b>		
<i>Aspergillus sydowii</i>	X <sub>T</sub>	1	<i>Exophiala dermatitidis</i>	X <sub>T</sub>	2
<i>Aspergillus sulphureus</i>	X <sub>T</sub>	-	<i>Exophiala pisciphila</i>	X <sub>T</sub>	1
<i>Aspergillus tamarii</i>	X <sub>T</sub>	1			
<i>Aspergillus terreus</i>	X <sub>T</sub>	2	<b>Microascales</b>		
<i>Aspergillus tubingensis</i>	X <sub>T</sub>	-	<i>Pseudallescheria boydii</i>	X <sub>T</sub>	2
<i>Aspergillus ustus</i>	X <sub>T</sub>	1			
<i>Aspergillus versicolor</i>	X <sub>T</sub>	1	<b>Onygenales</b>		
<i>Aspergillus wentii</i>	X <sub>T</sub>	-	<i>Chrysosporium farinicola</i>	X <sub>T</sub>	-
<i>Basipetospora halophila</i>	H <sub>P</sub>	-	<i>Chrysosporium fastidium</i>	X <sub>T</sub>	-
<i>Emericella nidulans</i>	X <sub>T</sub>	1	<i>Chrysosporium inops</i>	X <sub>T</sub>	1
<i>Emericella purpurea</i>	X <sub>T</sub>	-	<i>Coccidioides immitis</i>	X <sub>T</sub>	3
<i>Emericella rugulosa</i>	X <sub>T</sub>	-	<i>Coccidioides posadasii</i>	X <sub>T</sub>	3
<i>Emericella xerophila</i>	X <sub>P</sub>	-	<i>Gymnascella marismortui</i>	H <sub>T</sub>	-
<i>Eremascus albus</i>	X <sub>T</sub>	-			
<i>Eremascus fertilis</i>	X <sub>T</sub>	-	<b>Pleosporales</b>		
<i>Eupenicillium crustaceum</i>	X <sub>T</sub>	-	<i>Alternaria mouchaccae</i>	X <sub>T</sub>	-
<i>Eupenicillium egyptiacum</i>	X <sub>T</sub>	-	<i>Dendryphiella salina</i>	H <sub>T</sub>	-
<i>Eupenicillium sinaicum</i>	X <sub>T</sub>	-	<i>Ulocladium chlamydosporum</i>	X <sub>T</sub>	-
<i>Eurotium amstelodami</i>	X <sub>T</sub>	1			
<i>Eurotium chevalieri</i>	X <sub>T</sub>	1	<b>Saccharomycetales</b>		
<i>Eurotium desertorum</i>	X <sub>P</sub>	-	<i>Candida famata</i>	H <sub>P</sub>	1
<i>Eurotium echinulatum</i>	X <sub>T</sub>	-	<i>Candida magnoliae</i>	X <sub>T</sub>	-
<i>Eurotium halophilicum</i>	X <sub>T</sub>	-	<i>Candida parapsilosis</i>	X <sub>T</sub>	1
<i>Eurotium herbariorum</i>	X <sub>T</sub>	1	<i>Candida scottii</i>	X <sub>T</sub>	-
<i>Eurotium medium</i>	X <sub>T</sub>	-	<i>Candida versatilis</i>	X <sub>T</sub>	-
<i>Eurotium minor</i>	X <sub>T</sub>	-	<i>Metschnikowia bicuspidata</i>	X <sub>T</sub>	-
<i>Eurotium intermedium</i>	X <sub>T</sub>	-	<i>Pichia farinosa</i>	X <sub>T</sub>	-
<i>Eurotium repens</i>	X <sub>T</sub>	1	<i>Pichia membranifaciens</i>	X <sub>T</sub>	-
<i>Eurotium rubrum</i>	X <sub>T</sub>	-	<i>Pichia sorbitophila</i>	X <sub>T</sub>	-
<i>Eurotium umbrosum</i>	X <sub>T</sub>	-	<i>Pichia guilliermondii</i>	X <sub>T</sub>	1
<i>Penicillium adametzi</i>	X <sub>T</sub>	-	<i>Schizosaccharomyces pombe</i>	X <sub>T</sub>	-
<i>Penicillium albocoremium</i>	X <sub>T</sub>	-	<i>Zygosaccharomyces rouxii</i>	X <sub>P</sub>	-
<i>Penicillium antarcticum</i>	X <sub>T</sub>	-	<i>Zygosaccharomyces lentis</i>	X <sub>P</sub>	-
<i>Penicillium aurantiogriseum</i>	X <sub>T</sub>	1	<i>Yarrowia lipolytica</i>	X <sub>T</sub>	1
<i>Penicillium brevicompactum</i>	X <sub>T</sub>	1			
<i>Penicillium charlesii</i>	X <sub>T</sub>	-			

X<sub>T</sub> = Xerotolerant; X<sub>P</sub> = Xerophilic; H<sub>T</sub> = Halotolerant; H<sub>P</sub> = Halophilic. NB: most species are attributed to any of these categories on the basis of their general ecology, as growth curves at different levels of water activity / salinity have not been established.

## 2. Basidiomycota

### 2.1. FILOBASIDIALES, TREMELLALES, TRICHOSPORONALES

The small genus *Trichosporonoides* and its relative genus *Moniliella* comprise species (de Hoog, 1979) associated with industrial production of lipids and acids (Dakin and

Stolk, 1968). *Trichosporonoides* contains one species inhabiting pollen (Haskins and Spencer, 1967), and one from jelly (Hocking and Pitt, 1981). The genus was recently revised by de Hoog and Smith (1998), but the exact phylogenetic relation between *Trichosporonoides* and *Moniliella* has not yet been established. Members of the genus *Trichosporonoides* are too rare, both in nature and in the human-dominated environment, to speculate on any evolutionary tendencies. A relationship between these two genera and *Cryptococcus* (*Filobasidiales* / *Tremellales*) may be surmised. In *Cryptococcus* several species are known to occur in the osmotic environments of e.g. flower petioles (Sriburee et al., 2004) and in seawater (Nagahama et al., 2003b). Several species of *Cryptococcus* are known from the Antarctic (Vishniac and Onofri, 2003) and have been isolated with high frequency from the ice of polythermal glaciers in the Arctic (Gunde-Cimerman et al., 2003). Pathogenic potential is particularly found in the neurotropic *Cryptococcus neoformans* complex, which has an environmental niche in, among others, dried bird faeces (Gokulshankar et al., 2004).

TABLE 2. Correspondence of ability to tolerate low water activity\* and invasive potential in humans among the 106 currently recognized<sup>1</sup> orders of the fungal kingdom.

Halo- / xerotolerant:	Minimum $A_w$ <sup>2</sup> :	Medically significant:	Highest BSL level <sup>3</sup>
<i>Wallemiales</i>	0.77	<i>Wallemiales</i>	1
<i>Filobasidiales</i>	0.90	<i>Filobasidiales</i>	2
<i>Sporidiales</i>	0.90	<i>Sporidiales</i>	1
<i>Polyporales</i>	0.90	<i>Polyporales</i>	1
<i>Dothideales</i>	0.80	<i>Dothideales</i>	1
<i>Eurotiales</i>	0.80	<i>Eurotiales</i>	3
<i>Onygenales</i>	0.90	<i>Onygenales</i>	3
<i>Saccharomycetales</i>	0.80	<i>Saccharomycetales</i>	2
<i>Microascales</i>	?	<i>Microascales</i>	2
<i>Chaetothyriales</i>	?	<i>Chaetothyriales</i>	3
<i>Pleosporales</i>	?	<i>Pleosporales</i>	2
<i>Sordariales</i>	?	<i>Sordariales</i>	2
<i>Hypocreales</i>	?	<i>Hypocreales</i>	2
<i>Ophiostomatales</i>	?	<i>Ophiostomatales</i>	2

\*Most species are attributed to any of these categories on the basis of their general ecology, as growth curves at different levels of water activity / salinity have not been established.

<sup>1</sup>According to Kirk et al. (2001).

<sup>2</sup>Estimate, adapted from Pitt and Hocking (1997).

<sup>3</sup>Adapted from de Hoog et al. (2000).

The arthroconidial yeast *Trichosporon mucoides* was frequently isolated from hypersaline water of the salterns and the Dead sea (Butinar et al., 2005b). *Trichosporon* was recently segregated from the *Filobasidiales* / *Tremellales* on the basis of 26S rRNA gene phylogeny to be classified in the new separate order *Trichosporonales* (Scorzetti et al., 2002). *Trichosporon* contains mainly soil-borne species and human opportunists. Interestingly, several species are the exclusive agents of human white piedra, referring to

fungal colonization of mainly pubic and axillary hairs (Guého et al., 1992), which can be regarded to be a low  $a_w$  environment due to drying transpiration.

## 2.2. POLYPORALES

This order contains the single xerotolerant species *Sporotrichum pruinosum*, previously referred to as *Chrysosporium xerophilum* (Boekhout et al., 1989). The species is occasionally found on foodstuffs (Kinderlerer, 1997). It is the anamorph of *Phanerochaete chrysosporium*, which is one of the very few filamentous basidiomycetes with some invasive potential in warm-blooded animals (de Hoog et al., 2000).

## 2.3. SPORIDIALES

*Rhodosporidium*, *Leucosporidium* and *Sporidiobolus* are teleomorphs of several species of *Rhodotorula* and *Sporobolomyces*. Frequently isolated red yeast species are several *Rhodotorula* species from the deep sea and marine sediments (Nagahama et al., 2003a). The *Rhodotorula glutinis* and *R. minuta* species complexes are occasionally found in a clinical setting, but their virulence is low (de Hoog et al., 2000). *Rhodosporidium sphaerocarpum*, *R. babjevae* and *Rhodotorula laryngis* were repeatedly isolated from hypersaline water of salterns (Butinar et al., 2004b). *Leucosporidium* is consistently isolated from Antarctic marine waters (Fell et al., 1998).

## 2.4. WALLEMIALES

This order was recently introduced to accommodate the single genus *Wallemia*, a phylogenetic maverick in the *Basidiomycota* (Zalar et al., 2005). The genus contains only three species, all frequently involved in food spoilage. Members can be isolated from sweet (fruits, jams, cakes, pure sugar), salty (fish, meat, peanuts), or dried food (Samson et al., 2002). In nature they inhabit desiccated fruits and sea fish, and are common in hypersaline evaporation ponds at the Mediterranean, the Caribbean, as well as from the Dead sea (Wasser et al., 2003; Zalar et al., 2005). It is remarkable that the entire genus *Wallemia*, and therefore the entire order *Wallemiales*, are xerophilic or xerotolerant. Because of having their growth optimum in media with additional solutes and showing no growth without them, two out of three *Wallemia* species are considered to be xerophilic rather than xerotolerant. *Wallemia ichthyophaga* still shows growth at a water activity of 0.77, equivalent to 32% NaCl. It grows better with salt than with other solutes. *Wallemia ichthyophaga* is one of the most halophilic fungi known to date.

The combination of xerotolerance with human-opportunism is questionable in *Wallemiales*. *Wallemia sebi* infection (= 'hemisporiosis', named after the synonymous species *Hemispora stellata*), has only been reported in older literature, before the fifties in the previous century. Infections were reported to be cutaneous or subcutaneous, without specific clinical features. Indeed, the identity of one of these strains, CBS 196.56, has never been verified as *W. sebi* (Zalar et al., 2005). It is remarkable, however, that no modern case of any *Wallemia* infection has been reported after 1950. The cases all occurred well before the emergence of immunocompromised patients. We therefore doubt whether these cases were really caused by *W. sebi* as primary etiologic agent, and prefer to omit the *Wallemiales* as an order with dual behaviour. The group is

characteristic in its preference of environments with low water activity, but it is unlikely to have an opportunistic potential.

### 3. *Ascomycota*

#### 3.1. *DOTHIDEALES*

Remarkably, nearly all dothideaceous species growing at low water activity are halotolerant or halophilic (Table 1), whereas outside the *Dothideales* this property is rare. The natural habitat of the species concerned is the hypersaline coastal salterns worldwide (Butinar et al., 2005c). These fungi are practically unknown in non-natural environments – not even on salted fish such as their halophilic counterparts in *Eurotiales* and *Wallemiales*. Apparently the ecology of these fungi is not solely based on tolerance of high salt concentrations, and this is further evidenced by the properties of *Hortaea werneckii* (Petrovič et al., 2002). This species is the dominant fungal halophile in hypersaline evaporation ponds (Gunde-Cimerman et al., 2000; Zalar et al., 1999), and the only one causing human tinea nigra, a characteristic type of colonization of human hands (Severo et al., 1994). This disorder is caused by non-invasive colonization of the salty, dead keratin-layers of the hands of hyperhydrotic individuals after prolonged desiccation in the sun at the beach (Göttlich et al., 1995). These patients placed their exceptionally salty hands in seawater and particularly the hypersaline evaporation ponds around the world (Zalar et al., 1999). *Hortaea werneckii* seems to have taken accidental advantage of the environment of the human skin which is very different from its natural habitat, but resembles it in one essential regard, high salinity. This dependence of crucial factors enables a fungus to make an enormous ecological leap which might be the starting point for a process of sympatric speciation. However, the tinea strains do not show any further transmission and thus this new niche is an evolutionary dead end. The fungus is easily removed with ointments dissolving dead keratinous material (Rippon, 1988) and thus should not be regarded as a clinical problem. Therefore, it has been recommended to lower the biosafety level of this fungus to BSL-1 (de Hoog et al., 2000). Remarkably, two related species of *H. werneckii* with very similar ecology, viz. *Phaetotheca triangularis* and *Trimmatostroma salinum*, are not known as etiologic agents of human tinea nigra. Perhaps *H. werneckii* is exceptionally versatile, as it is widely dispersed in seawater-related environments and is also found on salty hands and in house-dust (Zalar et al., 1999).

The order *Dothideales* has a xerotolerant tendency as it contains a large number of extremotolerant species growing as epilithic or cryptoendolithic species at high temperature, such as on Mediterranean marble (Wollenzien et al., 1996), at low temperature in Antarctic sandstone (Selbmann et al., 2005) and Arctic glacier ice (Gunde-Cimerman et al., 2003). Nearly all species are heavily melanized, have thick cell walls, and frequently exhibit a meristematic, isodiametric type of thallus expansion, leading to highly resistant cell clumps (Sterflinger, 1998). *Aureobasidium pullulans* is an ubiquitous xerotolerant fungus on honeydew, and at lower environmental salinities in saltern evaporation ponds. It is common as well in the artificial environment on low-nutrient, moist surfaces such as glass where it exhibits its xerotolerant, meristematic ecotype (Schabereiter-Gurtner et al., 2001). *Aureobasidium pullulans* is a typical

opportunistic in causing insignificant, cutaneous infections after trauma in otherwise healthy patients. It very rarely shows systemic dissemination and only if the patient is severely immunocompromised (de Hoog et al., 2000). This species occurs commonly on damp inert surfaces such as stone, glass and metal including medical instruments.

Most members of the genus *Cladosporium* (section *Cladosporium*; David, 1997) have a cosmopolitan distribution as ubiquitous decomposers of dead plant material (David, 1997), therefore they are commonly found in indoor and outdoor air. Contrary to the ubiquitous distribution of saprobic Cladosporia, some species were consistently isolated from specific, extreme environments of salterns and salty lakes worldwide and they represented the most common and frequent fungal taxa in various hypersaline environments (1000-3600 CFU l<sup>-1</sup> for hypersaline saltern water) (Gunde-Cimerman et al., 2000). Cladosporia were reported from wood immersed in hypersaline water of Great Salt Lake in Utah, they were isolated from saline soils and salt marshes, the phylloplane of Mediterranean plants and the rhizosphere of halophytic plants (references after Gunde-Cimerman et al., 2004). The halotolerant *Cladosporium* saltern community was found to be composed of mainly two round-spored species, *C. sphaerospermum*, and a possibly new, yet undescribed species (Zalar et al., 2001). Common, frequently air-borne saprobic species, such as *C. herbarum* and *C. cladosporioides* were also detected, but showed only mild halotolerance, expressed as an ability to grow in the presence of 5% additional NaCl. Human *Cladosporium* infections are extremely rare, and all species reported from clinical cases are listed as BSL-1.

### 3.2. EUROTIALES

Tolerance for high salt concentrations is a property found in many members of *Eurotiales*. When a survey was made of the NaCl tolerance of 975 species of terrestrial fungi selected from the major taxonomic classes, species of *Penicillium* and *Aspergillus* were notably the most resistant, with the majority of their species able to grow in the presence of 20% or more of NaCl. Over 70% of the Penicillia could tolerate 20% NaCl and more than half survived at 25% or greater concentrations. The aspergilli appeared to be somewhat less tolerant; nevertheless, about 70% could tolerate 20% NaCl and nearly half survived at 25% (Tresner and Hayes, 1971). The teleomorphs in *Emericella*, *Eupenicillium*, *Eurotium* and *Talaromyces* were all represented in saline soil, arid areas and salt marshes, in the ectorrhizosphere-rhizoplane zone of different halophytic plants (El-Morsy, 1999).

In the *Eurotiales* xero- and halotolerance are recurrent phenomena (Table 1). To date, eleven species are known to be xerotolerant, three xerophilic, and two show better growth with salt than with any other solute (Butinar et al., in press). The species listed are located in remote clades within the order. Thus it is likely that growth and survival of low levels of water activity is a main, plesiomorphic trait shared by the entire order. Indeed, although the natural ecology has not been established for every individual species, we note that numerous taxa originate from desert soil, brines, desiccated fruits in arid climates, dry bird and bat guano, and similar substrates. In the human-dominated environment they are among the main spoilage organisms of food products (Pitt and Hocking, 1997). Since, however, foodstuffs are basically derived from nature, despite the addition of salts and sugars for preservation no dramatic shift in ecology is apparent. But the *Eurotiales* are also among the predominant indoor fungi as highly successful

colonizers of surfaces after slight moistening, and are able to inhabit a wide variety of toxic compounds such as paint, tannins and industrial fluids. Under such conditions they are frequently encountered as near-monocultures, underlining their successful occupation of these new habitats. It thus can be stated that the human-dominated environment has provided an enormous potential for growth and dispersal of such fungi. They have emerged with clonal offshoots, while related species that still inhabit their original stressed habitats mostly exhibit their teleomorphs (Grishkan et al., 2003).

The result of the introduction of new environments for rapid reproduction may explain the repeated loss of teleomorphs in some groups of eurotalian fungi (LoBuglio et al., 1993). The sudden preponderance of clonality has certainly led to natural selection of genotypes that are more suitable for survival in the indoor environment. Therefore, in *Eurotiales* accelerated evolution is expected in those species that are successful colonizers of the indoor environment.

The genus *Eurotium* is the teleomorph genus for *Aspergillus* sections *Aspergillus* and *Restricti*, that have a xero- and halophilic preponderance (Pitt and Hocking, 1997). These species are common in a diversity of substrates with low water activity including saline environments. Members of the genus have been reported to live in concentrated salt or sugar solutions at  $a_w$  as low as 0.75 (Martín et al., 1998). The genus *Eurotium* has been reported both in arid and saline soils. Six different species of the known teleomorphic food-borne xerophilic genus *Eurotium* were repeatedly isolated in a mycodiversity study of hypersaline waters. At salinities above 17% NaCl, *E. amstelodami* was detected most consistently, followed by *E. repens* and *E. herbariorum*. Spatiotemporal frequency of occurrence and *in vitro* determined adaptive ability of propagules to survive a prolonged exposure to hypersaline conditions indicate that *E. amstelodami*, *E. herbariorum*, and *E. repens* contribute to the indigenous fungal community in hypersaline water environments (Butinar et al., 2005a).

The genus *Aspergillus* and its teleomorphs currently contains 254 accepted species (Pitt et al., 2000). It can be divided into three main groups (Peterson, 2000b). The first group contains species that are particularly xerotolerant and associated to the teleomorphic genera *Eurotium*, *Chaetosartorya*, *Petromyces*, *Neopetromyces* and *Fennellia*. Species in *Chaetosartorya* are also highly xerotolerant, but it is only the anamorphic taxon *Aspergillus wentii* that has been repeatedly reported from low water activity environments. The second group comprises the ascomycete genera *Emericella* and *Sclerocleista* and the third contains species associated to the ascomycete genera *Neosartorya* and *Neocarpenteles*.

Species in the genera *Petromyces* (anamorph *Aspergillus* section *Flavi*), *Neopetromyces* (anamorph *Aspergillus* section *Circumdati* or formerly the *Aspergillus ochraceus* group) (Frisvad and Samson, 2000) and *Aspergillus* section *Nigri* are all common at low water activity conditions, especially in warmer climates. Species associated with the genus *Fennellia* (anamorphs *Aspergillus* sections *Flavipedes*, *Terrei*, *Candidi* and *Cervini*) are less frequent in dry habitats, but species such as *A. flavipes*, *A. terreus* and *A. candidus* are quite common in haline environments.

Species in *Emericella* and its anamorphs *Aspergillus* sections *Nidulantes*, *Versicolores* and *Usti* are common in marine environments and in dry foods and other low water activity environments. *Emericella nidulans*, *A. versicolor* and *A. sydowii* are particularly common in salterns. The thermophilic genus *Neosartorya* with the

anamorph *Aspergillus* section *Fumigati* is common in self-heated plant waste and also in desert soil. *A. fumigatus* is found repeatedly in salterns (Tepšič et al., 1997).

The genus *Penicillium* is associated to two teleomorph genera, *Eupenicillium* and *Talaromyces*. *Eupenicillium* is rather closely related to *Aspergillus* in a phylogenetic sense, while *Talaromyces* with its associated anamorphs in *Penicillium* subgenus *Biverticillium* may be a distinct genus closer to *Byssochlamys* and *Thermoascus* (Peterson, 2000a; Pitt, 1979; Stolk and Samson, 1972). *Eupenicillium* is uncommon in saline environments, but its *Penicillium* anamorphs are frequently food-borne (Frisvad et al., 2000) and soil-borne (Christensen et al., 2000). Many species in subgenus *Penicillium* grow well in salted foods, especially species in series *Chrysogena*, *Olsonii*, *Viridicata*, *Verrucosa*, *Camemberti*, *Expansa*, *Solita* and *Urticicola* (Frisvad and Samson, 2004). Many members of the soil-borne *Penicillium* subgenus *Furcatum* are also recovered from saline environments.

Species in the teleomorph genus *Talaromyces* in general thrive at higher water activities than *Eupenicillium* (Andersen and Frisvad, 2002; Pitt and Hocking, 1997). However, some species that have been recovered from haline environments are classified in *Talaromyces* and associated Biverticillia: *T. flavus*, *P. funiculosum*, *P. islandicum*, *P. purpurogenum* and *P. variabile*.

The *Eurotiales* is also one of the most significant orders with respect to pathogenicity to humans. It contains the biverticillate species *Penicillium marneffeii*, which is a BSL-3 pathogen emerging at very high frequency in the AIDS-patients of Southeast-Asia (Ukarapol et al., 1998). It produces a unique pathogenic phase, with arthroconidia actively reproducing within phagocytes and subsequent dormancy in healthy bamboo rats (Chariyalertsak et al., 1996). The infection kinetics of *P. marneffeii*, with endogenous reactivation at the moment of impairment of acquired cellular immunity, is identical to that of established systemic pathogens such as *Coccidioides immitis* and *Histoplasma capsulatum* (Schaffner, 1989). Its natural environmental niche is hitherto unknown with certainty, because all attempts to isolate the fungus from directly soil or air have failed, although PCR-based tests are positive (Vanittanakom et al., 2002). *Penicillium* subgenus *Biverticillium* contains a large number of thermophilic species from dry, heated soil. Outside *P. marneffeii* more species are encountered that have a pathogenic potential for humans and animal (G.S. de Hoog and R.A. Samson, unpublished data). The origin of AIDS-association in *Biverticillium* is hitherto not understood. In the second main group of potential human-invaders, the *Aspergillus* section *Fumigati* with its *Neosartorya* teleomorphs, a compromised innate immune system is a necessary condition for infection. Hence, such species are associated with e.g. leukemic patients and transplant recipients, and are rarely observed in AIDS patients (de Hoog et al., 2000). In contrary *A. fumigatus* is extremely common during self-heated phases of composting of plant material (Göttlich, 1995) and is ubiquitous in outside air. In susceptible humans it causes a typical inhalation mycosis, whereby colonization and invasion are usually accompanied by an allergic response from the host. *P. marneffeii* and *A. fumigatus* / *A. flavus* thus are ecologically fundamentally different.

### 3.3. CHAETOTHYRIALES

This small order of black yeast-like fungi and their teleomorphs (genus *Capronia*) is exceptional in the fungal kingdom in comprising a wide diversity of opportunistic human

pathogens. Slight xerotolerance is expressed in the fact that particularly the species with capsulate yeast cells (*Exophiala dermatitidis* and *E. spinifera*) are regularly encountered on sweet surfaces of fruits and flowers (G.S. de Hoog, unpublished data), and thus exhibit a dual tendency as observed in *Cryptococcus* above. Numerous species are oligotrophic, living e.g. in drinking water, while some are particularly found in sea water and are opportunists of fish. *Exophiala dermatitidis* is associated with human cystic fibrosis (Haase et al., 1991; Horr  et al., 2004), a genetic disorder in the transmembrane conductance regulator gene (CFTR) leading to a high degree of salt excretion in lungs, intestines and on the skin. The mucous lungs of a significant percentage of CF patients is asymptotically colonized with this otherwise uncommon fungus. Its natural niche probably is in association with tropical frugivorous birds and bats, while the fungus goes through a habitat shift in the human environment by occurrence in a high frequency in human bathing facilities (Matos et al., 2002). The frequencies of two main genotypes in natural and artificial environments are different (M. Sudhadham and G.S. de Hoog, unpublished data). CF patients probably acquire their infection from this pre-selected set of strains in the steam bath or ordinary bathrooms. Humans are colonized by a genotype that is rarely encountered in nature. This selection must be based on strains being differentially predisposed to survival in the human environment, and thus may be accompanied by changes in infectious ability. The trigger for this speciation process may be linked to the species' halotolerance.

### 3.4. MICROASCALES

The order contains a single species exhibiting halotolerance, *Pseudallescheria boydii*. *In vitro* it tolerates 5% additional NaCl (de Hoog et al., 1994). This property enables the fungus to reside in somewhat osmotic environments such as dry bat faeces (Ajello et al., 1977) and bird guano at their roosting sites (Brandsberg et al., 1969). It occurs in brackish and salt water, such as submerged wood in estuaria (Kirk, 1967), tide-washed areas and marine soil (Dabrowna et al., 1964; Pawar et al., 1963). This natural ecology predisposes the fungus for colonization of the mucous lungs of patients with cystic fibrosis (Cimon et al., 2000), very similar to the black yeast *Exophiala dermatitidis* mentioned above. Also *P. boydii* passes through a selective step during the transition from nature to the human-dominated environment, which is agricultural soil and polluted ponds (de Hoog et al., 1994). It is presently one of the important opportunistic, potentially fatal agents in the immunocompromised patients. It causes death with a frequency of 1:1000 in transplant recipients (Nucci, 2003) and 4:1000 in patients with hematological malignancies (Pagano et al., 2001). Its invasive potential is not emerging with this recent hospitalized patient population, but the fungus always has been a major cause of traumatic infection in otherwise healthy hosts, e.g. in mycetoma (Kemper, 2000) and arthritis (Ginter et al., 1995).

### 3.5. ONYGENALES

Several *Chrysosporium* species grow on dry foodstuffs, particularly *C. farinicola* having an optimum near  $a_w$  0.93. They grow poorly on media in which the controlling solute is something else than sugars. Species of the genus *Gymnascella*, such as *G. dankaliensis* and *G. hyalospora* naturally inhabit dung and soil including saline and desert soil

(Deshmukh, 2002). *G. marismortui* is found in water of the Dead Sea and was never recorded on other localities. It grows optimally at NaCl concentration between 3 and 10% NaCl (Buchalo et al., 1998).

The order *Onygenales* comprises the the main families of human-pathogenic fungi: viz. the *Arthrodermataceae* with the dermatophytes on humans and other mammals, and the *Onygenaceae* with the systemic pathogens such as *Histoplasma* and *Coccidioides*. Most species, cutaneous as well as systemic, are viewed upon as true pathogens, since they are poorly affected by the intact innate immune system but are typically controlled by acquired cellular immunity (de Hoog et al., 2000). The systemic species show endogenous reactivation when the acquired immune system is impaired, such as in the case of AIDS (Schaffner, 1989). The systemic species have a double life cycle, involving an invasive phase in an animal vector, and an environmental phase. The xerotolerant environmental phase resides in habitats that are hostile to most fungi, namely dried bat dung and guano in the case of *Histoplasma* (Lyon et al., 2004) and salty, alkaline desert soil in the case of *Coccidioides* (Maddy, 1957). Both presently recognized *Coccidioides* species tolerate 8% NaCl (Fisher et al., 2002).

### 3.6. PLEOSPORALES

Xero- and halotolerance in this order is moderate. *Dendryphiella salina* is a marine hyphomycete (Genilloud et al., 1994), although it was also recovered on driftwood immersed in hypersaline water of the Adriatic salterns, but failed to grow at concentrations higher than 17% NaCl in the medium (N. Gunde-Cimerman, unpublished data). It can utilize different nitrogen compounds, has a broad pH growth range, particularly at alkaline values and a broad temperature profile (Clipson et al., 1989, 1990; Galpin et al., 1977). Several species of *Alternaria* and *Ulocladium* are specialized to live in salt marsh soils in arid deserts (Ranzoni, 1968; Simmons, 1981), all having a preponderantly meristematic phenotype.

The pathogenic potential of the *Pleosporales* is also moderate. Two saprophytic *Alternaria* species, *A. alternata* and *A. infectoria*, are frequent cutaneous opportunists in immunocompromised humans (de Hoog and Horré, 2002). Several species causing the subcutaneous disorder mycetoma are also members of the *Pleosporales* (de Hoog et al., 2004).

### 3.7. SACCHAROMYCETALES

This order of ascomycetous yeasts contains numerous species associated with sugary plant saps and exudates. Osmotolerant yeast taxa are found mainly in the families *Saccharomycetaceae* and *Metschnikowiaceae*. They are known from honey, pollens, molasses, fresh and dried fruit (Prada and Pagnocca, 1997), and are associated with sugary plant saps and exudates (Hocking and Pitt, 1997). Tolerance to high concentrations of sugars is found in *Zygosaccharomyces rouxii*, *Z. bailii* and *Schizosaccharomyces pombe*. Osmophilic *Z. rouxii* and *Candida versatilis* are used for soya sauce fermentation. Yeasts preferring reduced  $a_w$  for growth, described as osmophilic, were known primarily as contaminants in the food industry, but they remained largely unknown in natural low water activity habitats. Yeasts isolated from hypersaline waters, having hemiascomycetous affinities, belong to the genera *Candida*,

*Debaryomyces*, *Metschnikowia* and *Pichia*. Different species of the genus *Pichia* are frequent saline aquatic isolates, with *P. membranifaciens* being the most common (Soares et al., 1997).

*Candida famata* is a halotolerant food-borne yeast and a model organisms for fungal halotolerant adaptation (Prista et al., 1997) has been found mainly in cold sea water (Norkrans, 1966) and recently in natural hypersaline habitats, particularly salterns at the Namibian Skeleton coast and Great Salt Lake, both exposed to low seasonal temperatures. Xerotolerant strains usually belong to the variety *famata*, which is a common contaminant of foodstuffs (Butinar et al., 2005b). The variety *flareri* is more thermotolerant and known as an opportunistic pathogen on humans (de Hoog et al., 2000).

In the waters of the Dead Sea diverse opportunistic pathogenic species of the genus *Candida* were found. These included *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*, occasionally known from the more polluted intertidal estuarine water and sediments (Soares et al., 1997), but isolated for the first time from hypersaline water of the Dead Sea (Butinar et al., 2005b). Among these only *C. parapsilosis* was known previously as a food-borne.

In the waters of the Dead Sea diverse opportunistic pathogenic species of the genus *Candida* were found. These included *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*, occasionally known from the more polluted intertidal estuarine water and sediments (Soares et al., 1997), but isolated for the first time from hypersaline water of the Dead Sea (Butinar et al., 2005b). Among these only *C. parapsilosis* was known previously as a food-borne halotolerant yeast, while others were not known for their halotolerance. *C. parapsilosis* is frequently introduced into hospitalized, immunocompromised patients due to biofilm formation inside catheters and on prosthetic materials (Branchini et al., 1994; Levin et al., 1998). *Candida albicans* and *C. dubliniensis*, which are among the main mycotic agents of disease in immunocompromised patients, do not have an appreciable xerotolerance.

#### 4. Discussion

The main purpose of this review is to reveal a possible link of xerotolerance with other ecophenotypes in the evolutionary history of fungi. This idea was motivated by the observation that xerotolerance and the ability to cause infection in humans – either having impaired immunity or otherwise healthy – show a similar distribution at the ordinal level in the fungal tree of life. Thus all orders including xerophilic species also comprised human pathogens and vice versa, despite the fact that each trait is fairly rare amongst fungal species and orders. Remarkably, however, at the species level the two properties seem to be nearly mutually exclusive. Pronounced xerotolerance thus does not seem to be directly associated or beneficial for pathogenicity, while lower levels of xerotolerance are promotive. We conclude that a moderate degree of xerostress-tolerance is likely be a plesiomorphic (ancestral) character in the orders with dual behaviour. Better survival of environmental stress resulting from the development of mechanisms for xerotolerance may have enhanced evolutionary fitness of environmental pathogens with low competitive ability such as *Coccidioides*. Cellular mechanisms put in action by xerotolerance are also helpful to overcome various other hostile conditions.

Also survival from phagocytosis and subsequent killing by oxygen-radicals of the innate immune system, cytotoxic activity from complement and killer cells, low pH, and iron deprivation may be promoted by the dramatic response by a complex gene expression underlying osmotic shock (Crespo et al., 2001; Gash et al., 2000). The few fungal orders where appreciable xerotolerance is encountered share other types of stress tolerance that are equally uncommon elsewhere in the fungal kingdom, suggesting that a general type of stress-response underlies both types of ecology.

Immediate responses to an increase in osmolarity involves shrinking of the cell, a loss of cell polarity, disassembly of the actin cytoskeleton, changes in the permeability of the plasma membrane, accumulation of polyols and a temporal arrest of the synthesis of the cell wall (Hernandez-Saavedra et al., 1995). As a consequence, shrinkage of the cytoplasm and local redistribution of periplasmic and/or matrix cell wall material takes place, while osmotic stress does not affect the synthesis of cell wall constituents. Cells become more isodiametric in shape. Similar processes take place in the human-invasive forms of *Coccidioides*, *Exophiala* and *Fonsecaea* as they establish themselves in the mammal host (Kurappayil and Szaniszló, 1997; Sun et al., 1986), triggering a change from longitudinal to isodiametric cellular expansion. Their invasive forms consist of isodiametrically expanding spherules and muriform cells, respectively.

A key factor in stress in clinical fungi is the ability to grow at elevated temperatures. This property is enhanced by mechanisms such as regulation of membrane fluidity which are also involved in xerotolerance. Changes in membrane composition and its properties represent an important factor in the adaptation to high salinity (Russell, 1989). It seems that salt-tolerant fungi show a broad spectrum of alterations in lipid composition as the response to increased external salinity. Sterols and phospholipids are the two major lipid constituents of eukaryotic membranes. Overall changes in phospholipid to sterol ratio, changes in the degree of unsaturation of fatty acids, changes in phospholipids as well as in membrane fluidity, have been demonstrated in salt-tolerant yeasts when exposed to higher salinities and compared to salt-sensitive *Saccharomyces cerevisiae* (Turk et al., 2004). At elevated salinities an increase in unsaturation of phospholipid-esterified fatty acids was shown in halotolerant black yeast *Aureobasidium pullulans*, although changes in the membrane fluidity over the tested salinity range resembled to that of salt-sensitive *S. cerevisiae*. In both cases the membrane fluidity decreased with the rise in NaCl concentration, while in halophilic fungi, such as *Hortaea werneckii*, fluidity is maintained over increasing salt levels. Therefore it was concluded that higher membrane fluidity is of crucial importance for halophily (Turk et al., 2004). On the other hand it seems that at elevated temperatures changes in the membrane composition have an opposite affect, by primarily decreasing the membrane fluidity, although very few studies have been performed (Hazel and Williams, 1990).

Extracellular glycoproteins have been isolated from many fungi grown in media containing elevated concentrations of salt. Glycoproteins produced under salt stress can differ (Breierova et al., 1997a). These glycoproteins are known to be associated with the ability of binding water and protection of water regime in the cell (Breierova et al., 1997b). Capsular polysaccharides of *Cryptococcus* delay drying processes in environments with extremely low humidity (Aksenov et al., 1973). Extracellular polysaccharides (EPS) of *Cryptococcus* have also been proven to be a major virulence factor using animal experiments (Chang and Kwon-Chung, 1994). Capsular glucuronoxylomannan (GXM) down-regulates cell-mediated protective immune response

by reducing the production of pro-inflammatory cytokines (Andrade et al., 2002). In the black yeast genus *Exophiala*, the two most pathogenic species are the capsulate ones, *E. dermatitidis* and *E. spinifera* (Yurlova and de Hoog, 2002). Remarkably, both genera *Exophiala* and *Cryptococcus* exhibit an association with occurrence on fruits and flowers as well as with dry bird and bat guano (Lopez-Martinez and Castanon-Olivares, 1995; Matos et al., 2002), the common factor being decreased water activity.

Melanin is a further, additive virulence factor, in that melanin-deficient mutants are less virulent in *Cryptococcus* (Noverr et al., 2004) as well as in *Exophiala* (Dixon et al., 1992; Peltroche-Llacsahuanga et al., 2003). Members of the *Dothideales* enhance their stress-tolerance with increased melanization (Figueras et al., 1996) combined with thick cell walls and meristematic growth. Ultrastructural studies of the halophilic black yeasts *Hortaea werneckii*, *Phaeothea triangularis* and *Trimmatostroma salinum* cell walls showed that the organisation of melanin granules is dependent on the concentration of salt in the medium (Kogej et al., 2001; Ravishankar et al., 1995). The granules in the outer part of the cell walls were loosely organised in medium without salt, but became more densely packed as the salt concentration in the medium increased. This kind of granular reorganisation at increased salinity was absent in xerotolerant *A. pullulans*. These results suggest a potential osmoprotective role of melanin in the cell wall of halophilic black yeasts, and a higher degree of specialization of the halophilic compared to xerotolerant species.

The EPS-producing black yeast *Aureobasidium pullulans* is very common in indoor and human-made environments, while the natural habitat predominantly is found on plant surfaces covered with honeydew. Similarly, many *Penicillium* species have shifted from natural to indoor environments and thus have made a considerable ecological leap. *Exophiala dermatitidis* is rarely found in nature but has a striking prevalence in hot steam baths (Matos et al., 2002). In all these fungi a high degree of fitness is reached in the new environment, and transmission is efficient. This shift is likely to be accompanied by a step of natural selection, as not all genotypes may be equally suited for growth and dispersal in the new environment.

The phenomenon of habitat-shift is pronounced in *Exophiala dermatitidis* and *Pseudallescheria boydii*. These species combine a (low) degree of halotolerance with an obvious clinical potential. In *E. dermatitidis* a jump from frugivorous animal faeces in the tropical rain forest to human-made steam baths is supposed (M. Sudhadham and G.S. de Hoog, unpublished data); in *P. boydii* the leap possibly concerns estuarine brackish muds to agricultural effluents (de Hoog et al., 1994). The two species have a marked association with the salty mucus of patients with cystic fibrosis (Haase et al., 1991; Horr  et al., 2004). Accelerated evolution through an enforced process of natural selection may be expected in these species, but the direction of evolution is unpredictable. The selective processes take place in the human-made environment rather than in the human host. Changes in virulence of the organism, either up or down, will be coincidental. The result of the process may nevertheless lead to an organism that is better equipped to infect the human host. If selection for temperature tolerance takes place, an increase in virulence may be expected.

Fungi growing indoors have an increased probability to be traumatically introduced into humans. However, since there is no further transmission, human infections are evolutionary dead ends and are unlikely to have any impact on the evolution of the fungus. The selective process apparently has led to a preponderance of genotypes that

are coincidentally more predisposed to cause human infection, e.g. by more pronounced thermotolerance, but that are not adaptive.

In this connection *Candida famata* is particularly interesting because the species comprises two varieties, var. *famata* and var. *flareri*, that show a predilection for dry foodstuffs and for human patients, respectively. It is tempting to speculate that in this species two evolutionary sympatric lines have developed by either a selection in dry environments, or in warm environments and thus leading to an entity that is better suited for human infection.

It seems that the truly halophilic / xerophilic species, such as *Trimmatostroma salinum* and *Wallemia muriae* are already too specialized for life in extreme ecological niches to be able to successfully survive an ecological shift, as described above for xerotolerant taxa. They seem to have adapted well to the special stress provided by their highly osmotic environmental niches. Species tolerating very low-water activities ( $X_p$  and  $H_p$ ; Table 2) are consistent in their ecology, both in natural and in artificial environments. They are unflexible from an evolutionary perspective.

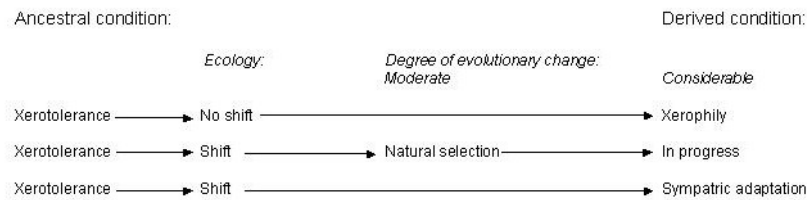
Habitat-shifts can be dramatic when an environment is accidentally colonized that is very different from the natural habitat – provided that further transmission takes place and fitness is not impaired. The evolutionary processes accompanying sudden leaps in ecology are conveniently monitored in artificial, human-made environments, such as indoor environments, because these are non-existent in nature. Any fungus that is overabundant in a fragmented artificial environment is subjected to dramatic natural selection leading to accelerated evolution (Vandergast et al., 2004). Species with less pronounced xero- or halotolerance ( $X_T$  and  $H_T$ ; Table 2) may be more prone to evolution in other directions.

Many  $X_T$  and  $H_T$  species, particularly members of *Eurotiales*, are found on foodstuffs, such as dry food, dry meat or sea fish. These habitats basically consists of materials that occur as well in nature, the main difference being that it is now being treated for preservation and subsequently eaten by humans. The occurrence of pronounced natural selection is thus less likely in food-borne fungi. Accelerated evolution thus is more likely to happen as a result of natural selection after an environmental shift. In the case of the systemic *Onygenales* such as *Coccidioides* the shift was accompanied by a plesiomorphic pathogenic potential, and thus has led to a process of adaptive, sympatric evolution, whereas the possibly enhanced virulence of black yeast-like fungi is more likely to be coincidental. The systemic *Onygenales* seem to have reached a high degree of adaptation, with an elaborate ecological strategy using rodents as vectors for dispersal, whereas the process of natural selection in indoor fungi has just begun after the introduction of suitable habitats by humans.

## 5. Conclusion

If xerotolerance is regarded as a general condition to cope with general stress, presence of properties underlying this ability provides the fungus with an armament to survive types of stressful conditions other than decreased water activity. Low degrees of xerotolerance therefore may mark different starting points of subsequent evolution, either into a direction of an even higher degree of stress tolerance (e.g., *Wallemia muriae*), or in another direction, leading to disruptive selection (e.g., *Exophiala dermatitidis*) or to

adaptive sympatric speciation (e.g., *Coccidioides immitis*). A diagrammatic representation of these evolutionary options is given in Fig. 2. Thus, xerotolerance is likely to be the closest evolutionary origin of dual capacities observed in the fungal orders of Table 2.



**Figure 2.** Diagram of hypothetical evolutionary options, with xerotolerance as a starting point.

## Acknowledgements

The authors are indebted to J.C. Frisvad, R.A. Samson, M. Sudhadham and C.S. Tan for constructive suggestions on the manuscript.

## References

- Abdel-Fattah, H.M., Moubasher, A.H. and Abdel-Hafez, S.I. (1977) Studies on mycoflora of saline marshes in Egypt. I. Sugar fungi. *Mycopathologia* **61**, 19-26.
- Abdel-Hafez, S.I. (1982) Survey of the mycoflora of desert soils in Saudi Arabia. *Mycopathologia* **80**, 3-8.
- Ajello, L., Kuttin, E.S., Beemer, A.M., Kaplan, W. and Padhye, A. (1977) Occurrence of *Histoplasma capsulatum* Darling, 1906 in Israel, with a review of the current status of histoplasmosis in the Middle East. *Am. J. Trop. Med. Hyg.* **26**, 140-147.
- Aksenov, S.I., Babyeva, I.P. and Golubev, V.I. (1973) On the mechanism of adaptation of micro-organisms to conditions of extreme low humidity. *Life Sci. Space Res.* **11**, 55-61.
- Amon, J.P. (1978) Thraustochytridis and labyrinthulids of terrestrial, aquatic and hypersaline environments of the Great Salt lake, USA. *Mycologia* **70**, 1299-1301.
- Andersen, B. and Frisvad, J.C. (2002) Characterization of *Alternaria* and *Penicillium* species from similar substrata based on growth at different temperature, pH and water activity. *System. Appl. Microbiol.* **25**, 162-172.
- Andrade, M.R., Almeida, M.G., Dos Reis, A.G. and Melo Bento, A.C. (2002) Glucuronoxylomannan of *Cryptococcus neoformans* exacerbates *in vitro* yeast cell growth by interleukin 10-dependent inhibition of CD4+ T lymphocyte responses. *Cell. Immunol.* **222**, 116-125.
- Andrews, S. and Pitt, J.J. (1987) Further studies on the water relations of xerophilic fungi, including some halophiles. *J. Gen. Microbiol.* **133**, 233-238.
- Boekhout, T., van Oorschot, C.A.N., Stalpers, J.A., Batenburg-van der Vegte, W.H. and Weijman, A.C.M. (1989) The taxonomic position of *Chrysosporium xerophilum* and septal morphology in *Chrysosporium*, *Sporotrichum* and *Disporotrichum*. *Stud. Mycol.* **31**, 29-39.
- Branchini, M.L., Pfaller, M.A., Rhine-Chalberg, J., Frempong, T. and Isenberg, H.D. (1994) Genotypic variation and slime production among blood and catheter isolates of *Candida parapsilosis*. *J. Clin. Microbiol.* **32**, 453-456.

- Brandsberg, J.W., Weeks, R.J., Hill, W.B. and Piggott, W.R. (1969) A study of fungi found in association with *Histoplasma capsulatum*: three bird roosts in S. E. Missouri, U.S.A. *Mycopathol. Mycol. Appl.* **38**, 71-81.
- Breierova, E., Stratilova, E. and Sajbidor, J. (1997a) Salinity affects fatty acid and extracellular glycoprotein composition of *Dipodascus australiensis*. *Food Technol. Biotechnol.* **35**, 237-241.
- Breierova, E., Kacurakova, M. and Stratilova, E. (1997b) The effect of high concentration of NaCl in the cultivation medium on changes in the composition of extracellular yeast glycoproteins. *System. Appl. Microbiol.* **20**, 348-355.
- Brock, T.D., Madigan, M.T., Martinko, J.M. and Parker, J. 1994. *Brock Biology of Microorganisms*, 7th ed., Prentice-Hall International, Upper Saddle River, NJ, 909 pp.
- Buchalo, A.S., Nevo, E., Wasser, S.P., Oren, A. and Molitoris, H.P. (1998) Fungal life in the extremely hypersaline water of the Dead Sa: first records. *Proc. R. Soc. Lond. B* **265**, 1461-1465.
- Butinar, L., Zalar, P., Frisvad, J.C. and Gunde-Cimerman, N. (2005a) The genus *Eurotium* – members of indigenous halophilic community in hypersaline waters of salterns. *FEMS Microbiol. Ecol.* **51**, 155-166.
- Butinar, L., Spencer-Martins, I., Oren, A. and Gunde-Cimerman, N. (2005b) Yeast diversity in hypersaline habitats. *FEMS Microbiol. Lett.*, in press.
- Butinar, L., Sonjak, S., Zalar, P., Plemenitaš, A. and Gunde-Cimerman, N. (2005c) Population dynamics of melanized halophilic fungi in solar salterns. *Botanica Marina*, in press.
- Chang, Y.C. and Kwon-Chung, K.J. (1994) Complementation of a capsule-deficient mutation of *Cryptococcus neoformans* restores its virulence. *Mol. Cell Biol.* **14**, 4912-4919.
- Cariyalertsak, S., Vanittanakom, P., Nelson, K.E., Sirisanthana, V. and Vanittanakom, N. (1996) *Rhizomys sumatrensis* and *Nomomyces badius*, new natural animal hosts of *Penicillium marneffei*. *J. Med. Vet. Mycol.* **34**, 105-110.
- Christensen, M., Frisvad, J.C. and Tuthill, D.I. (2000) *Penicillium* species diversity in soil and some taxonomic and ecological notes. In: R.A. Samson and J.I. Pitt (eds.), *Integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification*. Harwood Academic Publishers, Amsterdam, pp. 309-320.
- Cimon, B., Carrere, J., Vinatier, J.F., Chazalette, J.P., Chabasse, D. and Bouchara, J.P. (2000) Clinical significance of *Scedosporium apiospermum* in patients with cystic fibrosis. *Eur. J Clin. Microbiol. Infect. Dis.* **19**, 53-56.
- Clipson, N.J.W., Jennings, D.H. and Smith, J.L. (1989) The response of salinity at the microscopic level of the marine fungus *Dendryphiella salina* Nicot and Pugh as investigated stereologically. *New Phytol.* **113**, 121-127.
- Clipson, N.J.W., Hajibagheri, M.A. and Jennings, D.H. (1990) Ion compartmentation in the marine fungus *Dendryphiella salina* in response to salinity: X-ray microanalysis. *J. Exp. Bot.* **41**, 199-202.
- Crespo J.L., Daicho T., Ushimaru, T., and Hall M.N. (2001) The GATA transcription factors GLN3 and GAT1 link TOR to salt stress in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **276**, 34441-34444.
- Dabrowa, N., Landau, J.W., Newcomer, V.D. and Plunkett, O.D. (1964) A survey of tide-washed areas of Southern California for fungi potentially pathogenic to man. *Mycopath. Mycol. Appl.* **24**, 137-150.
- Dakin, J.C. and Stolk, A.C. (1968) *Moniliella acetoabutens*: some further characteristics and industrial significance. *J. Food Technol.* **3**, 49-53.
- David, J.C. (1997) A contribution to the systematics of *Cladosporium*. Revision of the fungi previously referred to *Heterosporium*. *Mycol. Pap.* **172**, 1-157.
- De Hoog, G.S. (ed.) (1979) *The Black Yeasts, II: Moniliella and Allied Genera*. *Stud. Mycol.* **19**, 1-90.
- De Hoog, G.S. and Horr , R. (2002) Molecular taxonomy of the *Alternaria* and *Ulocladium* species described from humans and their identification in the routine laboratory. *Mycoses* **45**, 259-276.
- De Hoog, G.S. and Smith, M.T. (1998) *Trichosporonoides* Haskins & Spencer. In: C.F. Kurtzman and J.W. Fell (eds.), *The Yeasts, A Taxonomic Study, 4th ed.* Elsevier, Amsterdam, pp. 873-877.
- De Hoog, G.S., Marvin-Sikkema, F.D., Lahpor, G.A., Gottschall, J.C., Prins, R.A. and Gu ho, E. (1994) Ecology and physiology of the emerging opportunistic fungi *Pseudallescheria boydii* and *Scedosporium prolificans*. *Mycoses* **37**, 71-78.
- De Hoog, G.S., Guarro, J., Figueras, M.J. and Gen , J. (2000) *Atlas of Clinical Fungi. 2nd ed.* Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands and Universitat Rovira i Virgili, Reus, Spain, 1124 pp.
- De Hoog, G.S., Adelmann, D., Ahmed, A.O.A. and van Belkum, A. (2004) Phylogeny and typification of *Madurella mycetomatis*, with a comparison of other agents of eumycetoma. *Mycoses* **47**, 121-130.
- Deshmukh, S.K. (2002) Incidence of keratinophilic fungi from selected soils of Kerala state (India). *Mycopathologia* **156**, 177-181.
- Dixon, D.M., Miglizzo, J., Cooper, C.R., Jr, Solis, O., Breslin, B. and Szanislo, P.J. (1992) Melanized and non-melanized multicellular form mutants of *Wangiella dermatitidis* in mice: mortality and histopathology studies. *Mycoses* **35**, 17-21.

- El-Morsy, E.M. (1999) Microfungi from the ectorrhizosphere-rhizoplane zone of different halophytic plants from the Red Sea coasts of Egypt. *Mycologia* **91**, 228-236.
- Fell, J.W., Blatt, G.M. and Statzell-Tallman, A. (1998) Validation of the basidiomycetous yeast, *Sporidiobolus microsporus* sp. nov., based on phenotypic and molecular analyses. *Antonie van Leeuwenhoek* **74**, 265-270.
- Figueras, M.J., de Hoog, G.S., Takeo, K. and Guarro, J. (1996) Stationary phase development of *Trimmatostroma abietis*. *Antonie van Leeuwenhoek* **69**, 783-791.
- Fisher, M.C., Koenig, G.L., White, T.J. and Taylor, J.W. (2002) Molecular and phenotypic description of *Coccidioides posadasii* sp. nov., previously recognized as the non-California population of *Coccidioides immitis*. *Mycologia* **94**, 73-84.
- Frisvad, J.C. and Samson, R.A. (2000) *Neopetromyces* gen. nov. and an overview of teleomorphs of *Aspergillus* subgenus *Circumdati*. *Studies in Mycology* **45**, 201-207.
- Frisvad, J.C. and Samson, R.A. (eds.) (2004) *Penicillium Subgenus Penicillium: New Taxonomic Schemes, Mycotoxins and Other Extralites*. *Stud. Mycol.* **49**, 1-251.
- Frisvad, J.C., Filtenborg, O., Lund, F. and Samson, R.A. (2000) The homogeneous species and series in subgenus *Penicillium* are related to mammal nutrition and excretion, In: R.A. Samson and J.I. Pitt (eds.), *Integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification*. Harwood Academic Publishers, Amsterdam, pp. 265-283.
- Galpin, M.F.J., Jennings D.H. and Thornton, J.D. (1977) Hyphal branching in *Dendryphiella salina*: Effect of various compounds and the further elucidation of the effect of sorbose and the role of cyclic AMP. *Trans. Br. Mycol. Soc.* **69**, 175-182.
- Gash, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., Eisen, M.B., Storz, D., Botstein, D. and Brown, O.P. (2000) Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Cell Biol.* **11**, 4241-4257.
- Genilloud, O., Pelaez, F., Gonzalez, I. and Diez, M.T. (1994) Diversity of actinomycetes and fungi on seaweeds from the Iberian coasts. *Microbiologia* **10**, 413-422.
- Ginter, G., De Hoog, G.S., Pschaid, A., Fellinger, M., Bogiatzis, A., Berghold, C., Reich, E.-M. and Odds, F.C. (1995) Arthritis without grains caused by *Pseudallescheria boydii*. *Mycoses* **38**, 369-371.
- Göttlich, E., (1996) *Untersuchungen zur Pilzbelastung der Luft an Arbeitsplätzen in Betrieben zur Abfallbehandlung*. Thesis, Erich Schmidt Verlag, Stuttgart, 244 pp.
- Göttlich, E., de Hoog, G.S., Yoshida, S., Takeo, K., Nishimura, K. and Miyaji, M. (1995) Cell surface hydrophobicity and lipolysis as essential factors in human tinea nigra. *Mycoses* **38**, 489-494.
- Gokulshankar, S., Ranganathan, S., Ranjith, M.S. and Ranjithsingh, A.J. (2004) Prevalence, serotypes and mating patterns of *Cryptococcus neoformans* in the pellets of different avifauna in Madras, India. *Mycoses* **47**, 310-314.
- Grishkan, I., Korol, A.B. and Nevo, E. (2003) Ecological stress and sex evolution in soil microfungi. *Proc. R. Soc. Lond. B* **270**, 13-18.
- Guého, E., Smith, M.T., de Hoog, G.S., Billon-Grand, G., Christen, R. and Batenburg-van der Vegte, W.H. (1992) Contributions to a revision of the genus *Trichosporon*. *Antonie van Leeuwenhoek* **61**, 289-316.
- Guiraud, P., Steiman, R., Seigle-Murandi, F. and Sage, L. (1995) Mycoflora of soil around the Dead Sea. II. System. *Appl. Microbiol.* **18**, 318-322.
- Gunde-Cimerman, N., Zalar, P., de Hoog, G.S. and Plemenitaš, A. (2000) Hypersaline water 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 Plemenitaš, 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., Frisvad, J.C., Zalar, P. and Plemenitaš, A. (2005) Halotolerant and halophilic fungi, In: S.K. Deshmukh and M.K. Rai (eds.), *The Biodiversity of Fungi: Their Role in Human Life*. Oxford & IBH Publishing Cp. Pvt. Ltd., New Delhi, pp. 69-127 (in press).
- Haase, G., Skopnik, H., Groten, T., Kusenbach, G. and Posselt, H.-G. (1991) Long-term fungal cultures from patients with cystic fibrosis. *Mycoses* **34**, 373-376.
- Haskins, R.H. and Spencer, J.F.T. (1967) *Trichosporonoides oedocephalis* n. gen., n. sp. I. Morphology, development and taxonomy. *Can. J. Bot.* **45**, 515-520.
- Hazel, J.R. and Williams, E.E. (1990) The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Progr. Lipid Res.* **29**, 167-227.
- Hernandez-Saavedra, N.Y., Ochoa, J.L. and Vazquez-Dulhalt, R. (1995) Osmotic adjustment in marine yeast. *J. Plankton Res.* **17**, 59-69.
- Hocking, A.D. and Pitt, J.I. (1981) *Trichosporonoides nigrescens* sp. nov., a new xerophilic yeast-like fungus. *Antonie van Leeuwenhoek* **47**, 411-421.

- Hocking, A.D. (1993) Responses in xerophilic fungi to changes in water activity, In: D.H. Jennings (ed.), *Stress Tolerance of Fungi*. Marcel Dekker, Inc., New York, pp. 233-243.
- Horré, R., Schaal, K.P., Siekmeier, R., Sterzik, B., De Hoog, G.S. and Schnitzler, N. (2004) Isolation of fungi, especially *Exophiala dermatitidis*, in patients suffering from cystic fibrosis. *Respiration* **71**, 360-366.
- Kemper, C.A. (2000) Eumycetoma. *Curr. Treatment Options Infect. Dis.* **2**, 533-538.
- Kinderlerer, J.L. (1997) *Chrysosporium* species, potential spoilage organisms of chocolate. *J. Appl. Microbiol.* **83**, 771-778.
- Kirk, P.M., Cannon, P.F., David, J.C. and Stalpers, J.S. (2001). *Ainsworth & Bisby's Dictionary of the Fungi*, 9th. ed. CAB International, Oxon, U.K.
- Kirk, P.W. (1967) A comparison of saline tolerance and sporulation in marine and clinical isolates of  *Allescheria boydii* Shear. *Mycopathol. Mycol. Appl.* **33**, 65-75.
- Kogej, T., Lanišnik-Rizner, T. and Gunde-Cimerman, N. (2001) Black yeasts from the salterns: The effect of salt on melanization, In: *Abstracts of the International Conference on Halophilic Microorganisms, 23-27 September 2001, Sevilla, Spain*, p. 24.
- Kurappayil, S.M. and Szanislo, P.J. (1997) Importance of calcium to the regulation of polymorphism in *Wangiella (Exophiala) dermatitidis*. *J. Med. Vet. Mycol.* **35**, 379-388.
- Kushner, D.J. 1978. Life in high salt and solute concentrations, In: D.J. Kushner (ed.), *Microbial Life in Extreme Environments*. Academic Press, New York, pp. 317-386.
- Lanyi J.K. (1979) Physicochemical aspects of salt dependence in halobacteria, In: M. Shilo (ed.), *Strategies of Microbial Life in Extreme Environments*. Verlag Chemie, Weinheim, pp. 93-107.
- Levin, A.S., Costa, S.F., Mussi, N.S., Basso, M., Sinto, S.I., Machado, C., Geiger, C., Villares, M.C.B., Schreiber, A.Z., Barone, A.A. and Branchini, M.L.M. (1998) *Candida parapsilosis* fungemia associated with implantable and semi-implantable central venous catheters and the hands of healthcare workers. *Diagn. Microbiol. Infect. Dis.* **30**, 243-249.
- LoBuglio, K.F., Pitt, J.I. and Taylor, J.W. (1993) Phylogenetic analysis of two ribosomal DNA regions indicate multiple losses of a sexual *Talaromyces* state among asexual *Penicillium* species in subgenus *Biverticillium*. *Mycologia* **85**, 592-604.
- Lopez-Martinez, R. and Castanon-Olivares, L.R. (1995) Isolation of *Cryptococcus neoformans* var. *neoformans* from bird droppings, fruits and vegetables in Mexico City. *Mycopathologia* **129**, 25-28.
- Lyon, G.M., Bravo, A.V., Espino, A., Lindsley, M.D., Gutierrez, R.E., Rodriguez, I., Corella, A., Carrillo, F., McNeil, M.M., Warnock, D.W. and Hajjeh, R.A. (2004) Histoplasmosis associated with exploring a bat-inhabited cave in Costa Rica, 1998-1999. *Am. J. Trop. Med. Hyg.* **70**, 438-442.
- Maddy, K. (1957) Ecological factors possibly relating to the geographic distribution of *Coccidioides immitis*. *Public Health Serv. Publ.* **575**, 144-157.
- Mahdy, H.M., El-Sheikh, H.H., Ahmed, M.S. and Refaat, B.M. (1996) Physiological and biochemical changes induced by osmolarity in halotolerant aspergilli. *Acta Microbiologica Polonica* **45**, 55-65.
- Martin, S., Companys, E., Sanchis, V. and Ramos, A.J. (1998) Effect of water activity and temperature on competing abilities of common maize fungi. *Mycol. Res.* **120**, 959-964.
- Matos, T., Haase, G., Gerrits van den Ende, A.H.G. and de Hoog, G.S. (2002). Molecular diversity of oligotrophic and neurotropic members of the black yeast genus *Exophiala*, with accent on *E. dermatitidis*. *Antonie van Leeuwenhoek* **89**, 293-303.
- Nagahama, T., Hamamoto, M., Nakase, T. and Horikoshi, K. (2003a) *Rhodotorula benthica* sp. nov. and *Rhodotorula calyptogenae* sp. nov., novel yeast species from animals collected from the deep-sea floor, and *Rhodotorula lysiniphila* sp. nov., which is related phylogenetically. *Int. J. Syst. Evol. Microbiol.* **53**, 897-903.
- Nagahama, T., Hamamoto, M., Nakase, T., Takaki, Y. and Horikoshi, K. (2003b) *Cryptococcus surugaensis* sp. nov., a novel yeast species from sediment collected on the deep-sea floor of Suruga Bay. *Int. J. Syst. Evol. Microbiol.* **53**, 2095-2098.
- Norkrans, B. (1966) Studies on marine occurring yeasts: Growth related to pH, NaCl concentrations and temperature. *Arch. f. Mikrobiol.* **54**, 374-392.
- Northolt, M.D., Frisvad, J.C. and Samson, R.A. (1995) Occurrence of food-borne fungi and factors for growth, In: R.A. Samson, E.S. Hoekstra, J.C. Frisvad and O. Filtenborg (eds.), *Introduction to Food-Borne Fungi*. CBS, Delft, pp. 243-250.
- Noverr, M.C., Williamson, P.R., Fajardo, R.S. and Huffnagle, G.B. (2004) CNLAC1 is required for extrapulmonary dissemination of *Cryptococcus neoformans* but not pulmonary persistence. *Infect. Immun.* **72**, 1693-1699.
- Nucci, M. (2003) Emerging moulds: *Fusarium*, *Scedosporium* and *Zygomycetes* in transplant recipients. *Curr. Opin. Infect. Dis.* **16**, 607-612.

- Oren A. (2002) *Halophilic Microorganisms and their Environments*. Kluwer Academic Publishers, Dordrecht, 575 pp.
- Pagano, L., Girmenia, C., Mele, L., Ricci, P., Tosti, M.E., Nosari, A., Buelli, M., Picardi, M., Allione, B., Corvatta, L., D'Antonio, D., Montillo, M., Melillo, L., Chierichini, A., Cenacchi, A., Tonso, A., Cudillo, L., Candoni, A., Savignano, C., Bonini, A., Martino, P. and Del Favero, A. (2001) GIMEMA Infection Program; Gruppo Italiano Malattie Ematologiche dell'Adulto. Infections caused by filamentous fungi in patients with hematologic malignancies. A report of 391 cases by GIMEMA Infection Program. *Haematologica* **86**, 862-870.
- Pawar, V.H., Padhye, A.A. and Thirumalachaer, M.J. (1963) Isolation of *Monosporium apiospermum* from marine soil in Bombay. *Hindust. Antibiot. Bull.* **6**, 50-53.
- Peltroche-Llacsahuanga, H., Schnitzler, N., Jentsch, S., Platz, A., De Hoog, G.S., Schweizer, K.G. and Haase, G. (2003) Analyses of phagocytosis, evoked oxidative burst, and killing of black yeasts by human neutrophils: a tool for estimating their pathogenicity? *Med. Mycol.* **41**, 7-14.
- Peterson, S.W. (2000a) Phylogenetic analysis of *Penicillium* based on ITS and *lsu*-rDNA nucleotide sequences, In: R.A. Samson and J.I. Pitt (eds.), *Integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification*. Harwood Academic Publishers, Amsterdam, pp. 163-178.
- Peterson, S.W. (2000b) Phylogenetic analysis of *Aspergillus* based on rDNA nucleotide sequences, In: R.A. Samson and J.I. Pitt (eds.), *Integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification*. Harwood Academic Publishers, Amsterdam, pp. 323-355.
- Petrović, U., Gunde-Cimerman, N. and Plemenitaš, A. (2002) Cellular responses to environmental salinity in the halophilic black yeast *Hortaea werneckii*. *Mol. Microbiol.* **45**, 665-672.
- Pitt J.I. (1979) *The Genus Penicillium and its Teleomorphic States Eupenicillium and Talaromyces*. Academic Press, London.
- Pitt, J.I. and Hocking, A.D. (1997) *Fungi and Food Spoilage*. 2<sup>nd</sup> ed. Blackie Academic & Professional, London.
- Pitt, J.I., Samson, R.A. and Frisvad, J.C. (2000) List of accepted species and their synonyms in the family Trichocomaceae, In: R.A. Samson and J.I. Pitt (eds.), *Integration of Modern Taxonomic Methods in Penicillium and Aspergillus Classification*. Harwood Academic Publishers, Amsterdam, pp. 9-47.
- Prada, G.M. and Pagnocca, F.C. (1997) Ascomycetous yeasts associated with naturally occurring fruits in a tropical rain forest. *Folia Microbiol. (Praha)* **142**, 39-46.
- Prista, C., Almagro, A., Loureiro-Dias, M.C. and Ramos, J. (1997) Physiological basis for the high salt tolerance of *Debariomyces hanseni*. *Appl. Environ. Microbiol.* **63**, 4005-4009.
- Ranzoni, F.V. (1968) Fungi isolated in culture from the Sonoran Desert. *Mycologia* **60**, 356-371.
- Ravishankar, J.P., Muruganandam, V. and Suryanarayanan, T.S. (1995) Isolation and characterisation of melanin from a marine fungus. *Botanica Marina* **38**, 413-416.
- Rippon, J.W. (1988) *Medical Mycology. The Pathogenic Fungi and the Pathogenic Actinomycetes*, 3rd ed. Saunders, Philadelphia, 797 pp.
- Russell N.J. (1989) Adaptive modifications in membranes of halotolerant and halophilic microorganisms. *J. Bioenerg. Biomembr.* **21**, 93-113.
- Samson, R.A., Hoekstra, E.S., Frisvad, J.C. and Filtenborg, O. (2002) *Introduction to Food- and Airborne Fungi*, 6<sup>th</sup> ed. Centraalbureau voor Schimmelcultures, Utrecht.
- Schabereiter-Gurtner, C., Pinar, G., Lubitz, W. and Rolleke, S. (2001) Analysis of fungal communities on historical church window glass by denaturing gradient gel electrophoresis and phylogenetic 18S rDNA sequence analysis. *J. Microbiol. Meth.* **47**, 345-354.
- Schaffner, A. (1989) Experimental basis for the clinical epidemiology of fungal infections. A review. *Mycoses* **32**, 499-515.
- Scorzetti, G., Fell, J.W., Fonseca, A. and Statzell-Tallman, A. (2002) Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. *FEMS Yeast Res.* **2**, 495-517.
- Selbmann, L., de Hoog, G.S., Mazzaglia, A., Friedmann, E.I. and Onofri, S. (2005) Fungi at the edge of life: cryptoendolithic black fungi from Antarctic deserts. *Stud. Mycol.*, in press.
- Severo, L.C., Bassanesi, M.C. and Londero, A.T. (1994) *Tinea nigra*: report of four cases observed in Rio Grande do Sul (Brazil) and a review of Brazilian literature. *Mycopathologia* **126**, 157-162.
- Simmons, E.G. (1981) *Alternaria* themes and variations. *Mycotaxon* **13**, 16-34.
- Soares, C.A.G., Maury, M., Pagnocca, F.C., Araujo, F.V. and Mendoca-Hagler, L.O. (1997) Ascomycetous yeast from tropical intertidal dark mud of southeast Brazilian estuaries. *J. Gen. Appl. Microbiol.* **43**, 265-272.

- Sriburee, P., Khayhan, S., Khamwan, C., Panjaisee, S. and Tharavichitkul, P. (2004) Serotype and PCR-fingerprints of clinical and environmental isolates of *Cryptococcus neoformans* in Chiang Mai, Thailand. *Mycopathologia* **158**, 25-31.
- Steiman, R., Guiraud, P., Sage, L., Seigle-Murandi, F. and Lafond, J.-L. (1995) Mycoflora of soil around the Dead Sea. I. Ascomycetes (including *Aspergillus* and *Penicillium*), Basidiomycetes, Zygomycetes. *System. Appl. Microbiol.* **18**, 310-317.
- Steiman, R., Guiraud, P., Sage, L. and Seigle-Murandi, F. (1997) Soil mycoflora from the Dead Sea Oases of Ein Gedi and Einot Zuqim (Israel). *Antonie van Leeuwenhoek* **72**, 261-270.
- Sterflinger, K. (1998) Temperature and NaCl-tolerance of rock-inhabiting meristematic fungi. *Antonie van Leeuwenhoek* **74**, 271-281.
- Stolk, A.C. and Samson, R.A. (1972) The genus *Talaromyces*. Studies on *Talaromyces* and related genera II. *Stud. Mycol.* **2**, 1-65.
- Sun, S.H., Cole, G.T., Drutz, D.J. and Harrison, J.L. (1986) Electron-microscopic observations of the *Coccidioides immitis* parasitic cycle *in vivo*. *J. Med. Vet. Mycol.* **24**, 183-192.
- Tepšič, K., Gunde-Cimerman, N. and Frisvad, J.C. (1997) Growth and mycotoxin production by *Aspergillus fumigatus* strains isolated from salterns. *FEMS Microbiol. Lett.* **157**, 9-12.
- Tresner, H.D. and Hayes, J.A. (1971) Sodium chloride tolerance of terrestrial fungi. *Appl. Microbiol.* **22**, 210-213.
- Turk, M., Méjanelle, L., Šentjerc, M., Grimalt, J.O., Gunde-Cimerman, N. and Plemenitaš, A. (2004) Salt-induced changes in lipid composition and membrane fluidity of halophilic yeast-like melanized fungi. *Extremophiles* **8**, 53-61.
- Ukarapol, N., Sirisanthana, V. and Wongsawasdi, L. (1998) *Penicillium marneffeii* mesenteric lymphadenitis in human immunodeficiency virus-infected children. *J. Med. Ass. Thai.* **81**, 637-640.
- Vandergast, A.G., Gillespie, R.G. and Roderick, G.K. (2004) Influence of volcanic activity on the population genetic structure of Hawaiian Tetragnatha spiders: fragmentation, rapid population growth and the potential for accelerated evolution. *Mol. Ecol.* **13**, 1729-1743.
- Vanittanakom, N., Vanittanakom, P. and Hay, R.J. (2002) Rapid identification of *Penicillium marneffeii* by PCR-based detection of specific sequences on the rRNA gene. *J. Clin. Microbiol.* **40**, 1739-1742.
- Vishniac, H.S. and Onofri, S. (2003) *Cryptococcus antarcticus* var. *circumpolaris* var. nov., a basidiomycetous yeast from Antarctica. *Antonie van Leeuwenhoek* **83**, 231-233.
- Wasser, S.P., Grishkan, I., Kis-Papo, T., Buchalo, A.S., Volz, P.A., Gunde-Cimerman, N., Zalar, P. and Nevo, E. (2003) Species diversity of the Dead Sea. In: E. Nevo, A. Oren and S.P. Wasser (eds.), *Fungal Life in the Dead Sea*. A.R.G. Ganter Verlag K.-G., Ruggell, pp. 203-270.
- Wollenzien, U., de Hoog, G.S., Krumbain, W.E. and Uijthof, J.M.J. (1996) *Sarcinomyces petricola*, a new microcolonial fungus from marble in the Mediterranean basin. *Antonie van Leeuwenhoek* **71**, 281-288.
- Yurlova, N.A. and de Hoog, G.S. (2002) Exopolysaccharides and capsules in human pathogenic *Exophiala* species. *Mycoses* **45**, 443-448.
- Zalar, P., de Hoog, G.S. and Gunde-Cimerman, N. (1999) Ecology of halotolerant dothideaceous black yeasts. *Stud. Mycol.* **43**, 38-48.
- Zalar, P., de Hoog, G.S. and Gunde-Cimerman, N. (2001) Fungi from the salterns: halophilic versus eurytolerant species in the genus *Cladosporium*, In: A. Ventosa and D.R. Arahall (eds.), *Abstracts of the International Conference on Halophilic Microorganisms, 23-27 September 2001, Sevilla, Spain*, p. 17.
- Zalar, P., de Hoog, G.S., Schroers, H.-J., Frank, J.M. and Gunde-Cimerman, N. (2005) Taxonomy and phylogeny of the osmophilic genus *Wallemia* (*Wallemiomycetes* and *Wallemiales*, cl. et ord. nov.). *Antonie van Leeuwenhoek* (in press).