BIOCHEMICAL AND MORPHOLOGICAL CHANGES IN SPIRULINA DURING SELENIUM NANOPARTICLE BIOSYNTHESIS

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Selenium is a trace element strictly necessary to ensure normal metabolism in the human body. Selenium deficiency in humans is associated with an increased risk of various diseases such as cancer, cardiovascular disease, type 2 diabetes mellitus, neurodegenerative diseases, and male reproductive disorders. The biological role of selenium is assigned to its antioxidant properties, which determine the protection of cells from oxidative lesions. In living cells, selenium is an essential component of selenoproteins through which it performs its function. There are currently 25 known selenoproteins, all of which are involved in metabolic processes aimed at preventing and fighting various diseases. The average daily recommended amounts of selenium for adults are about 55 μg. Athletes, pregnant women, nursing mothers, and people working in harmful conditions require higher doses of selenium. Selenium is predominantly present as selenomethionine in breadstuffs and grains, garlic, sea kale, olive oil, beer yeasts, legumes, olives, cocoa, pistachios, cashew, oat and buckwheat grain, and also in meat, seafood, milk, and dairy products. *Spirulina* biomass contains insignificant amount of selenium, 16.1 μg/g. At the same time, the biomass can be enriched with this element by the addition of various selenium compounds to the nutritive medium for cultivation of *Spirulina*. This parameter has increased 5–173 times and reached 2799 μg/g.

The major transformations of selenium by spirulina can be subdivided into three categories: oxidation-reduction, immobilization-mineralization, and methylation. The microbial reduction of oxidized, inorganic selenium compounds usually results in the incorporation into organic selenium compounds or, in some cases, the formation of well-defined nanoscale particles.

It was also found that the *Spirulina* biomass forms spherical selenium nanoparticles, which are localized extracellularly.

Biochemical analysis was used to assess the changes of spirulina biomass' main components (proteins, lipids, carbohydrates, and phycobilin) during nanoparticle formation. Since the spirulina biomass is rich in surface proteins, which interact with the selenite ions, the protein content was reduced by 32% in the first 24 h of the reaction, followed by a further protein reduction of 64% after 48 h. After 72 h of contact, the protein content in the biomass was 20 % of the original. Besides protein, the decrease of phycobiliprotein content in the spirulina biomass was observed. After 24 h of contact with the selenium ions, the phycobiliprotein content in spirulina biomass decreased from 6.9% to 2.4% for phycocyanin, and from 4.5% to 2% for allophycocyanin. The content of the polysaccharide decreased by 50%, and of lipids - by 23% during first 24 hours of reaction.

Selenium nanoparticles biosynthesis as result of spirulina exposure to inorganic selenium led to ultrastructural changes of the cells. Micrographs of control cells showed an intact cell wall, very thin capsule, compact thilakoids presented by a large number of dense lamellae, a large number of carboxysomes. Under the nanoparticles biosynthesis, the cell wall becomes diffuse. The contact with inorganic selenium and biosynthesis of nanoparticles produce a partial degradation of thilakoids. In spirulina cells with nanoparticles carboxysomes are missing. In normal cells carboxysomes contain the enzyme ribulose 1.5-diphosphate carboxylase/oxidase (RuBisCO), responsible for carbon dioxide fixation in spirulina. The absence of these inclusion bodies indicates on lower efficiency of carbon fixation. The interaction of spirulina cells with inorganic selenium caused a pronounced vacuolization of the cytoplasm. Also, the accumulation of nanoparticles leads to the release of a larger amount of extracellular polymers, which form a more pronounced layer of exopolysaccharides, damages of the cytoplasmic membrane and cell wall.