Discovering new inhibitors of the Alternative Oxidase Enzyme (AOX) from *Moniliophthora perniciosa*, cacao's pathogen, using extracts from *Neonectria ditissima*

Felipe Bisolo*, Bárbara A. Pires, Renata M. Baroni, Silvana A. Rocco, Mario R. O. Barsottini, Caline G. Ferraz, Gonçalo A. G. Pereira.

Abstract

The fungus *Moniliophthora perniciosa* is the causal agent of Witches' Broom Disease of cocoa. Its mitochondrial enzyme Alternative Oxidase (MpAOX) acts as a bypass to respiration inhibitors and confers fungal resistance to plant defense responses. Natural compounds with potential to inhibit AOX are produced by other fungi, such as *Neonectria ditissima*. Here, we describe the production of *N. ditissima* extracts with putative AOX inhibitors, which were obtained from freeze-dried mycelia.

Key words:

fungal extracts, AOX inhibitors, Witches' broom disease.

Introduction

The basidiomycete fungus *Moniliophthora perniciosa* is the causal agent of the Witches' Broom Disease of cocoa. The *M. perniciosa* mitochondrial enzyme Alternative Oxidase (MpAOX) confers resistance to inhibitors of the main respiratory pathway such as fungicides or metabolites produced during plant defense responses.¹ The ascomycete fungus *Neonectria ditissima* is known to produce natural isoprenoid compounds that inhibit plant and protozoan AOXs, such as Collectochlorin B.^{2,3}

The synthetic pathway for isoprenoid compounds production is not trivial, and a possible alternative is the extraction and isolation of those compounds from organisms that naturally produce them.⁴ An advantage of this method is the obtention of structurally related metabolites, which is useful for structure-activity relationships studies and the design of novel chemical entities. In this study, we aimed to obtain *N. ditissima* extracts and test their effect as MpAOX inhibitors and potential antifungal activity.

Results and Discussion

Extracts from three strains of *N. ditissima* were evaluated in order to detect their potential effect as inhibitors of the Alternative Oxidase enzyme (AOX) from *M. perniciosa*. Each of the three *N. ditissima* isolates were grown in three different culture media: 1) malt extract medium, 2) meat extract medium and 3) potato dextrose medium (BD) at 22 °C and 200 rpm in a rotatory shaker. Extractions were made using a series of different solvents and solvent mixtures, such as water, methanol, acetone, dichloromethane and hexane. However, liquid-liquid extraction from the culture media and solid-liquid extraction from the fungal mycelia were not efficient to provide bioactive compounds. Therefore, a new sample preparation and extraction method was tested: freeze-drying the *N. ditissima* mycelia grown in malt extract medium and extracting it with hexane and subsequently with methanol.

The new extracts were evaluated in biological assays using the yeast model *Pichia pastoris*, which has an endogenous AOX. Inhibiting the main electron pathway with Azoxystrobin (a known inhibitor of complex III) allowed us to evaluate the specific inhibition of *P. pastoris* AOX.

Image 1. Biological assay with malt extracts using the isolate 106LF from *N. ditissima* with different solvents. Optical density (600nm) is the measurement of the yeast growth. The presence of the extract without Azoxystrobin (left) and with Azoxystrobin (right).

The freeze-dried extract inhibited *P. pastoris* growth in combination with azoxystrobin (main respiration pathway inhibitor), indicating the specific inhibition of *P. pastoris* AOX and that the new extraction method was successful.

Conclusions

We conclude that freeze-drying the *N. ditissima* mycelia increases extraction efficiency for the obtention of bioactive metabolites and putative AOX inhibitors. This is probably due to an increase in surface area and in solubilization efficiency with sequential extractions with an apolar and a polar solvent. Further experiments are necessary to identify the metabolites present in that extract and to confirm MpAOX inhibition.

Acknowledgement

Thanks to CNPq and FAPESP for the financial support. And a special thanks to Ph.D. Juliana José for all the support during the project.

1. Thomazella, D. P. T. et al. *New Phytol.* **2012**, 194, 1025-1034.
2. Minagawa, N. et al. *Mol Biochem Parasitol.* **1997**, 84, 271-280.
3. Gutiérrez, M. et al. *J. Agric. Food Chem.* **2005**, 53, 7701-7708.
4. Mori, K. & Takechi, S. *Tetrahedron* **1985**, 41, 3049-3062.