MSc in Biochemistry for Health

Dissertation Project – 2nd Cycle

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TITLE: *Deciphering glycan-recognition systems of the Human Microbiome*

BACKGROUND

The human gut microbiota is a highly carbohydrate-active community with a broad capacity to metabolize dietary and host-derived glycans, which is essential to human nutritional balance, microbial-microbial and microbial-host cross-talk and immune system modulation. Thus, understanding carbohydrate recognition by the gut microbiota is of utmost importance for human health and nutrition.

Typically isolated gut strains exhibit a high number of gene clusters termed polysaccharide utilization loci (PULs) that respond and orchestrate the recognition and degradation of a specific glycan, allowing the bacteria to cope with the nutrient fluctuation. Intensive research on PULs characterization has shed light into their complex architecture, which includes modular carbohydrate-active enzymes with associated carbohydrate-binding modules (CBMs), starch-utilization system (Sus)-like proteins and surface glycan binding proteins (SGBPs). The number of newly identified PULs using genomic and transcriptomic analysis and bioinformatic prediction is growing fast and many of these proteins await elucidation and assignment of a carbohydrate-binding function.

We are currently investigating these complex glycan-recognition systems using an interdisciplinary approach combining bioinformatics tools, high-throughput molecular cloning and protein expression, glycan microarrays and protein X-Ray crystallography.

OBJECTIVES

The overall aim of the proposed project is to shed light over microbial PUL systems responsible for the degradation of diverse dietary and host glycans, in the human gut. It will be mainly focused on putative carbohydrate-binding proteins in PULs from strains of the Bacteroides genus, representative of the human gut microbiota – *B. thetaiotaomicron* and *B. ovatus*.

The project will allow the screening and identification of new protein binding specificities using glycan microarrays. Selected protein:glycan interactions will be further analysed and structurally characterized using X-ray crystallography. This integrative approach will contribute to functional analysis of PULs carbohydrate-binding proteins as well as to a broad understanding of the human microbiota metabolic capabilities.

PROJECT DESCRIPTION

Task 1: Protein expression and purification

Production of soluble recombinant protein targets selected from the protein library that includes CBMs, SusD-like proteins and SGBPs for their structure elucidation and biochemical and functional characterization. The selected proteins will be cloned in the pET system with histidine tags. Expression tests will be performed varying the *E. coli* strain, temperature, type of expression induction (auto- and IPTG) and time of induction. These clones will be then expressed in small scale cultures in order to have enough protein to be tested in the microarrays. The proteins will be purified using immobilized metal affinity chromatography (IMAC).

Task 2: Assessment of the glycan ligand specificities.

High-throughput screening analysis of the expressed proteins will be performed using state-of-the-art carbohydrate-microarray technology. The carbohydrate microarrays will comprise a diverse range of fungal-, plant- , bacterial- or mammalian-derived glycan probes. Binding to the glycan ligands immobilized into the microarray slides will be detected using a fluorescence immuno- and biotin-streptavidin detection systems.

Task 3: Structural studies and biophysical characterization

Here, two protein-ligand complexes will be selected with the aim to resolving the 3D-structure by X-Ray crystallography. The proteins of interest will be expressed in a larger scale for biochemical studies and preliminary crystallization and co-crystallization trials will be performed. The interaction of these proteins with their glycan ligands will be characterized using biophysical methods such as isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR).

It is intended that the student will receive training in different but complementary state-of-the-art methodologies in areas of Glycobiology, Structural and Molecular biology. The project is within the scope of funded research projects by the Portuguese Foundation for Science and Technology.

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	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Thesis										