

Cyanobacteria are unicellular microorganisms capable of photosynthesis that are mostly negatively recognised for their vigorous blooms in the water bodies. In addition to their harmful properties such as eutrophication and production of toxins, cyanobacteria also provide numerous benefits to the environment and human health. For example, cyanobacteria capable of nitrogen fixation can enhance rice productivity by 20%, whilst other strains are valuable sources of nutraceuticals among them phycobiliproteins (PBP). These protein structures participate in photosynthesis as antenna pigments that harvest wavelengths within 470-650 nm, thus complementing chlorophylls that cannot absorb in this range. Phycobilisomes contain phycobiliproteins, the only photosynthetic pigments that are water soluble. Among phycobiliproteins we can distinguish phycocyanin (blue) and phycoerythrin (red) which are used as food dyes, pigments in cosmetics, and as fluorescent reagents in clinical or research laboratories. Furthermore, C- phycocyanin (C-PC) plays an important role in coping with anemia, liver disease and is regarded as a nutraceutical which strengthens the immune system. There are also reports on its anticancer properties – interestingly it is toxic to cancer cells without exposing any toxicity to normal cells. Phycocyanins have numerous advantages as a target product from cyanobacteria most notably high price and abundance in naturally occurring organisms, but their efficient biosynthesis, and downstream processing: recovery from biomass, effective isolation remains a major hindrance. First, their isolation is problematic and suffers from low recoveries, and numerous protein contaminants, which may disqualify it from most high-end applications like biosensors or fluorescent labels. Secondly, lower purity phycocyanins like those currently used in food industry suffer from limited stability and need to be stabilized by additional agents that contribute to their final cost. This feature significantly limits their application in many food industries as their utilization is largely limited to the products that are not extensively processed thermally like ice-creams, chewing gums or sweets as such treatment results in their thermal decomposition and color fading due to the heat. An intrinsically stable phycocyanins from thermophilic organisms would have an advantage over currently used pigments and could have expanded utilization in food industry. Highly stable phycocyanins can then have significant advantages when compared to proteins from mesophilic organisms. Recently our research group characterized C-phycocyanin from a thermophilic cyanobacterium *Thermosynechococcus* 6715. The C-phycocyanin (C-PC) produced by this strain is one of the most thermostable proteins of this class reported to date, what makes it a very desired product. Thus, biosynthesis, isolation and purification of C-PC is the main goal of this research. Biosynthesis of C-PC requires optimization of numerous factors such as photobioreactor type, its hydrodynamic parameters, light regimes as well as selection of optimal carbon (also gaseous CO<sub>2</sub>) and nitrogen sources. The produced biomass must then be harvested and disintegrated to release the valuable phycobiliproteins that are bound to the thylakoid membranes of the photosynthetic apparatus. One of the methods of disintegration is homogenization that releases the cell components into the extraction medium. There are several other methods to achieve this goal (freeze-thaw cycles, milling, osmotic stress, detergent and organic solvent lysis, sonication etc.). The next step towards obtaining the pure bioproducts is their separation, extraction and purification. Selection of the purification method for these natural products is very important. Extensive research needs to be done that will determine characteristics of the purification process that are applicable for the bioproduct. Our goal is development of an integrated sequence of unit operations of DSP such as foam fractionation (FF), aqueous two-phase extraction (ATPE) and ATPE integrated with ultrafiltration (in one unit). The highest purification yield is achieved by chromatographic techniques that are expensive and time consuming. Therefore, the number of unit operations related to C-PC purification increases resulting in expensive final products and on each step of the process is associated with recovery losses. The reduction of unit operations is our main goal and can be achieved through process integration. For example, one of the innovative approaches is integration of membrane filtration (ultrafiltration) with aqueous two-phase extraction made of salt phase eg. sodium citrate and polymer phase composed of appropriately selected degree of polymerization. So far, PEG-inorganic salt systems have been used for the separation of C-PC by the ATPS method, which in large scale could bring a large load of pollutants to the environment. There are a few factors distinguishing our proposal, first of all, subject of our research is the thermophilic strain *Thermosynechococcus* 6715 and produced by it C-PC, which according to our previous study is one of the most thermostable once reported up to now. Next, growth optimization combined with insight into C-PC production as well as detailed study on the strain performance in two types of photobioreactors. Further, elaboration of procedure of C-PC isolation from cells and crude extract purification. According to our knowledge purification methods such as foam fractionation and ATPE combined with ultrafiltration (membrane extraction) have not been used for separation of C-PC.

Research results will expand the basic knowledge about phycocyanin biosynthesis, extraction, and purification in the environment-friendly way - thanks to the cyanobacteria ability to use CO<sub>2</sub> as a carbon source, oxygen production and through the use of biodegradable extractants. This can have a significant impact on the production and further use in the near future of this nutraceutical, not only as a thermostable food dye.