**Environmental conditions for fungal growth, and tolerance of extremes through physiological and morphological adaptations**

* **Introduction:**
* Almost all organisms can grow over a wide range of conditions, than will support their life cycle.
* Suboptimal factors combination (of 2) can prevent fungal growth. One suboptimal factor and other optimal factors can be tolerated by fungi.
* Competitive interactions can restrict growth of a fungus to much narrower range than lab.
  + Example: Wheat plant → grown on
    - Sterilized soil → inoculated with take-all fungus (aggressive root fungus) → caused root disease → when increased temperature 13-23-27 ° C → disease increased.
    - Natural non-sterilized soil → inoculated with take-all fungus → caused root disease → when increased temperature → disease decreased.
      * Why?? As increasing temperature favored other microorganisms rather than take-all fungus. Which have antagonism effect on the fungus. →→ Biological control.
* **Temperature and Fungal growth**

**Introduction:**

* Five groups of m.o. in terms of of their temperature ranges
  1. Psychrophiles (cold – loving).
  2. Mesophiles (moderate conditions.
  3. Thermophiles (heat – loving).
  4. Hyperthermophiles (over heat – loving).
  5. Psychrotolerant (cold – tolerant ≤ 5°C)
* Most fungi are mesophilic as few grow ≥ 37 °C. but bacteria can grow ≥ 37 °C.
* Upper limit for fungi or any other eukaryotic cells is almost 60-62 °C. but some bacteria thrive at 70 -80 °C. even some can grow at over 100 °C.
* Fungal types based on their temperature ranges
  1. Themophilic
     + Minimum growth at 20 °C.
     + Maximum growth at 50 °C.
     + Optimum 40 – 50 °C.
  2. Thermotolerant
     + Can grow at 12 °C.
     + Its optimum ≤ 40 °C.
     + Wide range 12 – 52 °C.
     + Wide range of habitats so wide range distribution.
  3. Mesophilic
     + 10 -40 °C.
     + Optimum range is 22 -25 °C.
  4. Psychrophilic
     + Optimum growth almost at 16 °C.
     + Maximum growth at 20 °C.
  5. Psychrotrophic
     + Can grow at low temperature
     + Can also grow above 20 °C.

**Physiological basis of temperature tolerance:**

* No single feature determine the different temperature ranges of fungi.
* Range of factors contribute to temperature tolerance in different organisms.
* **The upper temp. limits are set by**
  + One common theme is the ability to grow in more extreme environments involve adaptation of the whole organism → so the temp. limit will be set by the first cellular compound or process that break down → that is why eukaryotic cells upper temp. limit 60 -62 °C. while, prokaryotic cells and archea upper limit is ≥ 80 °C.
  + The enzymes and ribosomal components of thermophilic fungi were reported to be more thermo-stable than mesophiles.
  + Heat stability of enzymes is conferred by
    - Increased bonding between amino acids near enzyme active site.
    - Bonds other than heat-labile hydrogen bonds.
  + Heat stabilizing factors e.g. heat shock proteins→ can be synthesized at elevated levels in response to brief exposure to high temp. 45 – 55 °C. → they are stress proteins ubiquitous → act as chaperons helping to ensure cell’s proteins are correctly folded and damaged proteins are destroyed.

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Do thermophilic fungi benefit specifically from being able to grow at high temp.???

Comparisons of thermophilic and mesophilic fungi → showed no differences in their relevant growth parameter.

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It seems thermophilic fungi occupy their high temp. environment because

they are specifically adapted to do so. But they are no more efficient in

substrate utilization than are mesophiles.

* **The lower temp. limits are set by** 
  + Reduced rates of chemical reactions at low temp.
  + Increased viscosity of cellular water at subzero temp.
  + Excessive concentrations of cellular ions leading to protein inactivation.

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“ The lower growth temp. limit of psychrophiles is fixed not by cellular properties of cellular macromolecules, but instead by the physical properties of aqueous solvent system inside and outside the cell”

* Studies on bacteria, yeasts & filamentous fungi → revealed general phenomenon → changes in temp. lead to changes in the fatty acid composition of the membrane lipids → these changes help to ensure that membrane fluidity is optimal for functioning of membrane transporters & enzymes.►► Homeoviscous adaptation.
  + Some psychrophilic fungi
    - yeasts & filamentous fungi → fatty acids and membrane phospholipids are more unsaturated than in mesophiles. → degree of unsaturation increase at lower temp. → as saturated fatty acids are less fluid than unsaturated fatty acids at any given temp.
    - Consistent with this → an abundance of polyunsaturated fatty acids were reported in snow moulds.
    - Even thermophilic fungus has been found to have 2x (two folds) higher concentrations of linoleic acid (unsaturated) when grown at 30 °C than at 50 °C.
  + Some psychrophilic or psychrotrophic fungi
    - Contain higher concentrations of trehalose (disaccharide).
    - Fungi were reported to accumulate this sugar in response to low temp.
    - As trehalose act as general stress protectant in the cytosol and is known to stabilize membranes during dehydration.
  + Some psychrophilic or psychrotrophic fungi
    - Contain polyols (polyhydric alcohols) such as glycerol and mannitol.
    - Accumulate in response to stress conditions.
    - Mannitol act as cryoprotectant.
* **Hydrogen ion concentration and fugal growth**

**Introduction:**

* Responses of fungi to culture pH need to be assessed in strongly buffered media → why? → otherwise fungi can rapidly change the pH by selective uptake or exchange of ions.
* Mixtures of KH2PO4 & K2HPO4 are commonly used for this purpose.
* Many fungi grow over pH 4.0-8.5 or pH 3.0-9.0.
* Broad optimum pH range 5.0-7.0.
* Fungi grouped based pH tolerance to
  + Acid-tolerant
    - Several fungi
    - E.g. yeasts in animals stomach & mycelia fungi grow at pH 2.0.
    - But their optimum pH in culture is 5.5-6.0.
  + Acidophilic fungi
    - Able to grow down pH 1-2.
    - Found in few environments e.g coal refuse tips & acidic amine wastes.
    - Many yeasts.
    - Filamentous fungi isolated from media contain 2.5 N Sulfuric acid. e.g. *Acontium velatum* grow at pH 7.0 → but rapidly decreases pH to 3.0 which is close to its optimum pH.
  + Alkali-telerant fungi
    - Strongly alkaline environment pH 10.0. e.g. alkaline springs.
    - Specialized species of filamentous fungi and some yeasts.
    - They are rather alkalophilic → grow up to pH 11.0. e.g. fungi isolated from bird’s nests, which are specialized in degradation of keratin (which is present in skin, feathers, nails & hair).

**The physiological basis of pH tolerance:**

* In all cases have been investigated fungi grow at extremes of pH are found to have an internal, cytosolic pH of about 7.0.
  + Method of study: loading hyphae with pH-sensitive fluorescent dyes that are permeabilized through plasma membrane → these dyes show peaks of fluorescence at two wavelengths & the relative size of the two peaks changes with pH → enabling changes of less than 0.1 pH unit to be measured accurately.

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Results showed fungal cytosol has strong buffering capacity. Even when the external pH changed several units → cytosolic pH maximum change 0.2 – 0.3 units.

* + This pH homeostasis is achieved by several ways:
    1. Pumping H+ ions out through cell membrane to counteract inflow of H+ in acidic environment.
    2. Exchange of materials between cytosol & vacuoles (which normally have acidic contents).
    3. Interconversion of sugars & polyols such as mannitol. Which involves sequestering or release of H+.
* **Water availability and fungal growth**

**Introduction:**

* All fungi need physical presence of water for → uptake of nutrients through wall

& cell membrane.

→ release of extracellular enzymes.

* Need intercellular water as a milieu for metabolic reactions.
* ᵩ w = ᵩs + ᵩ m +ᵩ p +ᵩg. ( water potential = solute or osmotic potential + physical binding forces or matric potential + pressure potential + gravity potential)
* For a fungus to retain its existing water → must generate a potential equal to the external water potential.
* To gain water from the environment a fungus must generates a (-ve) lower water potential than the environment.
* How fungi response to water potential???
  + Most fungi are highly adept at obtaining water even in environment that exert a significant water.
  + Exceptions water moulds Oomycota as they have little ability to maintain turgidity against external forces they grow only or predominantly in fresh water habitats.
  + It used to be thought that fungi needed to remain turgid in order to grow.
  + But hyphae of water moulds can continue to grow even when lost turgor → why?? → because the extension hyphal tip is achieved by continous extension of cytoskeletal components (as extension of pseudopodia in the amoeboid organisms).
  + Nevertheless, even water moulds need to be turgid in order to penetrate solid surfaces.
* Almost all other fungi of soil & other terrestrial habitats can grow readily in media of water potential = -2 MPa.
* If water stress increased beyond this (more –ve) → then aseptate fungi & Oomycota are first to stop growing. → lower water potential limit is -4 MPa.
* But many septated fungal hyphae will grow in the range of water potentials

-4 MPa – -14MPa.

* Most water stress-tolerant fungi will grow at near maximum -20 MPa water potential & even will make at least some growth at -50 MPa water potential.
* Fungi as a whole can grow in environment few other organisms can grow.
* This ability to tolerate water stress is one of special features of fungi.
* But there is an important qualification because the response of a fungus to water stress depends on how this stress in general.
* Most fungi tolerate sugar imposed osmotic stress better than salt imposed stress → as they are inhibited by salt toxicity by osmotic potential as such.

**Physiological adaptations to water stress:**

* Fungi typically respond to low (-ve) external water potential → by generating an even lower internal osmotic potential. → so that cells or hyphae remain turgid. Which is achieved by:
  + Selective uptake and accumulation of ions from the environment. E.g. common accumulation of K+ by marine fungi. But high ionic levels are potentially damaging to cells. Even marine fungi rather accumulate K+ primarily to prevent more toxic Na+ ion from entering cells.
  + Accumulate sugars or sugar derivatives that do not interfere with central metabolic pathways → Compatible solutes → e.g. Glycerol.
    - Comparison between water stress-tolerant fungi and stress-intolerant fungi → shown both types produce compatible solutes in response to water stress.
    - But differ in their ability to retain the solutes → meaning that those stress-intolerant fungi, inspite of their synthesis for compatible solutes ( e.g. glycerol) → they leak from the cell into culture medium. Whereas, in stress-tolerant fungi retain the glycerol.
    - Insect pathogenic fungi spores → have potential to be developed as commercial biological control agents of insects, in place of some the toxic insecticides currently used. (limitations that the spores need a sustained high humidity in order to germinate & penetrate insect cuticle).
    - With this in mind, attempts are being made to increase levels of compatible solutes in spores of insect-pathogenic fungi.
    - These solutes can either be derived from nutrient – storage reserves or from nutrients taken up by cells.
  + Phyllospere fungi
    - In fungi grow as saprotrophs on surfaces of living or senescing plant leaves. → the environment termed phyllosphere.
    - Fungi with dark pigmentation (melanized) hyphae & spores are present in phyllospheres.
    - They donot tolerate low ( more -ve) water potential but they have remarkable ability to withstand periodic wetting and drying → which few other fungi can tolerate.
    - Phyllosphere fungi are naturally & specially adapted to fluctuating moisture conditions in their habitats.

**Light and fungal growth**

**Introduction:**

* Little effect on vegetative growth.
* But can stimulate pigmentation.
* Blue light in particular is the effective one.
* For example, it induces the carotenoid pigments in hyphae & spores of several fungi.
* Pigments serve to minimize photo-induced damage.
* Melanin protect cells against reactive oxygen species and ultra violet radiation.
* Light has much more profound effect on fungal differentiation acting as trigger for the production of asexual sporing structures or sexual reproductive structures in several fungi.
* The photoreceptors are elicited by NUV (near ultra violet) or Blue light → as Flavin-type photoreceptors.
* But there are considerable variation in photoresponces of different fungi, related to habitat requirements.
  + e.g. *Alteranaria spp* → UV → sporulation.
  + *Botrytis cinerea* → NUV → sporulation → Blue light → reverse sporulation.
  + Some fungi → red /far red light → sporulation regulation. (but less common than blue light responses.)
* Light eliciting phototropism of
  + Sporangiophores of some zygomycota.
  + Ascus tips of some ascomycota.

**Genetic dissection of blue light perception in Neurospora crassa:**

* It is important eukaryotic model.
* Due to
  + Its relatively small genome.
  + Its rapid growth by random & stable integration of foreign DNA.
  + Abundance of well-characterized mutants.
* Preferred model organism for investigating light perception as
  + It perceives light only in blue/UV range.
  + Its relative few genes to be involved in responses.
  + It shows a pronounced circadian rhythm (a molecular clock) → innate period length close to 24 hours. & that is compensated against temp. & nutrition. But can be reset by environment light cues.
* Fungal light response in recent series of papers

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Characterized the first fungal blue-light photoreceptors.

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Regulatory protein termed White Collar 1 (WC-1)

Linked to a chromophore (yellow pigmented flavin adenine dinucleotide FAD)

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The WC-1 protein interacts with DNA

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Initiate gene transcription.

* Another protein (WC-2) also act as transcription factor as it forms a complex with WC-1.

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WC-1/WC-2 complex is localized in the light signal to the promoters of Blue-light regulated genes.

* Wild –type genes for WC-1 & WC-2 proteins had been known.
* Strains carrying mutant WC genes were known to be” blind”, as unable to induce carotenoid synthesis in response to blue light.

Blue light regulated genes that induce carotenoids synthesis in *N. crassa* are down regulated after about 2 hours → Photoadaptation.

* Mutant gene = vivid → sustained expression of carotenoid genes in the light. → VIVID protein analysis (located in the cytoplasm) rather than nucleus → showed it binds to flavin-type chromophore.
* So it represent a second Blue light photoreceptor.
* It involved in responses to different intensities and in modulating the circadium clock.

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There seems to be a Dual –light perception system with at least 2 photoreceptors that serve different roles

* WC-1/WC-2 →Initiate light perception →responsible for dark to light transitions.
* VIVID →Detect changes in light intensity → regulate production of carotenoid protection against photodamage.

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