Created with the support of the project "Innovation of teaching natural science subjects at CTU FBMI"

Microscopic identification of Gram-negative bacteria – cyanobacteria – blue-green algae

Aim, principles, theory

a) To learn about the cellular organization of Gram-negative bacteria - cyanobacteria

b) For selected cultures (from the collection of the Faculty of Science of Charles University) without knowledge of the cultures, make identification and classification, determine the shape, cell size and colour of cyanobacteria

c) Make your own collection from the natural environment, try to identify and taxonomically classify cyanobacteria, determine the shape, cell size

Cyanobacteria/Blue-green algae

Cyanobacteria are a unique group of Gram-negative bacteria - autotrophic prokaryotic organisms capable of photosynthesis. DNA in cyanobacterial cells is stored freely in the cytoplasm, ribosomes 70S, storage substances - cyanobacterial starch, volutin, some representatives have gas bladders (so-called aerotypes). Main photosynthetic pigment - chlorophyll a - thylakoids - photosynthesis with PS I and PS II. PS II - water photolysis - oxygen is released - oxygenic photosynthesis. Origin of oxygen atmosphere on Earth. Phycobilisomes - light-harvesting antennae. Some ability to fix airborne nitrogen - heterocytes (not heterocysts!). Production of secondary metabolites - cyanotoxins. More toxic than curare! Cyanobacteria contain photosynthetic pigments (chlorophyll a, blue phycocyanins and red phycoerythrins, collectively called phycobilins and carotenoids). Their relative proportion depends on light and nutrition and determines the resulting colour, which ranges from olive green, grey-blue, red-violet, dark green-blue and black. Cyanobacteria may have cells or colonies of cells surrounded by mucus, movement. Cyanobacteria contain potent toxins, cyanotoxins (e.g. microcystins), which are allergenic, neurotoxic and hepatotoxic to humans. Cyanobacteria are highly observable by optical microscopy. In some species of cyanobacteria we find specialised heterocytes - thick-walled cells that serve to fix airborne nitrogen. Another distinct type of large "cell" is the akinetes - the formation of one or more vegetative cells, larger than heterocytes. Akinetes serve to survive in adverse conditions, through the winter. Occurrence - only in some species of cyanobacteria. A special type of filament is the so-called hormogonium, n.s. hormogonia is a mobile mucus-connected filament of cells, which is produced by some cyanobacteria of the family Nostocaceae, by which they can reproduce. Water bloom refers to the conspicuous green colouration of waters caused by an overgrowth of microorganisms in the water, especially cyanobacteria. It is most common in late summer in August and September, but due to current climate changes it can be seen as early as June. The main representatives of cyanobacteria in the water bloom are the genera Microcystis, Aphanizomenon and Anabaena. The water bloom is associated with the so-called eutrophication of waters, the process of enriching waters with nutrients, mainly nitrogen and phosphorus.

Material, laboratory glassware and plastic, equipment and more:

cyanobacterial cultures, environmental sampling, pipettes, microscope slides, preparation kits, distilled water, minicentrifuge, eppendorf tubes, optical microscope, QuickPHOTO and cellSens microphotography software.

Procedure

a) Prepare microscopic slides from the submitted cultures (at least 5 cultures) from the collection (without knowledge of the specific culture), take microphotographs. For each slide, propose a taxonomic classification according to the characteristic features.

b) Prepare microscopic slides from our own specimens from natural sources (without knowledge of the specific culture) in which we find at least (5 species of cyanobacteria). If we are unsuccessful and do not find cyanobacteria in our own samples, we will obtain samples from natural sites with cyanobacteria.

c) Prepare wet mount and select and photograph selected fields of view. For each cyanobacterial species, we will propose a taxonomic classification according to the characteristic features.

d) Using a mini-centrifuge and centrifugation, we can carry out the preparation of sediment samples.

e) Using software cellSens, we will measure the cell sizes in the slides and compare the different species.

f) Record all data in a table.

Sample number	Determination of cyanobacteria	Cyanobacteria type (cocoon/fibrous)	Cyanobacteria Colour	Cell size [µm]

Results

In the protocol, in a table, we will describe what species and genera of cyanobacteria we have obtained and observed, what shape and colour the cyanobacterial cultures have, what cell size we have measured for each cyanobacterium. We will describe the procedure of identification of cyanobacteria, according to which key we determined.

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Discussion and Conclusion

Find out how the identification of cyanobacteria went for other students in the group, discuss and describe the conclusion.