32° PhD CYCLE IN BIOTECHNOLOGY

PROPOSED TOPICS

SUPERVISOR: BARTOLUCCI

DEVELOPMENT OF BIOSENSORS FOR MONITORING OF ENVIRONMENTAL POLLUTANTS.

The project will be organized on the construction of whole-cell or enzyme-based biosensor for the detection of heavy metal pollution in order to screen for the presence of pollutants in different environments. To this purpose:

- Thermophilic microorganisms and their enzymes/proteins will be used as model systems
- Microorganism/s will be isolated from geothermal areas and adapted to grow in the presence of pollutants.
- Microbial communities spreading in different geothermal environments will be characterized.
- The characterization at molecular level of the defense strategies against environmental pollutants will be performed.
- Genetic systems for thermophilic microorganisms will be developed and optimized.
- Enzyme immobilization on different supports will be evaluated and characterized.

SUPERVISOR: GIARDINA/DE STEFANO

DESIGN, FABRICATION AND CHARACTERIZATION OF NANO STRUCTURED HYBRID BIO/NON-BIO INTERFACES FOR BIOMOLECULAR INTERACTIONS STUDY AND INDUSTRIAL APPLICATIONS.

Hybrid nano composites are made of a biological element, such as protein, enzyme, peptide, nucleic acid and so on, and a nano structured material which can be a simple physical support or exploits specific features due to its nature, organic or inorganic. The key point in design and realize these hybrid devices is the interface between the biological entity and the material surface. The activity of the biological component can be tuned to specific requirements by properly set the bio/non-bio interface, using chemical or physical treatment to functionalize the material surface. In this PhD research program, different support materials, with nano structured morphology, will be considered, namely metals, semiconductors and polymers in order to produce innovative devices for application in social interest fields like environmental monitoring, water purification and healthcare.

SUPERVISOR: PICCOLI

NOVEL HUMAN HOST DEFENSE PEPTIDES (HDPS) FOR BIOTECHNOLOGICAL APPLICATIONS.

The project is aimed at the development of new HDPs as potential bio-agents. Two novel HDPs, identified within specific sequence regions of a human protein (a polipo protein B) by a bioinformatic tool, will be produced through the development of a cost-effective fermentative strategy, and will be analyzed for their antimicrobial, anti-biofilm and anti-inflammatory activities. Specific nano particles and hydro gels will be tested as suitable vehicles for peptide assembly.

SUPERVISOR: PORTA/MARINIELLO

HYDROCOLLOID-BASED MATERIALS NANO-REINFORCED AND ENZYMATICALLY CROSSLINKED TO BE USED AS DELIVERY-SYSTEMS IN DIFFERENT INDUSTRIAL SECTORS

Hydrocolloids are natural polymers that have the characteristics to be both edible and biodegradable. Their use is recommended to obtain human and environmental friendly materials that could be used in several productive sectors. For example, in the food sector to prepare envelopes that convey antimicrobial or antioxidant molecules to extend the products half-life, but also in the pharmacological field to deliver molecules at the enteric level. Our group has been studying edible films since 2003 using mostly proteins and polysaccharides as hydrocolloids with and without the aim of the enzyme transglutaminase that catalyzes the formation of iso-peptide bounds among endo-glutamine and endo-lysine residues. The candidate will be dedicated to : i) prepare new materials made of an hydrocolloid matrix reinforced with

nanoclays and cross linked via transglutaminase; ii) investigate new material performances in terms of mechanical and barrier properties together with solubility and digestibility; iii) identify the best materials as delivery systems to convey molecules with special characteristics (antioxidants, drugs, antimicrobials, etc.).

SUPERVISOR: CAPPARELLI

BACTERIOPHAGE ENGINEERING FOR INDUSTRIAL APPLICATION.

Xylella fastidiosa is a source of important new diseases. The serious damage that is able to provide, make the bacterium of significative economic interest because it can attack and destroy important plants of agricultural interest. In agriculture, the use of bacteriophages (or phages) to control bacterial infections is becoming more widespread because these are eco-friendly and not harmful for the bacteria present in the soil. The research activity - given the importance of the Xylella fastidiosa - is focused on the development of a tool, able to recognize different *Xylella fastidiosa* strains. This objective could be achieved by the production of a bacteriophage that could be engineered, using CRISPR-Cas-Mediated genome engineering, or transported to the sites of infection by some molecules such as biomimetic hydroxyapatite.

SUPERVISOR: PICCIALLI

DNA G-QUADRUPLEXES: FROM NUCLEIC ACID INVOLEVED IN GENE CONTROL EXPRESSION TO HIGHLY ORDERED SUPRAMOLECULAR STRUCTURES

G-quadruplexes are unusual DNA secondary structures found in guanine rich oligonucleotide sequences having a natural propensity to self-associate in coplanar arrays of four guanines, known as G-tetrads. G-quadruplex structures have drawn the attention of researchers in medicinal chemistry, and more recently in supra molecular chemistry, and nanotechnology. It has been demonstrated that G-Quadruplex structures are present in the some tracts of the genome (for example in telomers and in several oncogenes) and that their stabilization could be a useful approach to control gene expression. Analogously, some G-Quadruplex structures have been selected as aptamers, i.e. molecular structures able to bind strongly and selectively a specific protein. Furthermore, G-quadruplexes can be used to build supramolecular structures. The overall G-quadruplex scaffold can exhibit several morphologies through the intramolecular or intermolecular organization of G-rich oligonucleotide strands. In particular, several G-rich strands can form higher-order supramolecular assemblies (nano-architectures) by the multimerization of G-quadruplex units. This research topic is centred on the production and on the study of new kind of G-Quadruplexes that could be useful to propose new efficient gene control strategies or to produce new supramolecular architectures based on the G-quadruplex scaffold.

SUPERVISORS: MONTI/OLIVIERI

MICROALGAE AS FACTORY FOR HIGH VALUE BIOPRODUCTS

Microalgae may be a continuous and eco sustainable resource of natural products, including pigments as antioxidants, and both polyunsaturated fatty acids (PUFAs) and proteins as food additives. The specific objectives of the PhD are: 1) screening and selection of potential micro algal species, 2) selection of optimal cultivation conditions, 3) antioxidant activity ad PUFA content characterization,4) development of a high efficient, biocompatible, and low cost processes for bio product extraction/purification according to the bio refinery concept.

SUPERVISOR: MARZOCCHELLA

PROCESS DEVELOPMENT FOR POSTBIOTIC FUNCTIONAL FOODS FOR THE DIET OF PEOPLE CHARACTERIZED BY HIGH PATHOLOGICAL VULNERABILITY

The aim of the research is to develop a process for a new category of functional food and/or functional ingredients to be used in the diet of people characterized by a high pathological vulnerability (e.g. infant, pregnant women and elderly).This new category of foods - also known as functional post biotics - can lead to a positive effect on human health, preventing diseases by increasing the immune response of the host. Post biotics are composed by “dead microorganisms” and by metabolites produced during the fermentation of selected substrates. Stability of post biotics may be enhanced by pasteurizing the fermented products by a mild thermal process to preserve functional metabolites from thermal degradation. Recent scientific evidences pointed out that these kinds of functional foods are a relevant

option for probiotics feeding, a category of food/ingredients that may induce a negative response in the host mainly due to live microorganisms. The main steps of the project will be focused in the election/characterization/optimization of the process to maximize the post biotic activity of functional foods. Process development for postbiotic functional foods for the diet of people characterized by high pathological vulnerability The aim of the research is to develop a process for a new category of functional food and/or functional ingredients to be used in the diet of people characterized by a high pathological vulnerability (e.g. infant, pregnant women and elderly).This new category of foods - also known as functional postbiotics - can lead to a positive effect on human health, preventing diseases by increasing the immune response of the host. Postbiotics are composed by “dead microorganisms” and by metabolites produced during the fermentation of selected substrates. Stability of post biotics may be enhanced by pasteurizing the fermented products by a mild thermal process to preserve functional metabolites from thermal degradation. Recent scientific evidences pointed out that these kinds of functional foods are a relevant option for probiotics feeding, a category of food/ingredients that may induce a negative response in the host mainly due to live microorganisms. The main steps of the project will be focused in the selection/characterization/optimization of the process to maximize the postbiotic activity of functional foods.

SUPERVISORS: SANNIA/VELOTTA/DIRK MAYER

QUARTZ-CRYSTAL MICROBALANCE (QCM) AND ELECTROCHEMICAL IMMUNE SENSORS FUNCTIONALIZED BY PHOTOCHEMICAL TECHNIQUE FOR FOOD SAFETY.

The surface functionalization is one of the fundamental step in the design and realization of a biosensor since any protocol must warrant the specificity of the sensor against the contaminant to be detected without affecting the inherent sensitivity of the platform. Recently, at the University of Naples Federico II (Department of Physics) a simple photochemical technique(Photonic Immobilization Technique, PIT)has been proposed as a valuable method to steer antibodies on gold surfaces so that at least one of their variable residue is oriented upright and, hence, exposed to the solution. This achievement has paved the way to a number of applications of QCM-based immunosensors aimed at the detection of contaminants in real food[R. Funari, et al. Anal. Chem. 85, 6392-6397 (2013) and R. Funari, et al. Biosens Bioelectron 67, 224-229 (2015)]. Since such a technique only requires that the sensor surface is thiol sensitive (whence the need for gold), we anticipate its effective application even to electrochemical sensors, the latter platform offering the important advantage of being miniature is able.

Industrial partner: **Promete SRL** (Napoli). This company is a CNR spinoff and has been active in the technological transfer from academy to industries for 20 years, thus its business consists of bringing the results of the research to market. In this project Promete will make available the expertise gained in last years on numerical approaches for modeling real systems to the optimization of the fluidic circuit of the immunosensor. Moreover, the company is also committed to bringing to the market the device we are going to realize.

Foreign partner: **Juelich Forshungzentrum** (Germany). This institute hosts a number of facilities (e.g. the Helmholtz Nanoelectronics Facility) and a research group on Bioelectronics headed by dr Dirk Mayer. The collaboration between the bioelectronics group and the group of prof. Velotta has recently led to the first images of the PIT effects on the antibody interaction with gold surface[R. Funari, et al Langmuir 32, 8084-8091 (2016)]. In this project we aim at the application of the PIT to the microelectrode arrays realized at Juelich by means of a printer with unique performances for multiplexing applications.