Model Answer
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M.Sc. (Botany) IV Semester
Paper – LBT 402
Cyanobacteria: Stress Biology and Molecular Genetics

Section - A

Ans. 1 (i) b - involved in photosynthesis

(ii) d – Phycobilisomes

(iii) d – all of the above

(iv) d – unicellular Cyanobacteria

(v) a – *Spirulina*

(vi) d – Triglycerides

(vii) a – Fatty acid desaturase

(viii) b – promoter region

(ix) b - Sig – G

(x) c – both of the above

Section – B

Ans. 2. The effect of water stress in Cyanobacteria:-

On nucleic acid- The water stress leads to accumulation of mutations, damage to DNA due to chemical modifications, cross linking and base removal. There may be single stranded breaks in DNA. Low water is responsible for causing conformational changes in DNA converting it from B to A form. It also induces oxidative stress related effects due to generation of ROS (reactive oxygen species) like OH radicals. DNA becomes more susceptible to UV damage.

On proteins – As the water is necessary for protein structure and folding, the deficiency of water considerably alters the structure of the protein. Proteins are also damaged by the ROS due to induced oxidative stress effect. The free thiol residues of cystein are oxidized to produce thiyl

radicals which can form the intra and inter molecular cross linking. The proteins become sensitive to proteolysis, inactivated and show reduced activity.

On lipid membrane – under water stress membranes changes their morphology and loss the Ca transport activity. The degree of saturation of fatty acid of plasma membrane increases. The membrane become more prone to damage due to ROS

Measures to counter the water stress:-

Protection of nucleic acid: -

Cyanobacteria have multiple genome copies. They have UV absorbing pigments. The histone-like DNA binding proteins like HNS, HU etc. in association of topoisomerase provide regulation on DNA repair. There are some 'Repair Ligases' that contribute a lot in repair of single strand damage. Cyanobacteria are capable of retarding the rate of depurination and other damages. Double helix structure and amount of bound water plays role in tolerance.

Protection of lipid membrane:-

There is accumulation of trehalose and sucrose sugars which stabilizes the lipid layer. Trehalose replaces shell of water around macromolecules circumventing damaging effects. Both sucrose and trehalose can replace the bound water and can form the aqueous glasses around the desiccated cells.

Protection of protein:-

The bound water plays important role in protein folding and structure which provides stability to it during desiccation. There is synthesis of Ubiquitin protein which plays important role in lysis and selective degradation of proteins. There is de-novo synthesis of water-stress proteins which accumulate in glycan sheath. These water stress proteins have structural role in cell stability. During desiccation Cyanobacteria accumulate huge amount of singular polypeptide 'cyanophycin' which contributes to protein stability. The amount of synthesis of enzyme 'catalase' is also increased which protects the damage repair. The carbohydrate trehalose protects the protein damage during the desiccation by accumulation. The protein Dna K reactivates the heat denatured RNA polymerase in presence of Ca^{++}.

Ans. 3. Effect of stress on proteins, nucleic acids and lipids-

Effect of stress on proteins:-

1. Decrease in rate of protein synthesis. Protein pool show considerable fraction of modified proteins on rehydration.

2. It induces the oxidative damage effects by generating ROS

3. There is change in conformation through the cross-links mostly due to oxidation of free thiol residue of cystein to thiyl radcals, which may link thiyl radicals forming double bonds.

4. There is loss of diffusion barrier to membrane impermeable markers and ultimately cell lysis as a result of oxidative damage.

5. There is enhanced sensitivity to proteolysis and inactivation reduced activity

6. The proteins get denatured at low temperature

Effect of stress on nucleic acid-

1. Accumulation of mutations, over the time, when there is no cell growth.

2. DNA damage due to chemical modifications (alkylation and oxidation), cross - linking, base removal by ionizing and non- ionizing radiation.

3. Occurrence of single stand breaks of genome.

4. Conformational changes in DNA, base methylation in major groove of DNA.

5. Conversion of B form to A form of DNA.

6. Alteration in DNA as a result of increased covalent cross - links between proteins and DNA that accumulate continuously during desiccation.

7. Susceptibility to U.V. damage depends on the secondary structure of double helix, which is further influenced by amount of bound water.

8. Ultimate loss of control mechanism that maintain low ROS concentration.

9. The OH - can tack and damage almost every molecule of living cell. In DNA it hydroxylates bases and enhances mutation rates.

Effect of stress on lipid membrane-

1. Vesicles fusion in absence of trehalose, change in morphology, loss of calcium transport activity.

2. Thermal upshift leads to rapid increase in membrane fluidity upon subsequent rehydration.

3. Damage by ROS (oxygen and Hydroxyl radical) initiates peroxidation.

4. Lipid peroxidases decompose to give volatile hydrocarbons and aldehydes.

Ans. 4.

Genome organization of *Synechocystis* PCC 6803

The nucleotide sequence of the entire genome of *Synechocystis* sp. PCC6803 was determined in 1996 as the first example of a photoautotrophic organism. The nucleotide sequence of the entire genome was 3,573,470 bp long, and the average GC content was 47.7%. One of the notable features of the *Synechocystis* sp. PCC6803 genome is its high content of two types of repetitive sequences, IS-like elements and a HIP1 (highly iterated palindromic) sequence. There are over 70 IS-like elements containing ORFs which show similarity to bacterial transposases spread all over the genome. They were classified into 9 groups on the basis of similarity and/or structure of 17-36 bp inverted repeats on both termini. Interestingly, only 26 of them seemed to hold the coding capacity of full-length transposase proteins. The remaining ORFs were disrupted by mutations such as frame-shift or deletion or by insertion of other IS-like elements.

Structural RNA genes: It has 2 copies of an rRNA gene cluster, 42 tRNA genes, and a gene for a RNA subunit of RNase P. Most of the genes are present in a single copy; the only exception is 2 copies of trnl-GAU genes located in the duplicated.

Protein coding genes: A total of 3,168 potential protein coding genes were assigned in the *Synechocystis* sp. PCC6803 genome. The gene density is 1 gene per 1.1 kb, a typical value for bacterial genomes. The average length of the translated gene products was 326 amino acid residues, and the potential protein coding regions, as a whole, occupied 87.0% of the genome. Of

the 3,168 potential protein coding genes, 145 (4.6%) were identical to the reported *Synechocystis* sp. PCC6803 genes, 935 (29.4%) showed high degree of similarity to reported genes, and 324 (10.2%) and 342 (10.8%) showed similarity to known and hypothetical genes, respectively. The remaining 1,424 (45.0%) did not show any apparent similarity to sequences in the public DNA and protein databases.

Photosynthetic genes: One hundred twenty eight genes involved in various stages of photosynthesis were identified on the basis of sequence similarity to the photosynthetic genes reported in *Synechocystis* sp. PCC6803 and other organisms. The most significant similarity as a whole genome was observed between *Synechocystis* sp. PCC68O3 and the plastid of *Porphyra purpurea* (red alga), in which 94% of the plastid genes showed homology to the genes in *Synechocystis* sp. PCC68O3 genome.

Genes for signal transduction system: In *Synechocystis* sp. PCC6803, a total of 80 genes for two component signal transducers, including 26 genes for sensory kinases, 38 genes for response regulators, and 16 genes for hybrid sensory kinases containing both transmitter and receiver domains, have been identified.

Ans. 5.

The direct conversion of solar energy into liquid fuel using photosynthetic microorganisms is an attractive alternative to fossil fuels. There are several advantages to using organisms such as microalgae and cyanobacteria: their readily available genetic tools and sequenced genomes; their higher growth rate compared to plants; and their ability to thrive in areas that cannot support agriculture. Utilization of these organisms can provide a way of resolving the potential conflict between the use of land for food or for biofuel production.

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Cyanobacteria are advantageous organisms for industrial applications as they present fast cell growth, have simple nutrient requirements (mainly water, sunlight and CO2 , are naturally transformable and thus have the potential to be genetically engineered. Cyanobacteria are well suited for synthetic biology and metabolic engineering approaches for the phototrophic production of various desirable biomolecules, including ethanol, butanol, alkylesters, and hydrocarbon biofuels. Two biofuels that are being considered in microbial production systems are alkanes and isoprenoids. Alkanes of defined chain lengths can be used as injection fuel similar to petrol and jet fuel.

Many cyanobacteria synthesize alkanes in minute quantities. Optimizing the expression of the alkane biosynthesis genes and enhancing the carbon flux through the fatty acid and alkane biosynthesis pathways should lead to the accumulation and/or secretion of notable amounts of alkanes. Cyanobacteria produce carotenoids and extending the carotenoid biosynthetic pathways by introduction of constructs for appropriate terpene synthases should allow the biosynthesis of selected mono- and sesquiterpenes. Thus, Cyanobacteria can be utilized to produce the biodiesel.

Cyanobacteria are known to produce the Hydrogen gas by direct biophotolysis by using enzyme Nitrogenase and / or bi-directional Hydrogense in both hetercystous and non-heterocystous forms. One of the most focused Cyanobacteria is Nostoc which is the representative genus in hydrogen research. The unicellular non-nitrogen fixing Cyanobacteria like *Spirulina platensis* and *Gloeocapsa apicola* show high activity of reversible Hydrogenase under dark anaerobic conditions. These properties of the Cyanobacteria can be harnessed to generate very efficient, carbon neutral biofuel.

Ans. 6.

Bioactive compounds:

A substance or a compound has a biological activity if it has direct effects on a living organism. These effects can both be adverse or beneficial depending on the substance, the dose or the bioavailability.

Cyanobacteria in production of bioactive compounds:

Pigments: The extensively utilized pigments in bioindustry, are the (PBS) phycobiliproteins (comprising phycocyanin, phyco-erythrin and allophycocyanin), The most common cyanobacterial strains utilized for this purpose are *Anabaena variabilis*, *Aulosira fertilissima*, *Hapalosiphon* sp. and *Tolypothrix tenuis.* A number of patents have been filed for use of these in such bioindustries. Other applications of the biliproteins include confectionaries, candied ices and sherbets. PBS, particularly PC (plastocyanin) and carotenoids have been reported to exhibit a variety of pharmacological properties. Romay et al. reported the antioxidant, anti-inflammatory, neuroprotective and hepatoprotective effects of C-phycocyanin. C-PC is also used for the treatment of diseases such as Alzheimer's and Parkinson's and prevents experimental oral and skin cancers. Strong antioxidant properties and anti-inflammatory properties have been ascribed to the blue colour pigment present in the extract of *Aphanizomenon flos-aquae* and *Spirulina platensis.* A novel online fluorescence monitoring system for marine cyanobacterial cultivation based on phycocyanin fluorescence was developed.

Carotenoids act as antioxidants, hence, protect cells from damage by unstable oxygen molecules. These pigments can boost the immune system and possibly lower the risk of heart disease,

prevent onset of cancers and protect against age related diseases such as cataracts and macular degradation, multiple sclerosis.

Extracellular growth promoting substances:

Cyanobacteria can benefit plants by producing growth promoting regulators/hormones, i.e. gibberellin like, cytokinin like, auxin like compounds or abscissic acids. The capacity for IAA (Indole acetic acid) biosynthesis was found in representatives of free living and symbiotic cyanobacteria of the genera *Nostoc*, *Chlorogloeopsis*, *Calothrix*, *Plectonema*, *Gloeothece*, *Anabaena*, *Cylindrospermum* and *Anabaenopsis.* A gibberellins-like substance has been isolated from the cyanobacterium *Phormidium foveolarum, Cylindrospermum* spp. and in *Anabaenopsis* spp. Selyakh and Semenova have reported the presence of cytokinins in culture liquids of *Chlorogloeopsis* and *Calothrix.*

Cyanobacteria are an excellent source of nutrition. *Spirulina* supplies all most of the vitamins like B-series vitamins, tocopherol, niacin; inositol and folic acid that all living beings need to carry on metabolic processes.

Cyanobacteria	**Vitamins reported**
Anabaena flos_aquae	B12
Chroococcus minutus	B12
Oscillatoria jasorvensis	Pantothenate, B12
Spirulina spp.	B12, pantothenate, inositol
Nostoc spp.	B12, B1, biotin, nicotinic acid

Exopolysaccharides:

Many species of unicellular and filamentous cyanobacteria produce large quantities of extracellular polymeric substances consisting mainly polysaccharides; especially edaphic forms which produce extracellular polymers of diverse chemical composition, especially exopolysaccharides (EPS) that enhance microbial growth and as a consequence, improve soil structure and fertility.

Allelochemicals:

Cyanobacteria are known to excrete bioactive compounds into the environment, which are important determinants of allelopathic activity in water and soil. *Nodularia harveyana* produce a

lipophilic bioactive compounds against cyanobacteria, eubacteria, rotifers and crustaceans. Most of the known cyanotoxins are photosynthetic inhibitors. Cyanobacteria are proving to be a rich source of a large number of novel compounds with biological activity. The microcystins are a group of cyclic heptapeptide (7 amino acids) hepatotoxins (liver toxins) produced by a number of cyanobacterial genera, the most notable of which is the widespread *Microcystis.* The anatoxins are a group of neurotoxic alkaloids produced by a number of cyanobacterial genera including *Anabaena*, *Oscillatoria* and *Aphanizomenon*. Like anatoxins, the saxitoxins are neurotoxic alkaloids, which are also known as PSP's (paralytic shelfish poisons) due to their occurrence and association with seafood. They block sodium channels in nerve cells, thus causing neurotoxic effects.

Ans. 7.

Many cyanobacteria are known to be able to synthesise outermost slimy investments which are called capsule, sheath, mucilage, slime or glycocalyx. This extra-cellular mucilaginous material is mainly polysaccharidic in nature.

Chemical properties of Cyanobacterial polysaccharide: Cyanobacteria have 3 main types of polysaccharides.

1. A sheath, a thin uniform structured external layer immediately next to the outer membrane.

2. A capsule or slime (capsular polysaccharide CPS), more outer unstructured zones. CPS is intimately associated with the cell surface.

3. Soluble polysaccharides or released polysaccharides (RPS) which are released by many Cyanobacteria into the media.

The neutral sugars xylose, arabinose, fucose, rhamnose, galactose, glucose, mannose and uronic acids are the major components of both the RSPs and CPSs.

The RSPs of most of the strains have been examined contain 0 to 9 different neutral monosaccharides, depending upon strain. Glucuronic and/or galacturonic acids are present in most cyanobacterial RPSs. Usually, glucose is the dominant monosaccharide of the RPS although uronic acid, xylose, arabinose, fucose , rhamnose and mannose are the dominant

monosaccharides in some cyanobacterial RPSs. Galactose is dominant sugar only in *Anabaena sphaerica* RPS. Ribose, osamine, methyl sugar and unidentified residues are also present in several cyanobacterial RPSs. In several Cyanobacteria RSPs contain protein also.

The cyanobacterial CSP consists of various neutral monosaccharides ranging from two to as many as nine depending on strain. In general, there is no noteworthy differences in the major monosaccharide compositions between the cyanobacterial RPSs and CPSs. But the composition of the slime polysaccharide of *Microcystis flos-aquae* resembles that of the plant polysaccharide pectin. The CSPs of 2 cyanobacteria also contain proteins. In most of the RSPs and CSPs the sulphate group is present which is rare in polysaccharides produced by eukaryotes.

Cyanobacterial RPSs and CPSs often contain one to three pentoses which are absent in most of polysaccharides from other prokaryote sources.

Potential biotechnological applications of the cyanobacterial polysaccharide:

As bioflocculant: The extracellular flocculants are known to be released from *Phormidium*, *Anabaena circinalis*. These flocculants are used in clarification of tap water, reduction of suspended solid matter in reservoirs.

As biopolymer: The cyanobacterial polysaccharide possess promising properties and potential for industrial exploitation as emulsifiers, stabilizers or thickening agents. The corboxylate groups present in most of the cyanobacterial exopolysaccharides might be used for linking to natural or synthetic polymers to generate new polysaccharides. Pectin like polysaccharides can be harvested from the *M. flos-aquae*.

As bioactive substances: Sulphated polysaccharides interfere with the absorption and penetration of viruses into host cells and inhibit various retroviral reverse transcriptase. These sulphated groups of polysaccharides possess promising potential applications in the pharmaceutical industry.

In removal and recovery of dissolved heavy metals: Some Cyanobacteria which produce large mounts of exopolysaccharides could be important in the removal and recovery of dissolved heavy metals. There are no significant differences in metal adsorption between living and dead cyanobacterial cell. The bio-absorption of metal ions by capsulated Cyanobacteria could be due

to mainly the presence of carboxyl groups and hydroxyl groups in the cyanobacterial CPSs. Mainly, Cu, Cd, Zn, Cr. And Pb are easily absorbed by the cyanobacterial sheath.

In adhesion: Cyanobacteria may be immobilized in columns for industrial processes due to their adhesion to a solid surface. The bioadhesive property of cyabobacterial exopolysaccharides is also of importance in creating novel associations between agronomically important plants and Nitrogen fixing cyanobacterial.

Ans. 8. Light stress management and Cyanobacteria:

1. Short term acclimation (State Transition): To accommodate the rapid fluctuations in light intensity or quality the photosynthetic apparatus can be modified within minutes. These short term modifications are referred as 'State Transition' and do not require protein synthesis. In many environment PAR is biased towards certain wavelength and LHC associated with PS II and PS I have different efficiencies in absorption of excitation energy. Illumination of cyanobacterial cell with ligh that preferentially absorbed by PBS results in excitation of PS II. This triggers rapid redistribution of energy that is transferred to PS I. This is called that cell are in State - II. While when illumination favours PS I excitation, causes relatively more of harvested light energy to be directed to PS II under these conditions the cells are said to be in State – I.

The state transition in Cyanobacteria require phosphorylation of components of LHC. According to 'Mobile Antenna' hypothesis PBS disconnect from the PS II and become physically associated with PS I. But, 'Spill over model suggests that excess excitation energy of PS II can spill over into reaction center of PS I in ill-defined manner. According to 'Detachment model' the excess excitation of PS II results in detachment of PBS, however, detached PBS does not necessarily reassociate with and donate energy to PS I. Some experiments suggest critical role of allophycocyanin in controlling and distribution of excitation energy between the PS.

2. Long term acclimation: In Cyanobacteria, the D1 protein has 2 distinct form which are encoded by a small psb-A multigene family. There are 3 psb-A genes viz. psb-A I, II, and III. The psb A I codes for form I of D1 protein while psb II and III genes code for form II of D1 protein. At low irradiance the transcription of psb-A I predominates if cells are transferred to high light intensity psb A II and psb A II transcripts accumulate. The D1 form II polypeptide

probably helps to protect the cells from photo-damage because D1 form II is not readily damaged in high light and form I does not turn over rapidly. But this form of D1-II is needed during initial exposure to high light and as the cells are acclimatized to the new light conditions.

3. Control of photosystem stoichiometry: Cyanobacteria have the mechanism to modulate PS I: PS II ratio in response to different wavelength of light which has helped to optimize photosynthetic efficiency. The changes in PS I: PS II ratio are mediated only by changes in amount of PS I present in cell while amount of PSII remains same. Under high white light intensity, both the PS are saturated and PS I: PS II ratio fall. The prolonged high light intensity may lead to decrease in total number of PS. It is suggested that redox state of electron transport system and especially cytochrome b6 is the signal that controls the level of PS I.

4. Control of phycobilisome biosynthesis by light quality: In Cyanobacteria the light quality and light intensity alter the PBS composition. The ability of Cyanobacteria to alter PC/PE composition (PC absorbs at red, PE in green) of PBS allows them to efficiently absorb the prevalent wavelength of light. Based upon the responses of Cyanobacteria to light quality, they have been divided in to 3 different groups-

Group 1 Cyanobacteria: can alter PBS size and number but do not markedly alter the absorbance characteristics of their PBS.

Group 2 Cyanobacteria: can alter level of PE in PBS

Group 3 Cyanobacteria can modulate both PE and PC level of PBS via process termed 'Complementary Chromatic Adaptation (CCA)'

These properties of Cyanobacteria not only protect their photosynthetic mahcinery against photo-oxidation but also enable them to be an efficient organism to harness the light energy in most efficient way.