ROLE OF Yap1 AND Sod1 IN ROS ACCUMULATION AND LOVASTATIN BIOSYNTHESIS REGULATION IN *Aspergillus terreus*

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Introduction. Lovastatin (LOV) is a secondary metabolite, produced by *Aspergillus terreus*. It has great commercial importance since it lowers cholesterol levels in blood[5]. Studies from our group, showed a link between reactive oxygen species (ROS) and LOV biosynthesis in submerged (SmF) and solid-state fermentation (SSF). Our results showed that *sod1* gene (oxidative stress defense enzyme) was intensely expressed during rapid growth phase (or trophophase) in LOV fermentations, but it was down regulated in idiophase. In that moment, ROS levels increased, generating an oxidative state during production phase[6]. In a subsequent work we showed that ROS regulated LOV biosynthesis (Miranda et al., in press).
Yap1 is a transcription factor that responds to oxidative stress[4] To study the mechanism by which ROS may regulate LOV biosynthesis, the expression of *yap1* during lovastatin SSF and SmF, was studied. Both culture systems were compared in an effort to detect differences that could account for higher production in SSF.

Methods. Analysis *in silico* of *lovE* promoter region (transcriptional factor of lovastatin biosynthesis) was performed using The MEME Suite and JASPAR software. SmF and SSF fermentation were performed with the high producing strain *Aspergillus terreus* TUB F-514 as describe Baños et al. (2009). Samples of different times were taken for RNA extraction and Northern-blot analysis, ROS and LOV determination.

Results.
The promoter analysis of *lovE* revealed the existence of one putative binding site for Yap1 (Fig.1). The transcription analysis indicated that *sod1* and *yap1* show similar patterns of expression during LOV fermentations: strong expression during trophophase and down regulation in idiophase, generating an increase in ROS concentration during production phase (Fig 2). On the other hand, higher ROS concentrations were detected in SmF, in relation to SSF.

Fig. 1 Analysis *in silico* of *lovE* promoter.

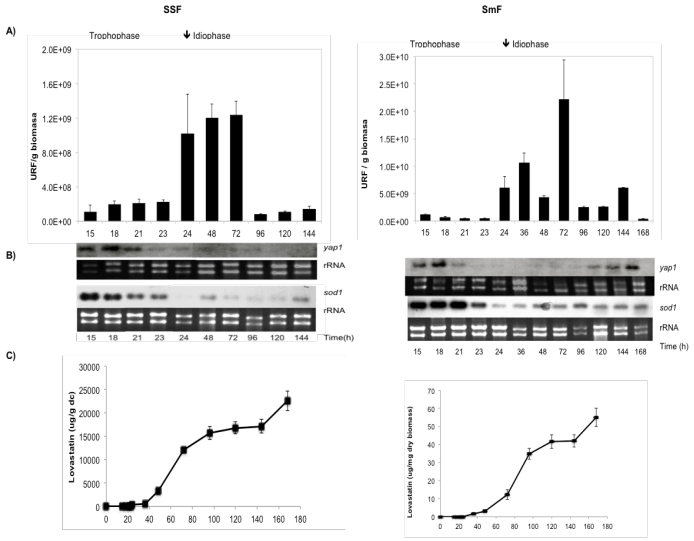

Fig. 2 A) ROS accumulation profile B) Northern analysis of *yap1* and *sod1* genes and C) lovastatin production during trophophase and idiophase of SSF (left) and SmF (right).

Conclusions.
1) *lovE* promoter has one putative binding site for Yap1.
2) Similar expression patterns of *yap1* and *sod1* genes during lovastatin fermentations, suggest that, in *A. terreus*, Yap1 regulates *sod1* gene, as has been shown in yeast and other fungi[4].
3) The down regulation of these genes in idiophase contributes to generate the ROS accumulation, which in turn induces lovastatin biosynthesis (Miranda et al, in press). Suggest that Yap1 acts as a negative regulatory protein, since its production is down regulated in idiophase. A similar proposal has been done by Reverbery et al (2008) for aflatoxin biosynthesis.

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