SCREENING OF SELECTED MICROORGANISMS FOR BIOCONVERSION OF (+)-VALENCENE TO (+)-NOOTKATONE.

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Introduction. Valencene is a low priced sesquiterpene found in orange essential oil, and it is a precursor of many flavors and fragrances, for instance (+)-nootkatone, a compound with higher added value. The use of microorganisms for these transformations has stimulated the biotechnology market [1,2].
This work is focused on a series of experiments to screening microorganisms capable of performing the bioconversion of (+)-valencene to (+)-nootkatone.

Methods. The experiments were conducted in serological flasks containing PDA culture medium. Which were inoculated with $1x10^6$ cells mL^{-1} and incubated at 30°C. After 7 days, three different combinations were tested: (a) 3.2 g/L (+)-valencene (aqueous phase), (b) orange essential oil (organic phase), and (c) oil-water (biphasic system) were added to serological flask and incubated at 30°C during 12 days. Samples were taken and substrate and product concentrations were measured by Gas Chromatography [3]. Substrate inhibition was determined for *B. theobromae* and *Y. lipolytica* in aqueous phase by increasing valencene concentration.

Results. Six microorganisms were tested for their ability to bioconvert the substrate; however, only three were able to bioconvert (+)-valencene to (+)-nootkatone in the biphasic system (Table 1).

Table 1. Microbial bioconversion from (+)-valencene to (+)-nootkatone in the biphasic system.

Microorganisms	(+)-nootkatone (mg/L)	% Bioconversion
Kluyveromyces marxianus	9.71 ±0.70	0.59%
Aspergillus tamarii	0.23 ±0.02	0.001%
Botryodiplodia theobromae	231.7±2.1	23.25%
Yarrowia lipolytica	216.9±5.8	29.5%
Phanerochaete chrysosporium	100.8±2.6	22.13%
Rhyzomucor sp	0.30 ±0.01	0.01%

Bioconversion kinetics of (+)-valencene to (+)-nootkatone in the biphasic system were obtained (Figure 1). After 12 days of bioconversion, *B. theobromae* y *Y. lipolytica*, yielded a conversion of 250.96±2.1 mg L^{-1} 241.80±5.8 mg L^{-1}, respectively.

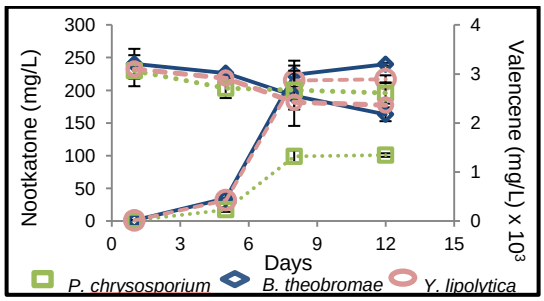

Figure 1. Bioconversion kinetics of (+)-valencene to (+)-nootkatone in the biphasic system.

Substrate inhibition was determined for *B. theobromae* and *Y. lipolytica* in aqueous phase (Figure 2). Both strains showed substrate inhibition above 3 g L^{-1}.

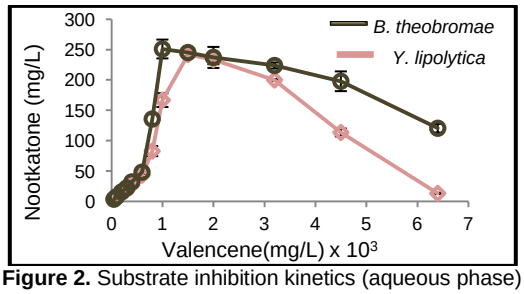

Figure 2. Substrate inhibition kinetics (aqueous phase) by *Y. lipolytica* and *B. theobromae*.

Conclusions. The regioselective synthesis method is naturally performed by the microbial pathway; therefore this method is to find alternatives in the industrial production of nootkatone.

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