# High-throughput screening for detection of monoamine oxidases and transaminases in fungi isolated from human skin.

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#### Abstract

High-throughput screening (HTS) assays were applied to screen enzymatic activity of monoamine oxidases and transaminases in fungi.

## Introduction

Enzymes have been widely used in the chemical industry, because they are versatile catalysts that operate under mild conditions and follow the Principles of Green Chemistry. Consequently, there is a huge demand for new sources of enzymes, which may be accomplished by the enzymatic screening of microorganisms.<sup>1</sup>

Such a screening when associated with fluorogenic substrates is a simple, low cost and sensitive technique, that allows a rapidly evaluation of a large number of samples.<sup>2,3</sup> Thus, HTS technique was applied to search monoamine oxidases (MAO) and transaminases (TA) in 39 fungi isolated from human skin<sup>4</sup>.

## Results and Discussion

Fungi were screened using the methodology described by Badalassi *et al.* (2000)<sup>5</sup> adapted for whole microbial cells<sup>6</sup>. Figure 1 shows the HTS assay scheme using the fluorogenic probe **1**.



Figure 1. Fluorogenic assay to detect MAO and TA activities.

Assays were performed in 96-well microplates (200 uL) and monitored by fluorescence ( $\lambda_{ex}$  460 nm) for 96h at plate reader (*PerkinElmer EnSpire*).

The concentration of the microbial suspension used was 50 mg/mL and for the probe 1 was 100  $\mu mol \ L^{-1}.$ 

Of the 39 fungi screened, 12 of them showed more than 15% of conversion of the probe **1** within 96h of reaction, as shown in Table 1.

**Table 1.** Enzymatic Conversion (%) of the fluorogenicprobe 1.

Code	Fungus	Enzymatic Conversion (%)			
		24h	48h	72h	96h
7M1	Epicoccum sp.	6	14	19	21
9M1	Epicoccum sp.	18	23	27	31
23M1-IS4	Scolecobasidium sp.	43	60	60	60
28M1	Epicoccum sp.	15	21	23	25
28M2	Epicoccum sp.	10	15	17	20
28M3-IS2	Epicoccum sp.	13	16	16	20
28M4	Phoma sp.	9	12	14	19
28M5	Massarina sp.	9	13	16	30
30M1-IS1	Phoma sp.	12	16	22	25
30M1-IS2	Aureobasidium sp.	16	18	19	22
37M-IS2	Marasmius sp.	22	29	35	40
43M1	NI	17	20	24	32

NI = Not Identified

## Conclusions

Monoamine oxidases/transaminases activities were detected in 12 fungi. After, these fungi will be evaluated by conventional biocatalysis assays with amines of synthetic interest.

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<sup>&</sup>lt;sup>1</sup> Reymond, J; Wahler, D. Curr. Opin. Chem. Biol. 2001, 5:152–158.

<sup>&</sup>lt;sup>2</sup> Reetz, M. T. Angew. Chem. Int. Edit. 2002, 41, 1335-1338.

<sup>&</sup>lt;sup>3</sup> Reymond, J.L. Ann. N.Y. Acad. Sci., 2008, 1130, 12-20.

<sup>&</sup>lt;sup>4</sup> Silva C.P. Potencial enzimático da microbiota da pele humana e sua ação sobre insumos de fragrâncias. *Tese de doutorado*, Unicamp, 2012.

 <sup>&</sup>lt;sup>5</sup> Badalassi, F. *et. al. Angew. Chem. Int. Ed.* 2000, *39*, 4067.
<sup>6</sup> Bicalho, B.; Chen, L-S.; Grognux J.; Reymond, J-L.; Marsaioli, A.J. *J. Brazil. Chem. Soc.* 2004, *15*, 911-916.

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