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.¹

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.^{2,3} Thus, HTS technique was applied to search monoamine oxidases (MAO) and transaminases (TA) in 39 fungi isolated from human skin⁴.

Results and Discussion

Fungi were screened using the methodology described by Badalassi *et al.* (2000)⁵ adapted for whole microbial cells⁶. 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 (λ_{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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