KINETIC STUDY OF THE STEREOSELECTIVITY REDUCTION OF ACETOPHENONE PROMOTED BY A STRAIN OF Geotrichum spp.

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Nowadays, biocatalytic stereoselective reduction of prochiral ketones is one of the most important methodologies in organic synthesis for the preparation of chiral alcohols. These enantiomerically pure alcohols are very important because they are key precursors in the obtaining of pharmaceutical compounds. Recently, a screening on fifty six strains of bacteria and fungi was made for our team toidentify microorganisms capable of performing the stereoselective reduction of acetophenone (AP) to 1-phenylethanol (1-PE). From this study a strain of *Geotrichum spp*.has been isolated and characterized, and this yeast has presented anexcellent conversion (>99%) and stereoselectivity (>99.9 e.e.%) in the preparation of (*R*)-1-PE from AP.

Objective: Determine the kinetic where by a strain of *Geotrichum spp.* transforms stereoselectively AP to (*R*)-1-PE and based on the results of this study propose a reduction mechanism.

Methods: The inoculum of *Geotrichum spp*. was developed in GPY broth (30 °C; 3 days) and the yeast cells were separated by filtration. Wet cells (≈2 g) were put in a 125 ml sterile conical flask containing 80 ml of 0.1M KH_2PO_4buffer with pH adjusted to 7.0. AP (50 mg) dissolved in 1 ml of dimethyl sulfoxide was added and the incubation was made on an orbital shaker at 100 rpm (30 °C). Samples at one hour intervals were taken during 48 hours.The assay was made in triplicate and the samples were analysed by chiral GC-FID and GC-MS.

Results: In the kinetic study, it can be observed that in the first 6h of reaction, the quantity of AP decreased and the two isomers (*R* and *S*) increased but in different proportions: the *S* isomer was present in greater quantity. From this time, another enzyme began to oxidize the *S* isomer to AP, so the AP concentration began to increase while a disminution in the S isomer could be observed. Meantime the R isomer continued increasing but in a major rate. From the 15h the *S* isomer disappeared completely, the amount of acetophenone decreased to be undetectable and the *R* isomer was the only product formed. In a period of 24h the reduction was completed and the % of conversion and e.e.% was maximum for the *R* isomer (>99% and >99.9% respectively).

In order to inhibit the oxidation of the *S* isomer to AP, a preliminary study was made with another set of reactions, using the same conditions as above but under Helium atmosphere. In these cases at the end of the reaction, the e.e.% reversed

and the *S* isomer was the main enantiomer formed (95% of reduction and 63 e.e.% for (*S*)-1-PE).

Conclusions: Based on the results obtained in this kinetic study and literature reports, it is possible to hypothesize a mechanism in which coexist at least three enzymes: an enzyme which converts irreversibly AP in (*R*)-1-PE, another second enzyme that transforms AP in (*S*)-1-PE and another third enzyme that is only capable of oxidizing (*S*)-1-PE to AP. However, further studies are needed to support these partial conclusions.