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# Kinetic resolution of chiral amines by endophytic fungi from *Humiria balsamifera*

**Maria Sandra R. Queiroz[1], Felipe K. Sutilli[2], Larissa Z. Pinheiro[1], Eunice Valduga[4], Mauricio M. da Silveira[5] Michelle F. Muzitano[3], Rodrigo O. M. A. de Souza[2], Denise O. Guimarães[3] and Ivana C. R. Leal[1]**

*[1]Laboratório de Produtos Naturais e Ensaios Biológicos (LaProNEB) DPNA -CCS- UFRJ, Rio de janeiro, Brazil- msandrarq@yahoo.com.br [2]Laboratório de Biocatálise e Síntese Orgânica (BOSS Group)- Bloco A- Instituto de Química- UFRJ [3]Laboratório de Produtos Naturais (LaProN)-UFRJ – Polo Macaé [4]Universidade Regional Integrada do Alto Uruguai e das Missões - URI. Erechim-RS [5]Universidade de Caxias do Sul. Caxias do Sul-RS*

Endophytic fungi are micro-organisms that can invade the tissue of living plants causing asymptomatic infections. They have been introduced in biotransformation studies of different drugs considering its versatility[1]. In this context, optically pure amines gained an increased significance as building blocks in organic chemistry[2]. In the biotransformation process, the energy is saved since it runs at low temperature, pressure and mild conditions.[2]. So, the present work aims to select endophytic fungi from *Humiria balsamifera* (Hb) able to conduct transamination reaction by optimized medium conditions and to promote enantioselective resolution of chiral amines. Endophytic fungi (20 micro-organisms; coded Hb) were cultivated in seed medium for mycelial mass growing and, later, in modified Czapek fermentative medium previously spiked with rac-phenylethylamine (6.45 µL). The culture was incubated at 30°C and 120 rpm. The culture broth (500 µL) were collected each 48 h during 8 days. So, this sample was partitioned into ethyl acetate (3 x 500 µL) for GC-MS analyses. The data showed 27.4 % of bioconversion after 48 h. Analytical chromatographic (HPLC-DAD) separations were carried out on a mobile phase consisting of heptane and isopropanol (9:1) (v/v) at a flow rate of 0.8 mL/min. The stationary phase consisted of a chiral column . A optimization of the parameters was performed by a fractionary factorial experimental design $2^{4-1}$(11 experiments) followed by a central composite rotatable design (CCRD) in order to achieve the best growing conditions (pH 5, 30°C, 120 rpm and 40 $gL^{-1}$ sucrose – 33 % conversion). The results showed an enantiomeric excess of 99% for the R enantiomer in 48 h (pH 5.0, 50°C, 120 rpm and 40 $gL^{-1}$ sucrose) suggesting the HB13 as a potential biocatalyst in the kinetic resolution of rac-1-phenylethylamine. Other chiral amines (Table 1) were then evaluated in the above described optimized conditions and, they showed to be biotransformed by HB13. Excellent enantiomeric excess were also observed for amines 1 and 3 (Table 1).

Table 1. Bioconversion rates for the correspondent acetones of different amines and kinetic resolution of chiral amine .

| Chiral amines | Ketone bioconversion (%) | enantiomeric excess (%) |
|---|---|---|
| **1**.tetrahydro-1-naphthylamine. | 47 | 95 *R* |
| **2**. *sec*butylamine | 25 | n.e.* |
| **3**.ethylbenzylamine | 46 | 97 *R* |

*None excess

The results indicated the relevance of endophytic microbiota of *Humiria balsamifera* as biocatalysts for transamination reactions, especially for converting to the *R* enantiomer of different chiral amines. An optimized condition was achieved for acetophenone production from rac-phenylethylamine and, the mathematical experiments accurately explained the reaction model.

**References:**

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