SCHEMA REDAZIONE PROGETTO DI RICERCA

CONCORSO PER L'ASSEGNAZIONE DI BORSE DI STUDIO DI DOTTORATO DI RICERCA

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Tematica vincolata Production of a protein-based biopolymer, through plant biotechnologies for the synthesis of environmentally friendly bioplastics.

PASQUALE CREANZA

Development of a protein-based edible biopolymer

a) state of the art:

Traditional polymers are a threat to the terrestrial ecosystem, a possible solution to this problem could be their replacement with environmentally friendly biopolymers. Unfortunately, the vast majority of biopolymers currently used show poorly performing chemical and physical characteristics. Protein-based biopolymers demonstrate a wide range of very interesting mechanical properties in many fields, ranging from medical to packaging to drug delivery.
The study of potentially usable biopolymers for this type of technology is constantly evolving. The idea behind this project is to use an edible protein (called phaseolin: Phs) derived from common bean seeds (Phaseolus Vulgaris), as a building block to produce a biopolymer that can be used in the food packaging industry. Normally, Phs are assembled as a homotrimer in the plant storage vacuole. Recently, we have genetically engineered the sequence of Phs in order to increase the degree of polymerization by inserting a cysteine residue at its C- terminal end (called Phs*).

b) research objectives:

The research objective is the production of biodegradable biopolymers using a vegetable protein (phaseolin) modified with genetic engineering techniques. The insertion of a cysteine residue in the amino acid chain of phaseolin should stimulate the polymerization of the monomers of the protein itself and at the same time increase its nutritional value by introducing an essential amino acid not present in its natural sequence. The synthesis and accumulation of this putative biopolymer will be attempted both in plant organisms within the chloroplasts, and in *e. coli* and the two systems will be compared.

c) theoretical and methodological framework:

The production of both transplastomic tobacco plants expressing the modified phaseolin gene called PHSL* and E. coli bacteria modified with the same gene is foreseen.
Western blot analyzes will be performed, using anti-phaseolin antibodies to select the plants and bacteria that accumulate the greatest amount of Phs*.
The selected plants will be grown under hydroponic conditions and in complete isolation inside growth chambers at constant temperature and humidity.
Chloroplasts containing PHSL* will be extracted and purified from transgenic tobacco leaves and then homogenated for subsequent extraction of the polymeric fraction.
The extraction and purification of PHSL* from bacteria will instead be carried out with the collaboration of an external operating unit with expertise in the biotechnology of prokaryotic organisms.

There is also the possibility of adding bioactive compounds extracted from vegetable matrices at various concentrations to the obtained polymeric matrix to improve the characteristics of the final product.
Subsequently, the purified PHSL* obtained will be solubilized in a suitable solution, then different parameters will be tested to induce an increase in the polymerization, for example, different redox systems, temperature, pH, and ionic strength of the solution.
The addition of additives such as detergents, osmolytes, small molecules, and cosolvents will be considered to identify the most effective cure enhancer. The potential use of less common oxidation strategies such as polymer-supported disulfide reagents (Clear-Ox™, tris(2-mercapto-acetamido ethyl)amine, or styrene-methacrylate beads) can be investigated.

d) research design:

The idea developed in this work is to use a plant storage protein, called phaseolin, to produce biopolymers.
Recent studies have shown that a genetically engineered phaseolin (Phs*), carrying a cysteine residue at its C-terminal end, can form interchain disulfide bridges and this bond is favored when the protein is expressed in an oxidizing compartment, such as the plant chloroplast.
The purpose is to use two expressing hosts for the production of Phs*:

1. plant model organisms (*Nicotiana Tabacum*), to produce polymeric Phs* inside the chloroplast. To this end, previously developed transgenic plants.
2. involve the production of Phs* as an N-terminal His-tagged monomeric protein in the cytoplasm of *Escherichia coli,* which is a well-known reducing environment.

e) expected results

This research project aims to develop biotechnology to produce eco-friendly bioplastics.
The project has translational potentiality since it aims at introducing an eco-sustainable approach to producing bioplastics and therefore reducing the impact of plastics pollution in the terrestrial ecosystem.
The new biopolymer is likely to generate substantial socio-economic benefits in terms of reduced external costs arising from the management of plastic materials as waste.
Moreover, the successful development of the biopolymer is expected to generate substantial private benefits in terms of market diffusion of the new product.

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