MSc Thesis Project Proposal

Dissertation Project

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Lab/Institution: Proteomics of non-model organisms - ITQB

TITLE: Desvendando o mecanismo de ação das nanopartículas de quitosano no combate ao patógeno alimentar *Listeria monocytogenes* - uma abordagem proteômica

Unravelling the mechanism of action of chitosan nanoparticles to combat the foodborne microbial pathogen *Listeria monocytogenes* - a Proteomic approach

RESUMO

In recent years, gastroenteritis cases resulting from *L. monocytogenes* infection have been reported [1]. This pathogen is responsible for a rare but fatal systemic disease called listeriosis, affecting primarily immune suppressed populations: pregnant women, infants, AIDS and oncologic patients, and organ transplant recipients. Among pathogenic bacteria, *L. monocytogenes* has become one of the most attractive model organisms for intracellular infection, ranks amongst the best-known invasive bacteria, being able to cross the intestinal, blood-brain and feto-placental barriers causing a disease known as listeriosis. The mortality rate is the highest of all foodborne pathogens, usually 20–30%. In addition to major health concerns, listeriosis outbreaks are associated with a severe economic burden related to food security, reaching an impressive 2 billion dollars per year in USA. Strategies to fight this infection are an imperious need in medical microbiology [2].

The known antimicrobial effects of chitosan nanoparticles (CS-NP) and their GRAS (Generally Recognized as Safe) status make them a promising adjuvant to act as a natural food additive in conservation to prevent the foodborne microbial pathogens contamination. In this research proposal we aim in the present study to depict the molecular effects implicated in the interaction of CS-NP against *L. monocytogenes* using omics approaches, in order to elucidate their antimicrobial mechanisms of action.

The differential *L. monocytogenes* proteomes sensitized to either CS-NP will be investigated through the combination of an integral proteome and bioinformatic analysis. A protein crude extract will be prepared from the *L. monocytogenes* sensitized by CS-NP, in four biological replicates. The quantitative proteomic analysis will be performed by micro-liquid chromatography–mass spectrometry (micro-LC-MS/MS), and the proteins with differential abundances will be identified by multivariate statistical analyses [3]. The differential protein expression signatures in *L. monocytogenes* exposed to the CS-NP will be analyzed by STRING in order to propose a putative molecular mechanism for CS-NP action in *L. monocytogenes* cells. By this approach is expected the identification of differentially expressed proteins in *L. monocytogenes* sensitized with CS-NP, and identification of its metabolic pathways affected by the nanoparticle's exposition. Understanding CS-antibacterial molecular effects can be valuable to optimize the use of CS-based nanomaterials in food decontamination and may represent a breakthrough on CS nanocapsules-drug delivery devices for novel antibiotics. This work plan offers the student the opportunity to work with a wide set of techniques, in the fields of Microbiology and Proteomics and aims to train the candidate to reach full autonomy and expertise in the analysis of scientific data through a multidisciplinary approach.

[1] Autoridade de segurança alimentar e económica (Portugal) https://www.asae.gov.pt/seguranca-alimentar/riscos-biologicos/listeria-monocytogenes.aspx Acessed: 06/05/2020

[2] (2008) Listeria monocytogenes. In: Foodborne Microbial Pathogens. Springer, New York, NY. https://doi.org/10.1007/978-0-387-74537-4_9

[3] (2020) Proteomic Analyses Reveal New Insights on the Antimicrobial Mechanisms of Chitosan Biopolymers and Their Nanosized Particles against *Escherichia coli*. International Journal of Molecular Sciences. https://doi.org/10.3390/ijms21010225

CALENDARIZAÇÃO

Month	1	2	3	4	5	6	7	8	9	10
Task 1 – Nanoparticle characterization	■	■								
Task 2 – Challenge *of L. monocytogenes* to nanoparticles		■	■	■						
Task 3 - Proteins extraction and Trypsin Digestion				■	■	■				
Task 4 - LC-MSMS analysis							■	■		
Task 5 - Differential proteomic analysis and writing of the dissertation								■	■	■

REGIME PREVISTO

Tempo integral

MÉTODO DE SELECÇÃO

Avaliação curricular e entrevista