POSSIBLE ROLE IN THE TRANSFORMATION OF ALPHA-PINENE TO CIS-VERBENOL OF THE CYP52PGU GENE OF *PICHIA GUILLIERMONDII* ISOLATED FROM GUT OF *DENDROCTONUS VALENS* BARK BEETLE.

Eneida Campos-Guzmán, Gerardo Zúñiga, Lourdes Villa-Tanaca, César Hernández R; Instituto Politécnico Nacional Escuela Nacional de Ciencias Biológicas, Departamento de Microbiología, Ciudad de México 11700. chdez38@hotmail.com

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Introduction. The bark beetles (Coleoptera: Curculionidae, Scolytinae) feed on phloem, a substrate rich in cellulose, hemicellulose, and several terpenes, but poor in assimilable nitrogen (Pureswaran and Sullivan 2012). With exception of the dispersion event, all stages of the life cycle are exposed to chemical defenses of conifers that generate a toxic environment containing volatile terpenes, and other secondary plant compounds. Monoterpenes, diterpenes, and sesquiterpenes are the most prominent resin compounds, but the alpha-,and beta-pinene are particularly abundant. These monoterpenes can destroy cellular integrity, reduce the mitochondrial activity, and inhibit the proton, and potassium pumps of several cells. Evidently, the concentration of these volatile substances in bark beetles environment is not enough to inhibit the growth of the bark beetles and their symbionts.
Bark beetles-yeasts symbiosis has been previously described, but no clear roles of simbionts have been defined. Particularly, members of the *Pichia guillermondii* yeast cluster isolated from *Dendroctonus* bark beetles gut are capable of utilizing hydrocarbons as a sole carbon source (Addams et al. 2011).

Methods. CYP52Pgu of *P. guilliermondii* 12. a fragment of 894 nt was amplified and sequenced from *P. guilliermondii* 12 genomic DNA (access number JQ838070)
In this work, the transcriptional expression of *CYP52Pgu* gene of *P. guillermondii* 12, a strain isolated from *Dendroctonus valens* midgut, was estimated by RT-PCR in presence of glucose and two pine phloem hydrocarbons (n-heptane and alpha-pinene).
Alpha-pinene biotransformation experiments were performed with *P. guilliermondii* non-proliferant cells permitted to observe the hydroxylation of alpha-pinene to cis-verbenol by gas chromatography coupled to mass spectrometry (GC/MS).

Results. The sequence of the *CYP52Pgu* gene of *P. guillermondii* 12 was identical to the homologous gene of *P. guillermondii* ATCC6260. The gene was expressed in the presence of both hydrocarbons (n-heptane and alpha-pinene), but the alpha-pinene expression level was higher than n-heptane as inductor. No gene expression was detected with glucose as a sole carbon source, and a catabolic repression effect on alpha-pinene and n-heptane induction was observed at low glucose concentrations. *In silico* analyses of the regulatory region confirmed that *CYP52Pgu* gene of *P. guilliermondii* is a catabolic repressible gene.
The highest transcriptional expression of the gene *CYP52Pgu* coincided with the times of maximum transformation of alpha-pinene to cis-verbenol. Transformation of alpha-pinene to cis-verbenol was observed in the control without cells, but the biotransformation with *P. guilliermondii* yeast in absence of glucose was significantly higher than autoxidation. No differences were observed between autoxidation and *P. guilliermondii* yeast incubated with glucose.

Conclusions. The gene CYP52Pgu is activate by the presence of alpha-pinene and is subject to catabolite repression, and this gene codes for a P450 monoxygenase.

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