#### NUTRITIONAL REQUIREMENTS OF MICROORGANISMS-

- All living organisms contain certain macromolecules, primarily proteins, nucleic acids, lipids, and carbohydrates → these macromolecules are composed of carbon (C), nitrogen (N), phosphorus (P), Sulfur (S), oxygen (O), and hydrogen (H).
- Cells also require- potassium, sodium, magnesium, calcium and iron (are the most abundant cations in microbial cells).
- Chloride (CI-) is the major anion. K+, Na+, CI- have important functions in controlling osmotic balance, and in charge dependent with macromolecules
- Mg2+ associates with ATP, and is a key component of many enzymes and protein-nucleic acid complexes, such as the ribosome.
- Several enzymes and cytochromes, the membrane-bound electron transfer protein that have critical roles in energy metabolism, require iron for their activity. Iron is frequently limiting for growth in natural habitats, where soluble iron can be in short supply. The mammalian body goes to great lengths to sequester iron in tightly bound protein complexes, both to minimise iron toxicity and to keep it away from microbial pathogens.
- Micronutrients- zinc, cobalt, molybdenum, copper and manganese are also needed for microbial growth. Certain enzymes and electron transfer protein complexes require small amounts of these metals. Most microbes need so little of these metals that they often are not intentionally added to culture media

Acquisition of nutrients

- Acquiring carbon and incorporating in into macromolecules is critical for a growing cell the process by which a cell imports a molecule and incorporate it into cellular constituents is called assimilation.
- Autotrophs assimilate carbon from inorganic sources. They convert the calor present in molecules such as carbon dioxide into organic molecules, this is a ded carbon fixation.
- Autotrophic organisms generate most of the Earth the targets, these organisms are generally responsible for the oxygen-rich atmosphere.
- Heterotrophs- can't assimilate carl of from inorganic carbol nolecules. They must obtain it in a pre-existing organic form. Heterotrophic microb is have evolved to acquire carbon from many kinds of Cleaules- carbohydrates am op acids, lipids, organic acids, alcohols and more.
- Nitrogen- abundant in biological macromolecules and actively growing microbes must acquire it in a usable form. Certain microbes can convert the dinitrogen gas (N2) present in the atmosphere into ammonia (NH3), through a process known as nitrogen in the form of ammonia (NH3). Still other microbes assimilate nitrate (NO3-) and nitrite (NO2-), which they then convert to ammonia. Once a cell obtains ammonia through any of these avenues, it can incorporate the nitrogen into the amino acids glutamate and glutamine. -> The molecules can serve as precursors for the generation of other amino acids and additional molecules that contain nitrogen.

Acquisition of energy

- Cells need energy to drive their metabolism
- Microorganisms require energy in various ways. Phototrophs capture light energy, or photons through p/s to generate chemical energy such as ATP. Many phototrophs, use the energy generated from p/s for carbon fixation, incorporating carbon dioxide into biological molecules such as carbohydrates.
- For these organisms an external source of organic carbon is not needed- they simply need access to adequate light and CO2 to acquire both carbon and energy.
- Chemotrophs- acquire energy through the oxidation of reduced organic or inorganic compounds they have acquired from the environment- in many cases where organic molecules are used as a source of energy, the same molecule serves both as a carbona energy source. E.g. glucose- heterotrophs.

it appears that no single factor or combination of factors together can be universally attributed to the provision of thermal stability in proteins.

Feller (2003) Cell Mol. Life. Sci. 60: 648-662.

- Psychrophiles are cold-loving organisms successfully colonize cold environments of the Earth's biosphere. To cope with the reduction of chemical reaction rates induced by low temperatures, these organisms synthesise enzymes characterised by a high catalytic activity at low temperatures associated, however, with low thermal stability.
- Emerging evidence suggests that psychrophilic enzymes utilise an improved flexibility of the structures involved in the catalytic cycle, whereas other protein regions if not implicated in catalysis may or may not be subjected to genetic drift.
- The most widely accepted hypothesis accounting for the dominant adaptive traits of cold-active enzymes, i.e. the high activity and the weak stability, suggests that there is a correlation between the activity, the flexibility and the stability of the enzyme molecule.
- The flexible structure of psychrophilic enzymes can provide enhanced ability to undergo discrete and fast conformational changes at low temperatures imposed by the catalytic events.

## Extreme Environments II: Acidophiles/ basophiles

Wessner et al pages 110-125

## Wessner et al page 171

### EFFECTS OF pH ON MICROBIAL GROWTH

- pH = expression of the hydrogen ion activity, or concentration of hydrogen ion a solution.
- The pH scale is an inverse logarithmic function (pH= log/H+Crawing from 0 to 14. pH 7 is neutral lower than pH 7 is acidic higher than pH 7 is alkaline orbasi

- each pH has a difference hy tenfold
- 18 of Microbial growbrates can be affected by the it affects the transmembrane electrochemical gradients that are necessary for A P sydness and many transport processes
- Neutrophiles grow optimally in environments with pH close to neutrality (5.5-8.5)
- Acidphiles prefer significantly acidic habitats (pH<5.5)
- Alkalophiles prefer alkaline habitats (pH>8.5)

The most thoroughly studied acidophilic microbes are the lactic acid bacteria of the genus Streptococcus. These aerotolerant anaerobes generate energy through fermentation, often producing lactic acid as a primary fermentation product. These bacteria have many nutritional requirements. Because their fermentative metabolism is so inefficient, they grow best in environments with high-sugar concentrations. Under these conditions, these bacteria produce abundant organic acids that can acidify the medium to pH4-5. These bacteria are often found in anaerobic habitats

Microbes also play an active role in altering the pH of their environment.

## Baker-Austin & Dopson (2007) TRENDS Microbiol. 15: 165-171.

- Extremely acidophilic microorganisms have an optimum growth of pH<3 and proliferate in</li> natural and anthropogenic low pH environments.
- Some acidophiles are involved in the catalysis of sulphide mineral dissolution, resulting in high concentrations of metals in solution
- Acidophiles have a variety of intrinsic and active metal resistance systems that likely combine to permit their growth in very high metal concentrations

# **Extreme Environments IV: Storage states**

Wessner et al. pages- 56-57

- Some Gram positive bacteria can undergo a form of cellular re-modelling called endospore formation, this is initiated by as a survival mechanism under stressful conditions, such as starvation. The endospores are largely metabolically inert structures that exhibit increased resistance to many harsh environmental conditions e.g. desiccation, UV light exposure and high temperatures.
- Endospores have dramatically thickened cell envelopes with additional layers of protein outside of the peptidoglycan → this makes them resistant to desiccation and chemical attack. Endospores shut down their metabolism completely and compact chromosomal DNA tightly with protective proteins. As a consequence they are incredibly durable and can remain viable for many years
- The cellular re-modelling involved in endospore formation requires major changes in gene expression

## ENDOSPORES:

# Errington (2003) Nature Rev. Microbiol. 1: 117-126.

- Spore formation in bacteria poses a number of biological problems of fundamental significance. Asymmetric cell division at the onset of sporulation is a powerful model for studying basic cell-cycle problems, including chromosome segregation and septum formation.
- Sporulation is one of the best understood examples of cellular development and differentiation. Fascinating problems posed by sporulation include the temporal and spatial control of gene expression, intercellular communication and or possaspects of cell morphogenesis.

## Knaysi (1948) Bacteriol. Rev. 12: 19-

- Endospore=intrace that spore formed by certail bacteria and capable of reaching a high degree on restaurce to deleteriouen cents
- In bacteria, endospore formation is nost prevalent among the rod-like forms and is the basis for the anaerobic genus *Clostridium* and the facultative *Bacillus*.

# HETEROCYSTS:

## Wolk et al. (2003) Ann. Rev. Gen:30: 59-78

- Many multicellular cyanobacteria produce specialised nitrogen-fixing heterocysts.
- Heterocyst structure and metabolic activity function together to accommodate the oxygensensitive process of nitrogen fixation
- Heterocyst- forming cyanobacteria differentiate highly specialised cells to provide fixed nitrogen to the vegetative cells in a filament
- Heterocysts are typically distinguishable from vegetative cells by their somewhat larger and rounder shape, diminished pigmentation, thicker cell envelopes, and usually prominent cyanophycin granules at poles adjacent to vegetative cells. The additional envelope layers surrounding heterocysts help to protect the enzyme nitrogenase from oxygen.
- Mature heterocysts provide the microoxic environment required for nitrogen fixation, spatially separating oxygen- evolving photosynthesis in vegetative cells from nitrogen fixation.

- Both of these pathways are found in many microbes. In general, glutamate dehydrogenase is used at high NH4+ concentrations, and GS-GOGAT is used during low ammonium availability. GS has a higher affinity for ammonium than glutamate dehydrogenase, so GS is more active at submillimolar NH4+ concentrations. In many microbes, these differences are reflected in the regulation of the enzymes, with GDH expression repressed and GS-GOGAT induced in low-nitrogen medium, and the converse when ammonium is abundant
- The amino group added to glutamate by GDH or GS-GOGAT can be donated to other molecules by enzymes called "transaminases".
- GDH reaction is reversible, so the enzyme can function for catabolic deamination of glutamate, generating NADPH and ά-ketoglutarate

### UTILISATION OF NITRATE AND NITRITE-

- Nitrate can be used as a terminal electron acceptor for anaerobic respiration → "dissimilative nitrate reduction"
  - "assimilative nitrate reduction" = nitrate is reduced to ammonia. This occurs in the cytoplasm and is not linked to energy metabolism
- In the cytoplasm, ammonia becomes ammonium and is then incorporated into biomass
- Cytoplasmic nitrate reductases usually use NADH or NADPH as electron donors (but there are others)
- Ammonis/ ammonium is the preferred nitrogen source since it needs no further reduction
- Nitrate reduction to nitrite is the first step in making ammonia/ammonium for assimilation. Production of nitrite sets the stage for nitrite reductase to go into action. Nitrite reductases used for nitrogen assimilation catalyse sequential transfer of three pairs of electrons using reduced ferredoxin as electron donors. Each transfer is accompanied by una ke of wo protons. The end result is ammonia that is incorporated into glutamate procescribed above.
  - protons. The end result is ammonia that is incorporated into gluar tensorescribed above.
    Denitrifying bacteria also generate nitrite from nitrate, but the dissimilatory nitrite reductase instead generates nitric or ide, white is rurther reduced to nitrous oxide and ultimately to dinitrogen. (None of the end end corporated into biomass)

#### SULFUR METABOLISM

- Biological to evalue s contain sulf or recredeced oxidation state. The most common inorganic form of sulfur in most environ in recredeced oxidation state. The most common inorganic extensively reduced before it can be incorporated into cysteine.
- Some habitats are rich in elemental sulfur or even hydrogen sulfide → so microbes undertake different assimilatory strategies
- Sulfate reduction also occurs in the cytoplasm and is used to incorporate sulfur into cellular components, is distinguished from dissimilative sulfate reduction – form of respiration coupled to energy metabolism
- Assimilative sulfate reduction is very common in microbes, dissimilative sulfate reduction is rare.
- Sulfate- is a very stable molecule, to which it is difficult to add electrons, to make it a better electron acceptor, sulfate is covalently attached to ATP by the enzyme ATP sulfurylase, generating adenosine 5' phosphosulfate (APS), this is converted into to phosphoadenosine 5'- phosphosulfate (PAPS). From PAPS, the sulfate group is reduced to sulphite with electrons from NADPH, and released from the rest of the molecule. The sulphite is reduced further to hydrogen sulfide potentially toxic intermediate that is quickly used to displace acetate in acetylserine to generate cysteine. The dissmilatory process also proceeds through a sulphite intermediate to hydrogen sulfide, which is excreted as a waste product from the cell. Sulfate reducers that use the dissimilatory pathway for energy metabolism can supply other microbes with reduced sulfur, and often live in close proximity to anaerobic phototrophs that consume hydrogen sulfide as an electron donor for p/s.