PiSolAdapt

Pisolithus croceorrhizus found in scrubby shrub moist soils (left) with small fruiting bodies (right). Photo credits: Jonathan Plett and Teresa Lebel.

Protein synthesis to cell detoxification: could diversification of eEF1Bγ roles in ectomycorrhizal fungi enable adaptation to environmental stress?

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Thematic actions concerned : WP1/WP2

Context —

Protein translation is a central process that governs the ability of an organism to develop. This process is highly controlled, mainly at the initiation and at the elongation steps. In fungi, the elongation complex eEF1 is composed of the guanidine nucleotide binding subunit eEF1A and eEF1B nucleotide exchange factor (itself a complex of eEF1Bα and eEF1Bγ subunits). While most of fungi exhibit only one or two eEF1Bγ isoforms, we showed that the ectomycorrhizal Pisolithus species exhibit an expansion of the eEF1Bγ-coding genes copy number, in particular species collected in highly disturbed environments in terms of temperature, nutrient levels and heavy metal concentrations.

The eEF1Bγ subunit is atypical in that it displays a glutathione transferase (GST) domain. The main described function of eEF1Bγ is to ensure the proper scaffolding of the different subunits in the eEF1 complex due to the GST domain which favors the protein-protein interaction in this multiprotein complex. However, GSTs are active detoxifying enzymes. Due to the presence of this GST domain in the elongation factor eEF1Bγ, we hypothesize that the function of eEF1Bγ in cells goes beyond the stabilization of the elongation complex, having a role in stress response. The gene duplication observed for fungi of extreme environment could be linked to their ability to resist stress and adapt to environmental constraints.

Objectives —

The main objective of this proposal is to define whether the duplicated isoforms still display a primary role in protein translation or whether they functionally diverged towards stress rescue. To do so, we propose to study some selected eEF1Bγ isoforms in both the process of protein translation and cell detoxification to evaluate the role of these enzymes in the resilience of the ectomycorrhizal Pisolithus species to environment constraints.

Approaches —
This project combines comparative genomics, and biochemical and physiological approaches.

1- Comparative genomics: the first step is a deep analysis of Pisolithus genomes that are available in the Joint Genome Institute database, to compare the eEF1Bγ sequences, correct some sequences based on RNASeq data that are available for some of the species, and locate the duplications in the genomes.

2- Biochemical characterization of eEF1Bγ proteins: The second step is to clone some selected eEF1Bγ genes, produce and purify the recombinant proteins and characterize them for their ability to interact with the other subunit eEF1Bα of the elongation complex, and for their ability to display glutathione transferase features.

3- Physiological approaches: This last part consists in analysing how the selected eEF1Bγ participate in the stress response *in vivo* by following their gene expression in various stress conditions.

Key results —

1- eEF1Bγ gene annotations are mostly incorrect especially for the genomes of the species collected in disturbed environment. Thus, we had first to correct the sequences and the annotations to establish a trustable list of all eEF1Bγ. We did the same analysis with all the GST from the other classes for 7 Pisolithus genomes. We found that the duplicated eEF1Bγ genes are either under their full-length form and/or a shorter form devoid of the elongation factor domain and thus constituted of only the GST domain.

2- Nucleic acid extraction is not an easy task in the environmental Pisolithus species collected in extreme environment. We manage to optimize protocols to obtain RNA and genomic DNA for some of the strains.

3- In parallel, to be able to compare the biochemical properties of the Pisolithus eEF1Bγ isoforms with the eEF1Bγ from a fungal model, we produced, purified and characterized the two full eEF1Bγ isoforms TEF3 and TEF4 (either full or truncated for their elongation factor domain) and the single YGR201C short isoform of the lab strain *Saccharomyces cerevisiae.* The obtained results show that all the proteins are active as GST and only the short isoform does not interact with eEF1Bα.

Main conclusions including key points of discussion —
The genomic analysis took more time than expected but was crucial to correct annotations and thus unable us to work with trustable sequences. In parallel to this analysis, we finished the biochemical characterization of all the eEF1Bγ from *S. cerevisiae* that will serve as a reference to compare the properties of the eEF1Bγ from the Pisolithus species.

Perspectives —
The next steps of the work are the production of the selected eEF1Bγ of Pisolithus for characterization. We are also waiting for a response from the PHC FASIC program for a grant mobility to Australia in our partner's lab, to set up the ectomycorrhizal microcosm to follow gene expression under stress.

Leveraging effect of the project—
The proposed research will benefit to the scientific community by the understanding of how fungi can adapt and evolve to bypass the toxicity of some compounds and the stress generated by extreme and/or disturbed environments. This could help predict the structuring of microbial communities and adaptation to constraints in the future.
Moreover, since *Pisolithus* sp are relevant fungal models in forestry, due to their role in promoting plant health and productivity under different environmental constraints, identifying functional markers of stress resistance could be a good way of selecting isolates that could be used as inoculum in seedling production.