MICROBIAL BIOTECHNOLOGY

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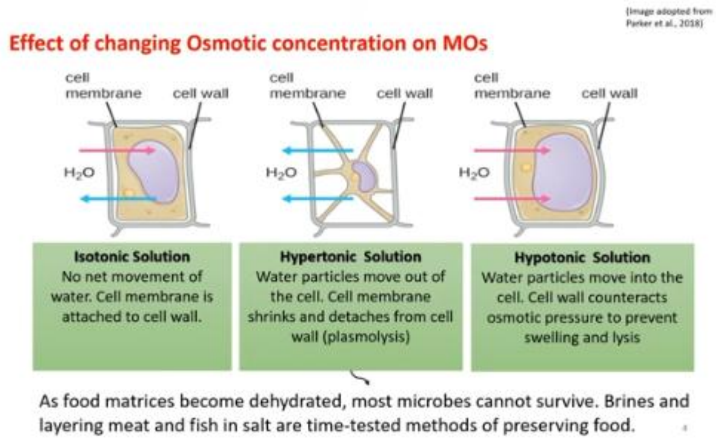
Lecture-12

Lec 12: Physiology of Extremophiles and adaptation-halophiles, xerophiles, radiophiles & mettalophiles

Hello friends, welcome to my course on microbial biotechnology. We are in module 3, currently discussing the physiology of extremophiles and their adaptations. In today's lecture, we will learn about halophiles, xerophiles, radiophiles, and metallophiles. So, let us start with adaptability to variable saline or osmotic conditions. You can see in this picture a saline environment.

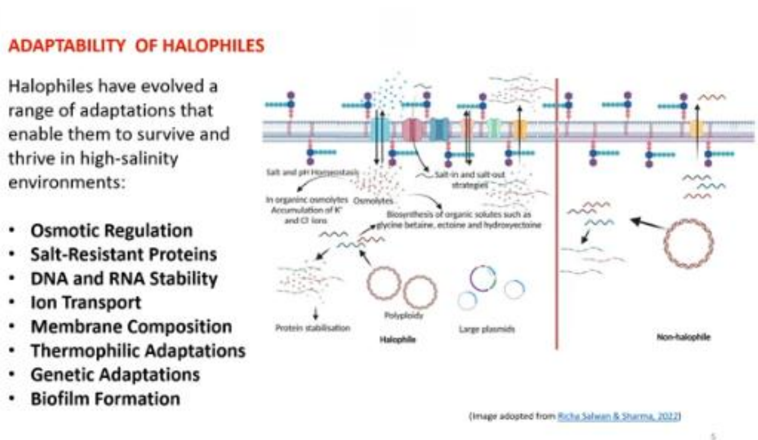
Salinity strongly influences microbial communities, ranging from marine environments to soda lakes and salt inclusions. Halophiles tolerate high salinity levels by achieving osmotic balance through the accumulation of potassium ions or the synthesis of compatible organic solutes. Water activity can be regulated by producing metabolites capable of storing or attracting water. Microbial life has been observed in brines with high concentrations of chaotropic salts, such as magnesium chloride and calcium chloride. Now, what are the effects of changing osmotic concentration on microorganisms?

Here is an isotonic solution, a hypertonic solution, and a hypotonic solution. You can see here, in the first case, there is no net movement of water. The cell membrane is attached to the cell wall. In a hypotonic solution, water particles move into the cell. As you can see, the cell wall counteracts osmotic pressure to prevent swelling and lysis.



In the hypertonic solution, water particles move out of the cell. The cell membrane shrinks, as you can see the cell membrane is shrunken and detaches from the cell wall. So this is called plasmolysis. As food matrices become dehydrated, most microbes cannot survive. Brines and layering meat and fish in salt are time-tested methods for preserving food due to these reasons.

What are the adaptability mechanisms employed by halophiles? They have evolved a range of adaptations that enable them to survive and thrive in high-saline environments. For example, they deploy osmotic regulation and salt-resistant proteins. DNA and RNA stability help in this adaptability. They also have regulation of ion transport.



And the membrane composition is also modified. There are thermophilic adaptations, genetic adaptations, and in many cases, biofilm formation. Salt and pH homeostasis are very important, which leads to protein stabilization. Here, one thing observed is that they have large plasmids and also engage in polyploidy. Salt-in and salt-out strategies are among the key factors that help in adaptability.

And then the presence of osmolytes and biosynthesis of organic solutes such as glycine, betaine, ectoine, etc. helps in this kind of adaptability. So, here are some examples. This is Salinibacter ruber. This is a red bacterium found in saltern crystallizer ponds in Alicante, Spain. This is a red bacterium found in saltern, Chrysalis japonis in Alicante, Spain. And this is Wallemia, found in hypersaline water of solar salterns, bitterns, and salted meat. This is a green algae, Dunaliella salina.

So, let us discuss these various strategies of osmotic regulation and others one by one. So, in osmotic regulation, halophiles use compatible solutes such as glycerol and trehalose to balance osmotic pressure, prevent dehydration, and stabilize cellular structures. Additionally, some employ a salt-in strategy, accumulating potassium ions within the cell to match the high salt concentration in their surroundings. Salt-resistant proteins.

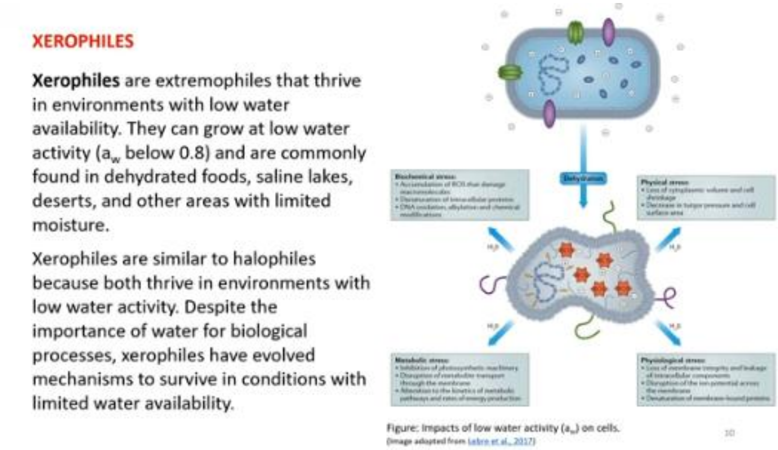
The enzymes and proteins of halophiles are specially adapted to function in saline conditions. These proteins often feature a high proportion of amino acids and flexible structures that help maintain stability and activity despite the high salt content. DNA and RNA stability, as one of the strategies, is very, very important. These halophiles typically have high G plus C content in their DNA, which contributes to greater stability under extreme conditions. They also produce protective proteins that seal both DNA and RNA from salt-induced degradation.

Then there are ion pumps and transporters. These are specialized ion pumps, such as the sodium-hydrogen antiporters, which help regulate internal salt levels, while potassium transporters are crucial for maintaining osmotic balance by accumulating potassium ions inside the cells. The cell membrane composition preserves membrane integrity and fluidity in saline environments. Halophiles adjust the composition of their cell membranes by increasing the proportion of unsaturated or branched-chain fatty acids. Then there are certain thermophilic adaptations. Many halophiles are also thermophiles, meaning they can survive at high temperatures.

Their proteins are adapted to function effectively in both high-salt and high-temperature conditions. Then there are certain genetic adaptations, which arise from genetic diversity in halophiles. These enhance mechanisms like polyploidy—having multiple copies of the genome—and horizontal gene transfer, which allow them to rapidly adapt to changing conditions. Then there is biofilm formation. Some halophiles form biofilms, which offer added protection against environmental stresses like high salinity and UV radiation. Let us now discuss adaptability to variable water activity, or AW.

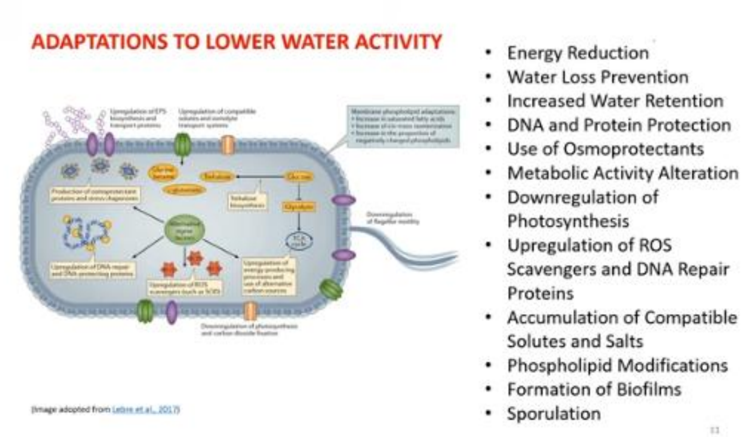
Water activity indicates the amount of available water in a substance that can support biological functions, with values ranging from 0 to 1. Water in food can be bound through solutes, ions, hydrophilic colloids, or water of crystallization, and microorganisms require available free water for growth. Most microorganisms grow well at water activities around 1 or 0.8. This is why drying food or adding large quantities of salt and sugar It is effective at preventing food spoilage by lowering water activity and limiting microbial growth.

So, xerophiles are extremophiles that thrive in environments with low water availability. They can grow at low water availability, below 0.8, and are commonly found in dehydrated foods, saline lakes, deserts, and other areas with limited moisture. Xerophiles are similar to halophiles because both thrive in environments with low water activity. Despite the importance of water for biological processes, xerophiles have evolved mechanisms to survive in conditions with limited water availability. So whenever there is dehydration due to high salt, high sugar, or any other conditions, the cell will undergo various stresses like physical stress, physiological stress, metabolic stress, and biochemical stress.



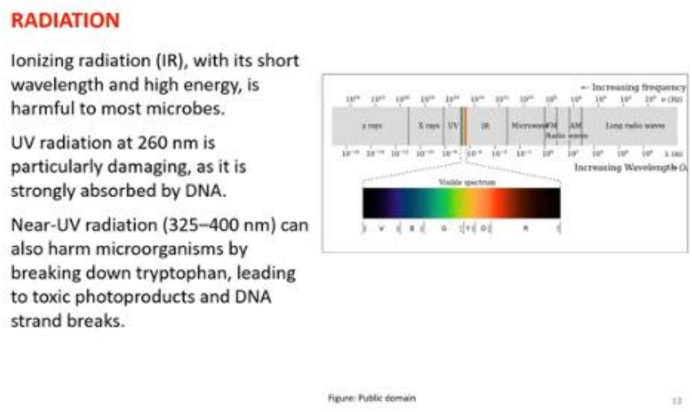
So here, in physical stress, there is a loss of cytoplasmic volume and cell shrinkage, a decrease in turgor pressure, and cell surface area. Then, under physiological stress, there is a loss of membrane integrity, leakage of intracellular components, and disruption of the ion potential across the membrane. The initiation of membrane-bound proteins and, under metabolic stress, there will be inhibition of photosynthetic machinery, disruption of metabolite transport through the membrane, alteration to the kinetics of metabolic pathways, and rates of energy production. It will also face certain biochemical stress where there is an accumulation of ROS that damages macromolecules, denaturation of intracellular proteins, deamination, alkylation, and chemical modification. So this is the impact of low water activity on microbial cells.

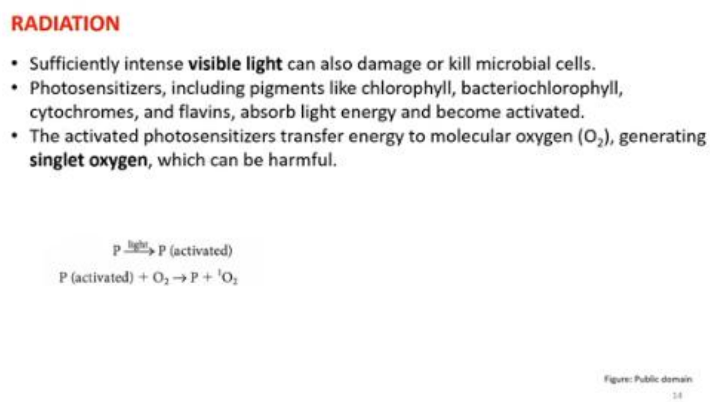
So how does a xerophile address this kind of stress or challenge? So, for these, various mechanisms are there. For example, energy reduction, water loss prevention, increased water retention, DNA and protein protection, use of osmoprotectants, metabolic activity alteration, downregulation of photosynthesis, upregulation of ROS scavengers and DNA repair proteins, accumulation of compatible solutes and salts, phospholipid modifications, formation of biofilms, and also sporulation. So these figures shows adaptation mechanisms overall. Now there is another kind of extremophiles known as radiophiles.



These are microorganisms which can adapt to high radiation. For example, Deinococcus radiodurans is highly resistant to gamma radiation due to its efficient DNA repair mechanisms. When exposed to radiation, it experiences severe DNA damage, but multiple copies of its chromosomal DNA facilitate repair and replacement of fragmented sections, maintaining cell viability. In addition to radiation, Deinococcus radiodurans is resistant to UV radiation, mutagenic chemicals, and desiccation. Its multigenomic structure with several copies of its genome enhances DNA repair and contributes to its exceptional resilience.

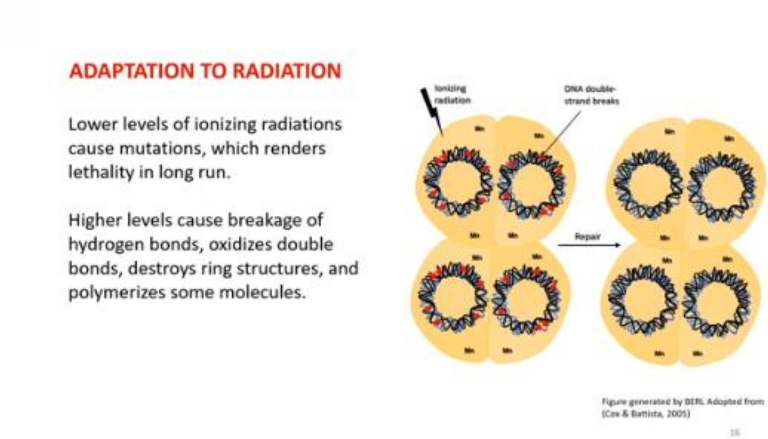
Hyperthermophilic archaea such as Thermococcus littoralis and Pyrococcus furiosus also survive high levels of gamma radiation. So this is the overall radiation spectrum, and you can see the visible spectrum over here, and then you have the ionizing radiation with its short wavelength, which has high energy and is harmful to most microbes. UV radiation at around 260 nanometers is particularly damaging as it is strongly absorbed by DNA. Near-UV radiation between 325 to 400 nanometers can also harm microorganisms by breaking down tryptophan, leading to toxic photoproducts and DNA strand breaks. Sufficiently intense visible light can also damage or kill microbial cells.



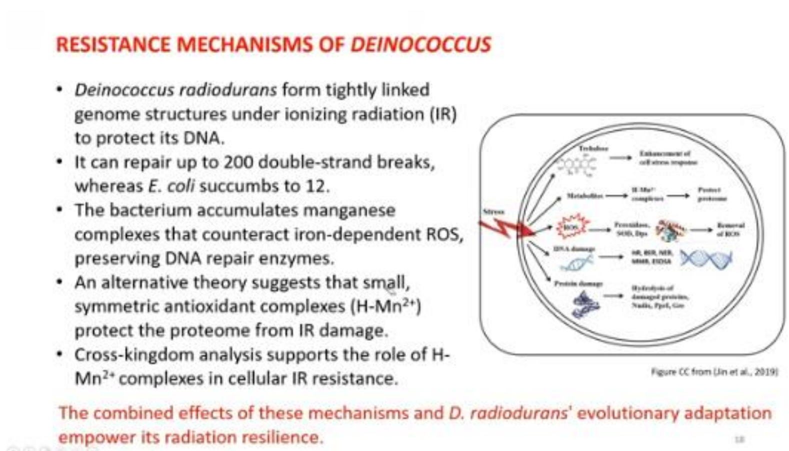


Photosensitizers, including pigments like chlorophyll, bacteriochlorophyll, cytochromes, and flavins, absorb light energy and become activated. The activated photosensitizers transfer energy to molecular oxygen, generating singlet oxygen, which can be harmful. So we see that radiation is a big challenge for microbes to survive. So, there is an example here: Sulfolobus tokodai increases expression of RadB and RadA in response to UV radiation, which is a survivable strategy. Then you have Dictyostelium, which uses translesion synthesis, the Fanconi anemia pathway, and nucleotide excision repair to survive radiation and DNA cross-linking agents.

Then you have others like Chlamydomonas and then D. radiodurans, which have been studied for their extremophilic traits, such as extremolites, extremozymes, and unique proteins like histone-like DNA-binding proteins (HU), which aid in survival under radiation. How do microorganisms adapt to radiation? Lower levels of ionizing radiation cause mutations, which render lethality in the long run. Higher levels cause breakage of hydrogen bonds, oxidize double bonds, destroy ring structures, and polymerize some molecules. So here, you can see the DNA double-strand breaks occurring over here, and then there are repair mechanisms that restore these back.



D. radiodurans stands out prominently due to its remarkable ability to reconstruct the genome following extensive radiation exposure, a phenomenon that captivates microbiologists and sparks intense scientific curiosity. What are the resistance mechanisms of these D. radiodurans? These form a tightening genome structure under ionizing radiation to protect their DNA. It can repair up to 200 double-stranded breaks, whereas E. coli succumbs to as few as 12 double-stranded breaks. The bacterium accumulates manganese complexes that counteract iron-dependent ROS, preserving DNA repair enzymes.

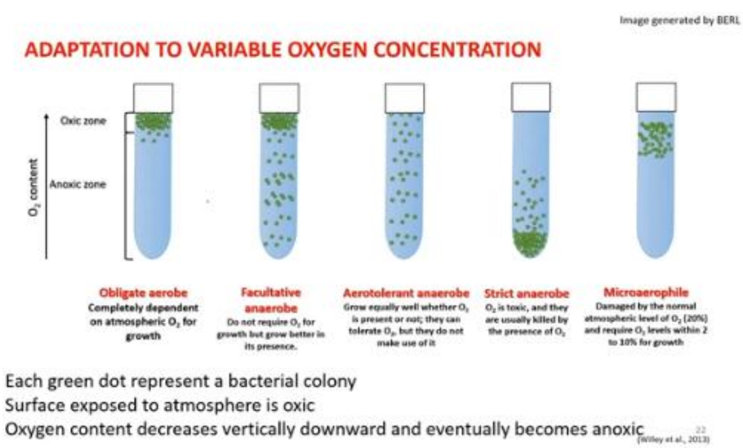


An alternative theory suggests that small, symmetric antioxidant complexes protect the proteome from IR damage. Cross-kingdom analysis supports the role of hydrogen and manganese complexes in cellular IR radiation resistance. The combined effect of these mechanisms and D. radiodurans' evolutionary adaptation empowers its radiation resistance.

Another type of extremophile is the metallophile. As the name suggests, these microbes can adapt to high metallic ion concentrations in their habitat.

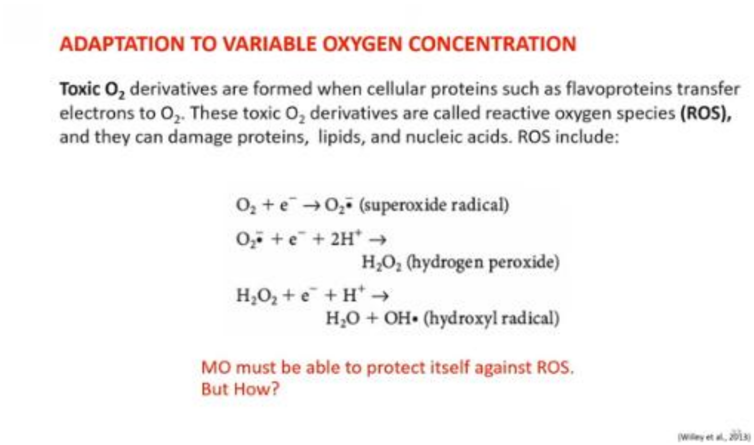
So, the adaptation mechanisms for metallophiles include conversion. The metallophiles utilize enzymes to convert these metals into less toxic or less bioavailable forms, reducing the metals' harmful effects on the cell walls. Then efflux, active removal of metals from the cell through specialized efflux systems or pumping systems. These adaptations allow metallophiles to survive and function in extreme metal-rich environments. Now let us also discuss some other adaptations apart from the ones studied, particularly adaptation to variable oxygen concentration.

So here you can see the various tubes. And you can see that there are two zones. On the top of the tube, you have an area which is rich in oxygen, and below you have an area which is poor in oxygen. So this is known as the anoxic zone, and this is the oxic zone in terms of oxygen content. So if we grow certain bacteria, some will grow at the top, some will grow at the bottom, and some will be dispersed throughout with some concentration at the top, some will be very uniform, and some will not grow at the top nor at the bottom but just a little submerged.

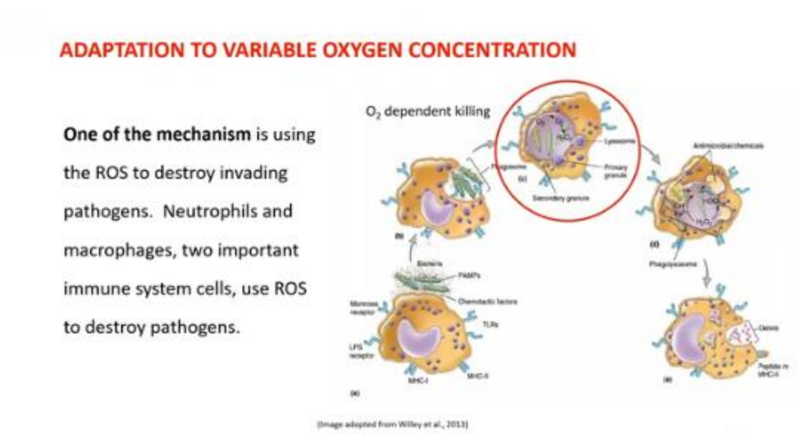


So this is a very interesting phenomenon. So, accordingly, they have been classified as obligate aerobes, facultative anaerobes, aerotolerant anaerobes, strict anaerobes, and microaerophiles. So, these obligate aerobes are completely dependent on atmospheric oxygen for growth. They cannot survive without oxygen; facultative anaerobes do not require oxygen for growth. They grow better in its presence, and aerotolerant anaerobes grow equally well whether oxygen is present or not. They can tolerate oxygen but do not make use of it.

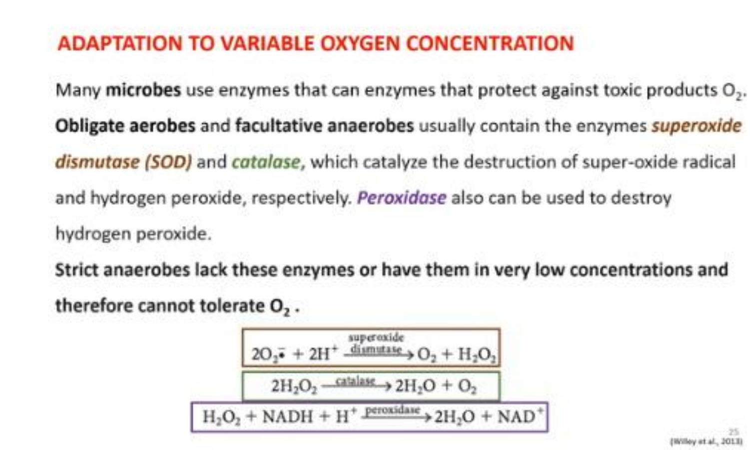
For strict anaerobes, oxygen is toxic, and they are usually killed by its presence. Microaerophiles are damaged by the normal atmospheric level of oxygen (around 20%) and require oxygen levels between 2% and 10% for growth. Thus, they survive well under slightly submerged conditions. What are the adaptation mechanisms to variable oxygen concentrations? Toxic oxygen derivatives form when cellular proteins, such as flavoproteins, transfer electrons to oxygen.



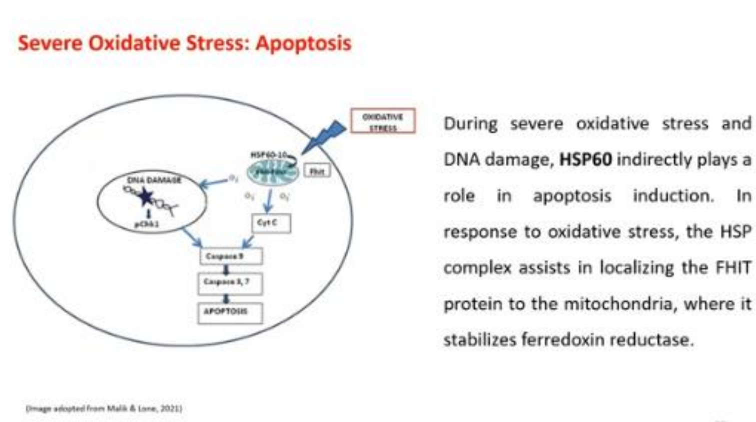
These oxygen derivatives are called reactive oxygen species, and they can damage proteins, lipids, and nucleic acids. Examples include the superoxide radical, hydrogen peroxide, and hydroxyl radical. Microorganisms must protect themselves against reactive oxygen species. But how do they do it? One mechanism is using ROS to destroy invading pathogens.



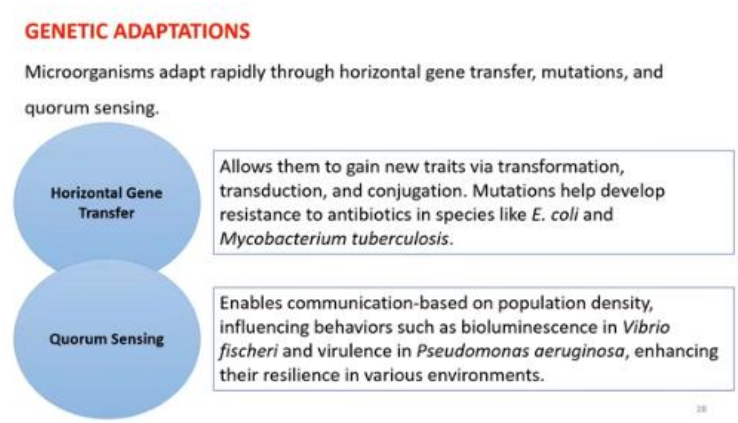
Neutrophils and macrophages, two important immune system cells, use ROS to destroy pathogens. This is called oxygen-dependent killing. Many microbes use enzymes that can protect against toxic products of oxygen. Obligated aerobes and facultated aerobes usually contain the enzyme superoxide dismutase and catalase, which catalyze the destruction of superoxide radical and hydrogen peroxide respectively. Peroxidase can also be used to destroy hydrogen peroxide.



Strict anaerobes lack these enzymes or have them in very low concentrations and therefore cannot tolerate oxygen. Severe oxidative stress or apoptosis occurs during severe oxidative stress and DNA damage. Heat shock protein 60 indirectly plays a role in apoptosis induction. In response to oxidative stress, the HSP complex assists in localizing the FHIT protein to mitochondria, where it stabilizes ferredoxin reductase, as you can see here. This leads to increased production of reactive oxygen species.



Many of them are getting produced, triggering the release of Cytochrome C and subsequent activation of caspase, ultimately leading to the apoptosis. In the case of genotoxic stress, FHIT functions as a part of the checkpoint response to DNA damage through Chk1 resulting in cell cycle arrest and potentially apoptosis of the DNA damage is severe. Now there are certain generic adaptations through horizontal gene transfer, mutations and quorum sensing which helps in this adaptation. So, horizontal gene transfer allows them to gain new traits via transformation, transduction and conjugation. Mutations help develop resistance to antibiotics in spaces like E. coli and Mycobacterium tuberculosis.



And then, in quorum sensing, which enables communications based on population density, influencing behaviors such as bioluminescence in Vibrio fischri and virulence in Pseudomonas aeruginosa, enhancing their resilience in various environments. So, conjugation is a type of horizontal gene transfer which involves direct contact between a donor and recipient cell via filamentous protein, a sex pilus, pulling them together. The donor transfers all or part of its plasmid through the pilus to the recipient, as you can see in this picture. Then there is transduction in B, which involves a bacteriophage vector delivering genes between bacteria. When the phage attaches to a bacterial cell, its DNA gets incorporated into the host chromosome.

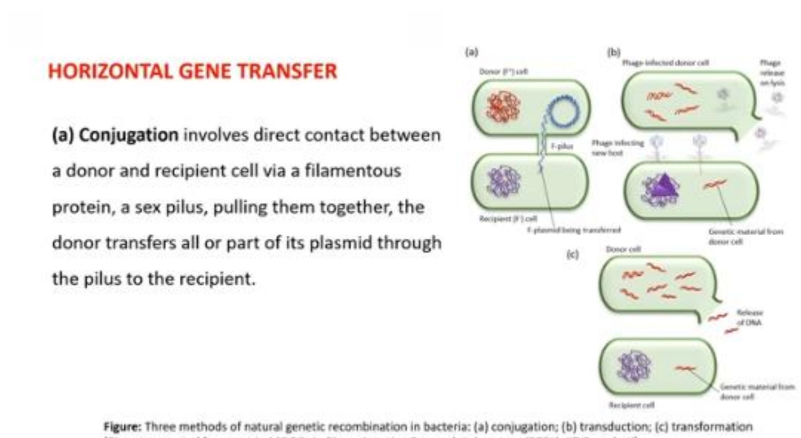
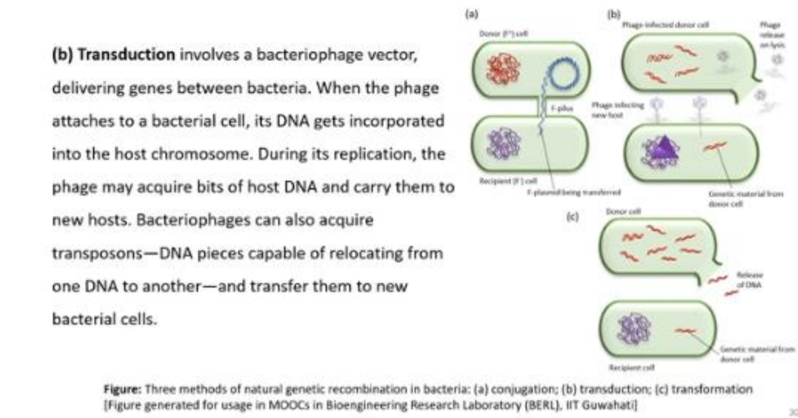


Figure: Three methods of natural genetic recombination in bacteria: (a) conjugation; (b) transduction; (c) transformation [Figure generated for usage in MOOCs in Bioengineering Research Laboratory (BERL), IIT Guwahati]



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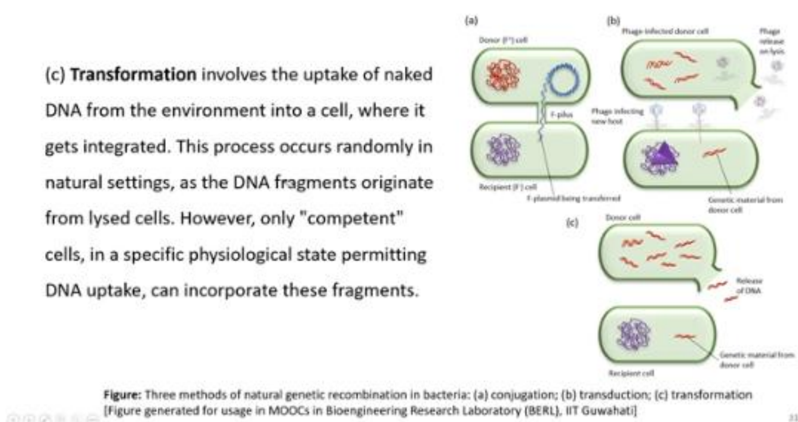
During its replication, the phage may acquire bits of host DNA and carry them to a new host. Bacteriophages can also acquire transposons, which are DNA pieces capable of relocating from one DNA to another, and transfer them to new bacterial cells. Then, finally, we have transformation, which involves the uptake of naked DNA from the environment into a cell, where it gets integrated. This process occurs randomly in natural settings, as the DNA fragments originate from lysed cells. However, only competent cells in a specific physiological state permitting DNA uptake can incorporate these fragments.



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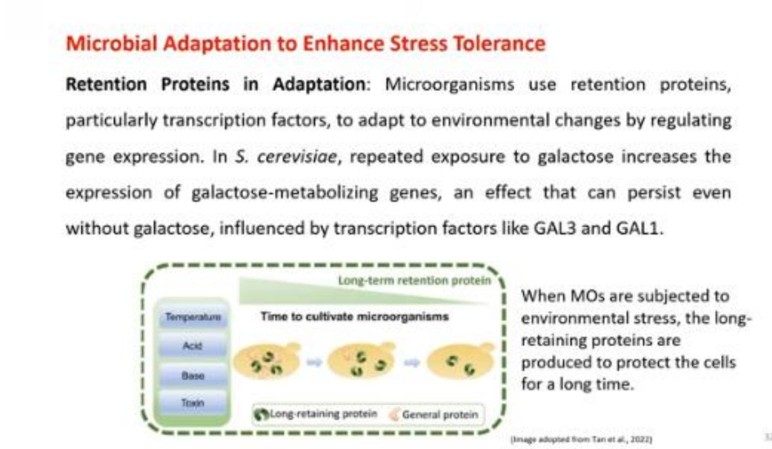
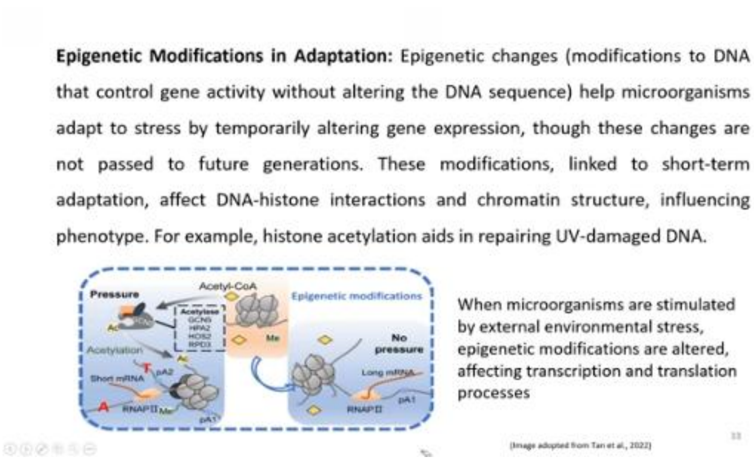
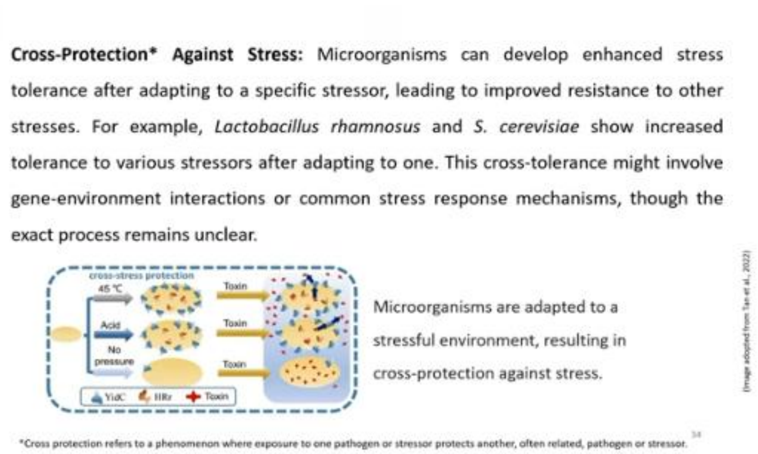
What are the microbial adaptations to enhance stress tolerance? Retention proteins in adaptation. Microorganisms use retention proteins, particularly transcription factors, to adapt to environmental changes by regulating gene expression. In Saccharomyces cerevisiae, repeated exposure to galactose increases the expression of galactose-metabolizing genes, an effect that can persist even without galactose, influenced by transcription factors like GAL3 and GAL1. When microorganisms are subjected to environmental stress, long-retaining proteins are produced to protect the cell for a long time.



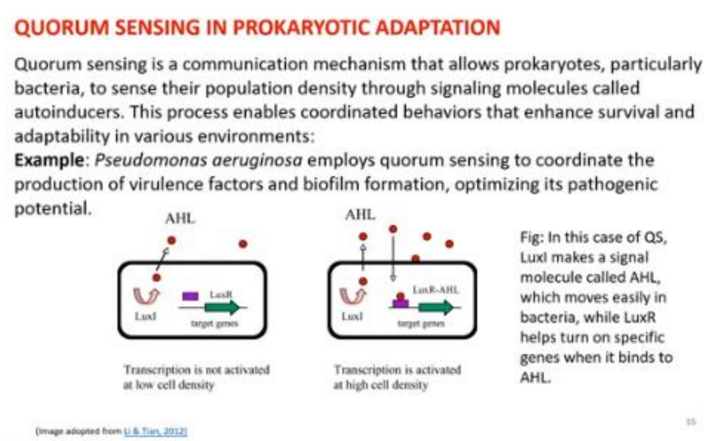
So you can see here these long-retaining proteins and the general proteins present over here. Epigenetic modifications also help in adaptation. Epigenetic changes, modifications to DNA that control gene activity without altering the DNA sequence, help microorganisms adapt to stress by temporarily altering gene expression, though these changes are not passed to future generations. These modifications linked to short-term adaptations affect DNA-histone interactions and chromatin structure, influencing phenotype. For example, histone acetylation aids in repairing UV-damaged DNA.



In this picture, you see when organisms are stimulated by external environmental stress, epigenetic modifications are altered, affecting transcription and the translation process. Then there is also cross-protection against stress. Cross-protection basically refers to a phenomenon where exposure to one pathogen or stressor protects against another, often related pathogen or stressor. Microorganisms can develop enhanced stress tolerance after adapting to a specific stressor, leading to improved resistance to other stresses. For example, Lactobacillus rhamnosus and Saccharomyces cerevisiae show increased tolerance to various stressors after adapting to one.



The cross-tolerance might involve gene-environment interactions or common stress response mechanisms, though the exact process remains unclear. So in this picture, we can see microorganisms are adapted to a stressful environment, resulting in cross-protection against stress. Then, quorum sensing is an important adaptation strategy in prokaryotes. Quorum sensing is a communication mechanism that allows prokaryotes, particularly bacteria, to sense their population density through signaling molecules called autoinducers. This process enables coordinated behavior that enhances survival and adaptability in various environments.



For example, Pseudomonas aeruginosa employs a quorum-sensing mechanism to coordinate the production of virulence factors and biofilm formation, optimizing its pathogenic potential. So, in this case of quorum sensing, LuxI makes a signaling molecule called AHL, which moves easily in bacteria, while LuxR helps turn on specific genes when it binds to AHL. So this is one of the methods. Here, the transcription is not activated at low cell density. The transcription is activated at higher cell density.

So, we come to the end of this part of the lecture. Thank you for your patient hearing.