Aspergillus terreus HIGHER LOVASTATIN PRODUCTION IN SOLID-STATE FERMENTATION CORRELATES WITH HIGHER EXPRESSION OF GENES *laeA* AND *lovE*

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Introducción. In a previous work we developed a high lovastatin producing system based on solid-state fermentation (SSF) on artificial inert support. In this culture system, *A. terreus* showed higher production, in relation to submerged fermentation (SmF) (1). Recent studies indicate that the fungus receives environmental signals, indicating the culture system, which generate a differential gene expression (2, 3 and 4). LaeA is a global regulator of secondary metabolism in *Aspergillus sp.* and other filamentous fungi, and part of the cAMP-PKA signaling pathway. This pathway transduces environmental stimuli, such as nutrient availability and others, and regulates genes associated with growth. Under stress conditions, this pathway activates genes related to secondary metabolism, sporulation, and stress resistance (5).

In the present work we studied the behavior of *laeA* and *lovE* regulatory genes during lovastatin SSF and FL, to determine molecular differences that help explain the higher production of lovastatin obtained in FS, in relation to the FL.

Methodology. Expression of genes *laeA* and *lovE* was determined (by qRT-PCR) during SSF with polyurethane as inert support, impregnated with liquid medium, and in SmF with *Aspergillus terreus* TUB F-514, a lovastatin producing strain (1). In SmF, biomass was quantified by dry weight. During SSF, biomass was determined by glucosamine content through a colorimetric method. Lovastatin concentration was quantified by HPLC (1). Total RNA was extracted using Trizol and treated with RQ1 RNase-Free DNase. The qRT-PCR was carried out using One-Step kit EXPRESS SYBR ® GreenER ™. A relative quantification was performed using a standard curve of *laeA* gene cDNA cloned in pGEM-T. These values were normalized with *H4* gene expression, which was used as endogenous control.

Results. As in previous works, we observed higher lovastatin production in SSF, in relation to the SmF. On day 5, the SSF specific production was about 9 times higher than SmF (Fig 1).

To study the potential role of genes *lovE* y *laeA* in the higher lovastatin production observed in SSF, their expression was quantified during SSF and SmF. As expected, no *lovE* transcripts were detected during growth phase (trophophase). However, in idiophase its expression was 3.2- and 2.4-fold higher in SSF (42 h and 72 h), in relation to SmF.

Surprisingly, *laeA* expression started during trophophase (18 h), and continued through out idiophase, in both culture systems. As in the previous case, at 72 h, *laeA* expression was 6.2-fold higher in SSF than in SmF.

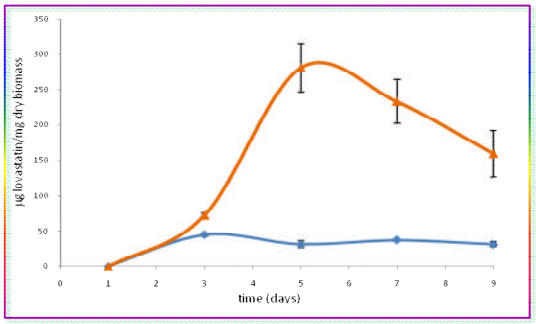

Figure 1. Lovastatin specific production by *A. terreus*, in SSF(▲) and SmF (◆).

This is consitent with the higher *lovE* expression and higher lovastin production found in SSF. These results also agree with the report of Barrios-González *et al.* (2008) (3), where the authors calculated a 4.6-fold higher *lovE* expression in SSF, by using Northern analysis.

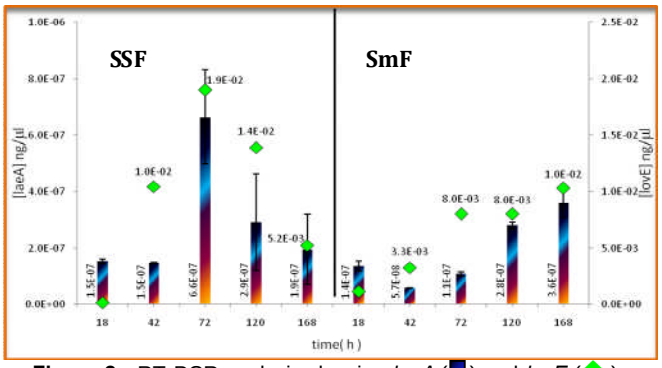

Figure 2. qRT-PCR analysis showing *laeA* (■) and *lovE* (◆) expression during the course of lovastatin SSF and SmF with *A. terreus*.

Conclusions. Results show that higher lovastatin production in SSF is, at least partially, due to higher expression levels of regulatory genes *laeA* and l*ovE*. Results also indicate a role of signaling pathway cAMP-PKA in transducing environmental cues indicating it is a solid medium. This, in turn, results in differential genes expression, giving rise to a different physiology in SSF.

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