Dissertation Project – 2nd Cycle

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TITLE: Unraveling phenolics with potential therapeutic application for neurodegeneration

BACKGROUND

Plants synthesize a staggering variety of secondary metabolites whose biodiversity provides a pristine pool of high-value compounds with potential application to human health. One of the largest classes of such phytochemicals are the phenolic compounds, which are present in high contents, great diversity and unique profiles in berry fruits. Indeed, the health benefits of these plant metabolites as well as their functional properties towards the prevention of many disorders, including neurodegenerative diseases and the associated chronic inflammatory processes, have been extensively described. Thus, it becomes imperative the identification of new bioactivities and the characterization of the molecular mechanisms underlying cellular protection. After the bioprospection of a worldwide berry germplasm using a yeast SMART platform, the extract from *Rubus genevieri* was identified as a potent protectant against pathological processes associated to Amyotrophic lateral sclerosis (ALS) whereas *Rubus idaeus* var Prestige displayed protective effects for Huntington's disease (HD) and inflammation. Extract fractionation and re-testing identified the fractions and potential compounds conferring protective activities. It remains to elucidate the molecular mechanisms behind the protective potential of these

OBJECTIVES

The main aim of this project is to investigate the molecular mechanisms underlying protection mediated by polyphenols using yeast and mammalian cellular models of these diseases.

The specific objectives are:

- To identify the phenolics conferring protective activities in the yeast models of ALS, HD and inflammation;
- To characterize the underlined molecular mechanisms of protection;
- To validate the results in mammalian cellular models of neurodegeneration and inflammation.

PROJECT DESCRIPTION

The present project is aligned with the aim of European project FP7-KBBE BacHBerry and will involve collaborative work with the consortium partners.

Task 1: Identification of protective phenolics from *Rubus genevieri* and *Rubus idaeus* var Prestige extracts

Potential compounds relieving proteotoxicity of FUS (FUsed in Sarcoma), whose aggregation is a pathological hallmark of ALS, will be identified using a yeast model of ALS encoding human FUS. Protective activities will be inferred by growth curve analysis and extrapolation of parameters such as lag phase, doubling time and final biomass of the cultures. A similar procedure will be used to identify compounds with protective activities towards pathological processes of HD, which include aggregation and toxicity of poly-glutamin (polyQ) expanded huntingtin. The yeast model for these analysis express the polyQ103-HTT-GFP fusion. In both cases, the models recapitulate the molecular aspects of the diseases. Identification of phenolics potentially attenuating inflammatory processes will be performed by the measurement of beta-galactosidase activity, using a reporter strain encoding the *lacZ* gene under the control of a promoter containing Crz1 recognition *cis*-elements. Crz1 is the yeast orthologue of NFAT, which controls pro-inflammatory responses in mammals, and shares with this transcription factor conserved mechanisms of activation.

Task 2: Investigation of the molecular mechanisms underlying protection by *Rubus* phenolics

Once identified the compounds displaying protective activities, the next step will be the investigation of the molecular mechanisms associated to each specific response. The ALS model will be subjected to a dose-response analysis to establish the protective concentration range of *R. genevieri* phenolics. An additional strain, encoding a GFP-FUS chimera, will be used to evaluate how they affect protein levels, clearance, subcellular localization and aggregation by means of immunoblotting, fluorescent microscopy and flow cytometry. Similar approaches will be performed to evaluate the activity of *R. idaeus* var Prestige phenolics towards polyQ103-HTT pathological processes using the GFP fusion described in Task 1. Also, a dose-response analysis will be done to establish the concentration range in which phenolics from the same matrix control Crz1 activation by enzymatic activity assays. These data will be strengthened by qRT-PCR to monitor transcriptional activation of endogenous Crz1 target genes such as *PMR1* and *PMC1*. A strain encoding *CRZ1*-GFP driven by the native *CRZ1* promoter will allow following Crz1 nuclear translocation upon stimulation and the influence of identified *Rubus* phenolics in this process.

Task 3: Validation of key results in mammalian cell models of neurodegeneration and inflammation

Final validation of previously identified compounds will be done in mammalian cell models. In particular neuroprotection will be evaluated in a human neuroblastoma cell injured with an oxidant agent by flow cytometry. Molecular targets of the identified compounds (Task 2) will be approached in the human cell model either by immunological or qRT-PCR techniques. A N9 murine microglia cell line will be used as a neuroinflammation cell model. The potential of identified phenolics for attenuating neuroinflammatory process in ATP-stimulated cells will be evaluated as regard calcineurin activity, NFAT phosphorylation status and subcellular compartmentalization using enzymatic assays, immunoblotting and fluorescence microscopy, respectively.

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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										