



# EFFECT OF SILENCING GENE *yap1* IN *Aspergillus terreus*



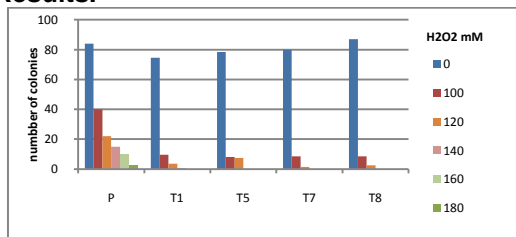
Ailed Pérez, Roxana U. Miranda, Javier Barrios-González. Universidad Autónoma Metropolitana Unidad Iztapalapa UAM-I Depto. de Biotecnología, Iztapalapa, C.P. 09340, México, D.F. jbg@xanum.uam.mx.

Keywords: Oxidative stress, lovastatin, *A. terreus*.

**Introduction.** Oxidative stress is defined as an imbalance between the generation of reactive oxygen species (ROS), and the intracellular antioxidant defenses (1). To keep healthy ROS levels, cells have developed mechanisms to detect and respond to oxidative events (2). Yap1 is a transcription factor, which acts as a redox sensor that is activated directly by higher levels of ROS (3). Approximately half of the 71 proteins induced by oxidative stress depend Yap1 (4). In previous work, we found a link between lovastatin production by *Aspergillus terreus* and ROS production (5). In order to study the molecular mechanism, *yap1* orthologue in *A. terreus* was silenced and transformants characterized.

**Methodology.** Vector pGdpPkiRNAi-*yap1* was constructed and transformed into *A. terreus* TUB F-514, a high lovastatin producing strain. Transformants were characterized in relation to sensitivity to H<sub>2</sub>O<sub>2</sub>, conidiation and lovastatin production. Sorulation kinetics was obtained by quantifying with a Neubauer chamber. Lovastatin was quantified by HPLC in samples from solid-state fermentation (SSF) and from submerged fermentation (SmF).

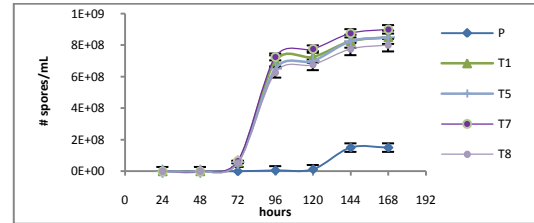
## Results.



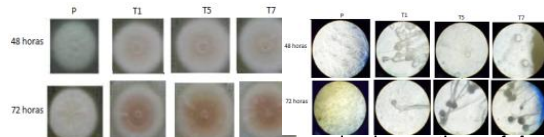
**Fig1.** Sensitivity of *A. terreus*: spores to H<sub>2</sub>O<sub>2</sub>. P: parental, T1 to T8: transformants(silenced *yap1*).

**Table 1.** Mycelium sensitivity to H<sub>2</sub>O<sub>2</sub>, (P: Parental, T1 to T8 transformants). 5 to 2, represent exponent of spore concentration: 10<sup>5</sup> to 10<sup>2</sup>.

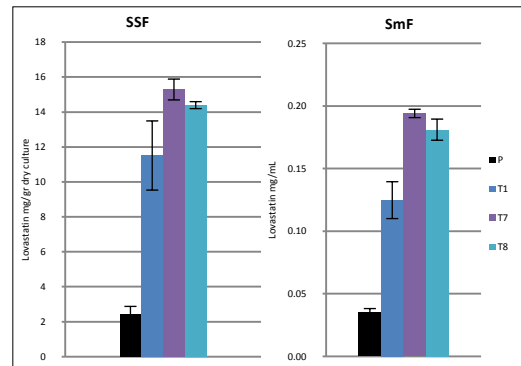
Strain	H <sub>2</sub> O <sub>2</sub>	0mM		10mM		20mM		30mM		40mM		50mM	
		5	4	3	2	5	4	3	2	5	4	3	2
P		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
T1		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
T5		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
T7		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
T8		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓



**Fig2.** Sporulation kinetics of *A. terreus*: P: parental, T1 to T8: transformants (silenced *yap1*).



**Fig3.** Macroscopic and microscopic observations of *A. terreus*: P: parental, T1 to T8: transformants (silenced *yap1*).



**Fig4.** Lovastatin production by *A. terreus* after 48 hours: P: parental, T1 to T8: transformants (silenced *yap1*).

**Conclusions.** Yap1 silencing in *A. terreus* caused a marked sensitivity to H<sub>2</sub>O<sub>2</sub> in the transformants, which appears to be related to an early start of lovastatin biosynthesis in both, SSF and in SmF. Also, to a precocious sporulation, together with higher spores production.

**Acknowledgements.** A. Pérez thanks CONACYT for scholarship No 2324-8508.

## References.

- Zjalic S., Ricelli A., Punelli F., Camera E., Fabbri C., Picard M., (2008). *Eukaryotic Cell* 7 (6), 988-1000.
- Scott W. Rowley M. (2003), *Eukaryotic Cell*, 2,(3): 381-389.
- Vargas P., Sanchez O., Kawasaki L., (2007), *Eukaryotic Cell*, 6 (9), 1570-1583.
- Mulford K. E. † Fassler J. S., (2011) *Eukaryotic Cell*, 10, (6): 761-769.
- Barrios G., J., & Miranda, R. (2010), *Appl Microbiol Biotechnol* 85 (4), 869-883.