

Functional characterization of the enzyme Alternative Oxidase (AOX) in yeasts and screening novel AOX inhibitor candidates for use against cocoa pathogens

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Abstract

In Brazil, the most harmful pathogens to cocoa culture are the fungi *Moniliophthora perniciosa* and *Ceratocystis cacaofunesta*, causal agents of Witches' Broom Disease and Wilt Disease of cocoa, respectively. To date there are no effective ways to control them. However, the fungal enzyme Alternative Oxidase (AOX) is a potential target for new fungicides, because AOX is crucial to the life cycle and infection process in both pathogens. Aiming to identify potential AOX inhibitors, experimental models have been developed and were used to characterize potential bioactive molecules, whose results will be presented.

Key words:

Enzyme Alternative Oxidase, Enzyme inhibitor, Fungicide

Introduction

Fungal diseases are the main cause of losses in cocoa culture. In Brazil, the most prominent pathogens are the fungi *Moniliophthora perniciosa* and *Ceratocystis cacaofunesta*, causal agents of Witches' Broom Disease and Wilt Disease of cocoa, respectively. Studies on the subject led to the identification of the mitochondrial enzyme Alternative Oxidase (AOX) as an important target for the control of these pathogens (1), from which new AOX inhibitor candidates were planned and synthesized. Our group have developed a functional assay in yeast (*Pichia pastoris*) model to evaluate AOX activity (2), allowing us to screen the candidate inhibitors for biological activity. On the other hand, further characterization of *M. perniciosa*'s and *C. cacaofunesta*'s AOX is necessary. Therefore, we also aimed to transform the CcAOX-coding gene in *Saccharomyces cerevisiae*.

Results and Discussion

AOX inhibitor candidates were screened for biological activity in *Pichia pastoris*, as described (2). Growth assays were conducted in liquid culture medium, in the presence of each candidate (0,5 mM) alone or with further addition of azoxystrobin (0,5 mg/L). In the presence of azoxystrobin, an inhibitor of the main respiratory pathway, AOX's activity comprises the sole pathway able to sustain *P. pastoris*'s growth. We observed that some planned candidate molecules reduced *P. pastoris*'s growth only when combined with azoxystrobin, indicating specific inhibition of AOX. Other molecules were effective in either the presence or absence of azoxystrobin, meaning that their activity is not targeted to AOX.

The yeast *S. cerevisiae* lacks an aox-coding gene and was, therefore, selected for genetic manipulation. The CcAOX-coding gene was cloned and transformed into *S. cerevisiae*, which was subjected to growth assays in the presence of azoxystrobin (0,5 mg/L). As opposed to results obtained with *P. pastoris*, azoxystrobin completely inhibited the growth of aox-transformed *S. cerevisiae*. However,

oxygen consumption measurements, combined with inhibitors of the main and alternate respiratory pathways (KCN and SHAM, respectively), indicated that CcAOX was indeed expressed and active.

Conclusion

Using an experimental model previously developed with *P. pastoris*, we were able to screen AOX inhibitor candidates and found seemingly promising molecules specific for AOX. Additional studies should be conducted to evaluate their true effect on this enzyme, as well as their fungicide activity against *M. perniciosa* and *C. cacaofunesta*. *C. cacaofunesta*'s AOX was successfully transformed into *S. cerevisiae* and expressed. In spite of being active, as assessed by oxygen consumption measurements, CcAOX did not promote growth when *S. cerevisiae*'s main respiratory pathway was blocked by azoxystrobin. This will limit the use of *S. cerevisiae* as a platform to characterize fungal AOX.

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