

Identification of new inhibitor molecules of the enzyme Alternative Oxidase with potential antifungal activity against cocoa pathogens

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Abstract

Fungal diseases are one of the major sources of losses for the cocoa culture (*Theobroma cacao*) worldwide. In Brazil, the most important fungal pathogens are *Moniliophthora perniciosa* and *Ceratocystis cacaofunesta*, causal agents of the Witches' Broom Disease and the Wilt Disease, respectively. To date, there are no efficient ways to control them. However, it was identified that the enzyme Alternative Oxidase (AOX) plays an important role in the life cycle of these pathogens, thus representing a potential target for the development of new fungicides. Aiming to identify novel molecules capable of inhibiting AOX, we developed new experimental models for screening and characterizing bioactive molecules against the enzyme of interest.

Keywords: Enzyme Alternative Oxidase, Inhibitor Molecules, Witches' Broom Disease.

Introduction

The cocoa tree (*Theobroma cacao*) provides the raw material for the production of chocolate and other products, and fungal diseases are the main cause of losses in cocoa farms (1). The pathogens that are present in Brazil are *Moniliophthora perniciosa* and the *Ceratocystis cacaofunesta*, causal agents of Witches' Broom Disease and Wilt Disease, respectively. To date, there is no effective way to control them. However, the mitochondrial enzyme Alternative Oxidase (AOX) is of great importance for the survival and virulence of these pathogens, mainly granting resistance against inhibition of the mitochondrial respiratory pathway. As such, AOX represents a potential target for controlling these fungi (2). New molecules candidates as AOX inhibitors were synthesized and are currently awaiting for functional evaluation. Therefore, the aim of this project is to develop experimental models suitable for characterizing the activity of these molecules. Our results show that the yeast *Pichia pastoris*, which carries an endogenous *aox* gene, can be useful for identifying specific inhibitors of AOX, providing relevant information for the rational design of potential antifungal agents against pathogens of cocoa.

Results e Discussion

Pichia pastoris growth conditions were initially standardized in the presence of azoxystrobin, an inhibitor of cellular respiration. AOX was then inhibited by the known drug salicylhydrxamic acid (SHAM).

Growth assays of *Pichia pastoris* on solid medium showed that without any drugs, as well as in the

presence of vehicle solution and SHAM alone, there is a good growth rate, indicating no effect on *P. pastoris* under these conditions. Azoxystrobin alone (0.5mg/L) leads to a decrease in the yeast growth rate, thus increasing the time necessary for the formation of visible colonies. Notably, the combination of azoxystrobin (0.5mg/L) and SHAM (1mM) prevented the development of the yeast altogether. The same results were observed in experiments performed in liquid medium.

In accordance with previous observations, AOX allowed fungal survival and growth after inhibition of cellular respiration by azoxystrobin. The inhibition of AOX by SHAM broke this resistance, abolishing fungal growth.

Conclusions

The experimental model presented here is able to discriminate the activity of a drug on AOX. Thus, growth rates of *P. pastoris* can be evaluated in the presence of candidate AOX inhibitors, revealing those that are active against this enzyme.

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