Fungi in Extreme Habitats
A SURVEY OF FUNGI IN A MILITARY AIRCRAFT FUEL SUPPLY SYSTEM

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Summary

More than thirty fungi were classified into four groups according to their ability to grow or not in the fuel. Over twenty-three isolates grew.
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Fungi in extreme environments

Classification by Temperature:

Psychrophiles: fungi adapted to growth at low temperatures, have optimal growth at 0-10° C

psychro tolerant can grow at or near 0 C

Mesophiles: the majority of fungi, grow in the range +5-35° C, have optimal growth at 18-25° C

thermo tolerant can grow above 40 C

Thermophiles: adapted to growth at high temperatures, have growth minima above 20° C, optimal growth at 40-50° C, maxima up to 60° C
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<th>Taxon</th>
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<td><em>Thermomyces lanuginosus</em></td>
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<td><em>Chaetomium thermophile</em></td>
<td>30–50 °C</td>
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<td><em>Humicola insolens</em></td>
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<td><em>Thermomucor pusillus</em></td>
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<td><em>Aspergillus flavus</em></td>
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<td><em>Penicillium chrysogenum</em></td>
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<td><em>Cladosporium herbarum</em></td>
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<td><em>Botrytis cinerea</em></td>
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<td><em>Rhizopus nigricans</em></td>
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<tr>
<td><em>Penicillium expansum</em></td>
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<td><em>Penicillium brevicaulis</em></td>
<td>30–60 °C</td>
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<tr>
<td><em>Sclerotinia borealis</em></td>
<td>20–60 °C</td>
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<tr>
<td><em>Typhula idahoensis</em></td>
<td>20–60 °C</td>
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**Psychrophiles**

Psychrophiles do not grow above 20 °C, optimum 0–17 °C

**Mesophiles**

Mesophiles have minima above 0 °C, maxima below 50 °C

**Thermophiles**

Thermophiles have minima above 20 °C, maxima above 50 °C

**Thermotolerant mesophiles**

**Psychrotolerant mesophiles**
Succession of fungi on compost:

Initially various soil and leaf inhabiting mesophiles
As temperature rises to above 40° C, germination of spores of thermotolerant species increases and they replace mesophiles as dominant fungi

Natural heating from decomposition is used to sterilize mushroom compost for Agaricus cultivation

Mesophiles are limited to cooler outer surfaces
Mesophiles increase again as interior of compost cools as decay slows
Habitats of thermotolerant and thermophilic fungi

Includes temperate regions where sun can warm soil to above 20 C

More common in tropics, subtropics, esp. sun exposed substrates, but mesophiles are also common

Occur in decomposing organic matter where natural heating occurs
Relatively few species of **thermophilic** fungi:

- Talaromyces thermophilus
- Thermomyces lanuginosus
- Thermoascus auranticus
- Rhizomucor miehei
- Humicola insolens
- Chaetomium thermophile

but many more spp are thermotolerant

The majority are cellulolytic, saprobic, often common in soils of temperate and tropical regions affected by solar heating
Phanerochaete chrysosporium, a thermotolerant basidiomycete white rot fungus.

Bioconversion of lignocellulosic wastes requires efficient removal of lignin—polyphenol oxidases.

Tolerates the high temperatures of composting, some industrial wastes.

Grows on lignin and cellulose wastes from paper manufacturing, fungal protein can be used as animal feed.

Phanerochaete the most intensively studied white rot fungus, first basidiomycete genome sequenced.
Phanerochaete and bioremediation

Enzymes of lignin degradation
   PODs strongly oxidative
   not substrate specific (like e.g. cellulases)

So fungal lignases, PODs can also be used to detoxify toxic wastes --
   polychlorinated biphenyl (PCB)
   pentachlorophenol ("penta", wood preservative)

Being explored for use in remediation of wastes from various types of manufacturing
Phanerochaete chrysosporium
Guayule, *Parthenium argentatum*
A source of hypoallergenic latex rubber

Guayule a native desert shrub, produces latex similar to *Hevea brasiliensis*, the main source of industrial rubber.

Guayule industry developed during WWII.

Quality of guayule rubber improved by composting chopped shrub prior to rubber extraction.
Thermophilic, thermotolerant fungi adapted to growth at higher temperatures in masses of rotting vegetation, compost

Thermophilic fungi first well characterized from the process of “retting” chopped guayule before extracting rubber. Allowing chopped plant material to stand in a mass before rubber extraction improved the yield. This was shown to be due to removal of resins by thermophilic microflora, including several fungi able to grow at up to 60°C.

Natural heating of compost during decomposition is used to partially sterilize the compost used for mushroom cultivation. Mushroom spawn is added to compost after it begins to cool, competition from other mesophiles is reduced.
Thermomyces lanuginosus, to date the fungal record for high temperature growth at 62° C
Thermophilic, thermotolerant fungi also known from geothermal soils

Redman et al 1999 reported 16 fungi with growth between 20-55° C isolated from Yellowstone geothermal soils with temperatures up to 70° C.

Redman et al also report that a symbiotic, endophytic fungus enabled the grass *Dicanthelium lanuginosum* to survive constant soil temperatures of 50° C and intermittent soil temperatures up to 65° C. Non symbiotic plants did not survive 65° C.

The mechanism for thermal protection is not known.
Fig. 1. Representative symbiotic (with Curvularia sp.) and nonsymbiotic D. lanuginosum plants with rhizosphere temperatures of 50°C for 3 days or 65°C for 8 hours/day for 10 days under laboratory conditions (A) and in 40° or 45°C soil under field conditions.

Cold tolerant fungi, psychrophilic fungi

Many fungi known from Arctic/Antarctic environments
summer temps > 0°C for brief time
survive at -50°C
All major phyla of true fungi represented
Macrolfungi known from Antarctica, sub antarctic islands
mainly associated with bryophytes

Psychrophilic yeasts common in antarctic soils
freeze-thaw cycle releases sugars from plant cells

Temperate/boreal zones
Snow molds, grow under snow pack, cause snow blight
disease of cereals
Snow mold caused by *Typhula* spp and *Microdochium nivale*
Fungi grow under snowpack
Phacidium, Herpotrichia are snow molds and snow blights of conifers, colonize foliage covered by snow.
Herpotrichia “felt blight”
Various fungal contaminants of frozen food products

Penicillium spp can grow at -3 C also various Mucorales (Zygomycota) Penicillium spp., yeasts, Cladosporium spp. can contaminate meat in cold storage.

e.g. Kuehn and Gunderson, 1963, reported on psychrophilic fungi from frozed pastries containing blueberries, cherries, apples. Found 350 – 1500 psychrophiles per gram in blueberry pastries. Mainly Aureobasidium pullulans, Botrytis, Penicillium spp., Mucor spp.
Robert Blanchette, U Minn is studying deterioration of the historic huts of the Scott expedition in Antarctica.
Decay of wood in Antarctica caused by soft-rot fungi

Waterlogged wood in contact with ground
Only active during summer when ground thaws
*Cadophora* spp. (anamorphic ascomycetes)
Probably endemic to Antarctica
Physiology of cold tolerance:

cryoprotectants – trehalose, glycerol, mannitol membrane stabilization during water and cold stress: accumulate in psychrophilic fungi grown at low temperatures

lipids, fatty acids – more unsaturated bonds in membrane lipids in cold adapted fungi, unsaturated lipids increase in fungi grown at lower temperature, remain fluid at lower temps

antifreeze proteins – extracellular and intracellular, produced by snow mold fungi, *Typhula* spp. alter nucleation and growth of ice crystals to prevent membrane damage, also slows ice formation in substrate

enzymes – various enzymes, cellulases, proteases, lipases with high activity at low temperatures
Schadt et al 2003 report that microbial activity is high under snowpack in subalpine soils, and microbial biomass reaches its peak under snow.

Fungi are the dominant component of the biomass.

DNA sequence analysis revealed at least three major clades of previously unknown classes of fungi active under snow in Colorado.
Fig. 1. Neighbor-joining phylogram of all 125 large-subunit ribosomal DNA clone sequences (green lines) and previously described fungal taxa (black lines). Symbols at the ends of branches indicate sample origin (red, undersnow; open circle, snowmelt; filled circle, summer). Major fungal phylogenetic lineages are abbreviated AP, Ascomycota, Pezizomycotina; AS, Ascomycota, Saccharomycotina; AT, Ascomycota, Taphrinomycotina; B, Basidiomycota; and Z, Zygomycota. Roman numerals indicate lineages known only from these soils. Scale bar equals branch length of 1% sequence change in substitutions per site. Blastocladiella emersonii (Chytridiomycota) and Thaumatomyra ruffoi (Metazoa, Insecta) were used as outgroup taxa. For a larger version of this tree, complete with taxon names and bootstrap values, refer to fig. S1.

Antarctic endolithic fungi

Microbial communities under the surface of porous rock
winter temperatures at rock surface 2° lower than air
summer temperatures 20° higher than air
Outer few mm of rock helps mitigate climate extremes
Simple communities of only a few spp.
Lichen dominated community about 10 mm below rock
surface the most common and best studied

Distinct layers in the rock dominated by particular microbial
groups is recognized
black and white zones contain fungi, endolithic lichen
green zone contains unlichenized green algae and
cyanobacteria
Endolithic fungi in Antarctica

Stratified microbial communities in porous sandstone

Fig. 1. Landscapes at sample locations. A. Timber Peak, Northern Victoria Land, Antarctica. B. Layered Beacon sandstone and dolerite in the University Valley, Southern Victoria Land, Antarctica. C, D. Sandstone sculptured by the activity of cryptoendolithic microrganisms in Battleship Promontory. E. Patchwork-like effect of sandstone surface resulting from biogenous weathering. F. Typical stratification in sandstone colonised by lichen dominated community: (1) silicified, reddish brown crust; (2) black zone colonised by lichenised and non-lichenised fungi; (3) white zone colonised by lichenised fungi and lichenised algae; (4) green zone colonised by non-lichenised algae and cyanobacteria.
Antarctic endolithic fungi

Fig. 9. Cryomyces antarcticus. A, CCFEE 534 grown on different media. B, C. CCFEE 453, yeast-like organisation (B) and thick-walled, enteroblastic, germinating mother cell (C). D, CCFEE 534, yeast-like organisation and thick-walled, cross-decorated enteroblastic germinating mother cell. E, CCFEE 515, monoluid hyphae. F–H, CCFEE 534, yeast-like organisation and enteroblastic germination (F), monoluid hyphae (G) and seceded cells showing scars (H). D–E. Light microscopy; F–H. SEM. Bars indicate 10 μm.

Fig. 11. Unidentified meristematic species from Antarctic rock. A. CCFEE 451, SEM of hyphal growth and swelling cell at terminal position. B. CCFEE 457, SEM of monoluid hyphae and conidia produced by arthric secession. C. CCFEE 507, SEM of monoluid, cylindrical hyphae and EPS. D. CCFEE 5211, light microscopy of monoluid, branched hyphae and bitruncated conidia. E. CCFEE 5018, light microscopy of monoluid hyphae. F. CCFEE 502, SEM of monoluid and cylindrical hyphae. G, H. CCFEE 5176, coupled cells, observed with scanning (G) and light microscope (H). Scale bar = 10 μm.
Xerotolerant and osmotolerant fungi

xerotolerant fungi grow in material with low water content

osmotolerant fungi grow in material with low osmotic potential

water activity $a_w = \text{equilibrium relative humidity (\%)}$

$a_w$ is a measure of water concentration

water potential ($\psi$) is a measure of energy, relates to the potential for water to move into the fungus from the environment
Xerotolerant, osmotolerant fungi grow at water activities below 0.85 (85% RH) or -24.5 Mpa at 25 C

Fungi capable of growth at very low $a_w$ are uncommon mainly yeasts, Saccaromycetes also Aspergillus (Eurotium) spp. & Penicillium spp.

Osmotolerant fungi can grow in high solute concentrations (very low water potential)

- honey, jams, syrups, nectars, dried fruits can grow at $a_w < 0.6$ or about -70 Mpa at 25 C
- group includes some marine yeast species
- some yeast species traditionally used in soy fermentations
Xerotolerant fungi

Fungi of very dry environments

Aspergillus (Eurotium) contains the most xerotolerant species also a few Penicillium spp.

Xerotolerant fungi important as food spoilage agents
Grow in dried foods at above 12% water content (aw > 0.60)

Xerotolerant Aspergilli mainly use lipids, simple sugars, not starch

Xerotolerant fungi also produce mycotoxins, so food may be unusable even if not obviously deteriorated
Physiology of xerotolerance

Fungi growing at low water potential must modify internal cell environment so water will not be lost to environment.

Accumulate polyols (glycerol) when growing at low water potentials.

- Polyols do not interfere with cell metabolism. They can be tolerated in cells at high concentrations.
- Synthesis of polyols increases in all fungi under water stress.
- Also help to protect enzymes from low internal water potential.

Spores protected from dessication in dry environments.

Trehalose accumulates in spores during maturation and helps protect membranes.