

Preparation of enantiopure 1-arylethanols using microorganisms and enzymes by tandem process

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Abstract

Enantiopure 1-arylethanols were prepared in high yield and ee by OKR catalyzed by *C. albicans* and by stereoinversion using two microorganisms with opposite stereoselectivity via a tandem process.

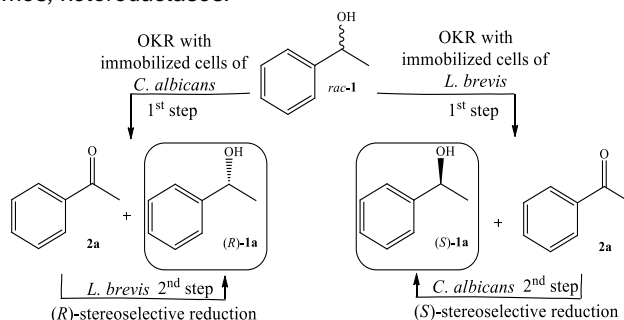
Introduction

Obtaining enantiopure compounds through biocatalysis has been widely studied, since they are important chiral building blocks for the synthesis of natural products, pharmaceuticals, and agricultural chemicals. Biocatalytic desymmetrization using prochiral derivatives has shown enormous potential and is highly significant in several interesting asymmetric processes. However, racemic substrates are more readily available starting materials than prochiral substrates.^{1,2}

Results and discussion

C. albicans CCT 0776 has shown a relevant potential to catalyze the oxidation of *para*- and *meta*-substituted 1-arylethanols and promote an Oxidative Kinetic Resolution (OKR) to produce 50 % of (*R*)-enantiomer and 50% of the correspondent ketone. Combine the OKR potential of *C. albicans* with an anti-Prelog ADH enzyme or a microorganism was the aim to increase the efficiency of the process. Several yeasts, filamentous fungi and bacteria were screened and it was found that *Lactobacillus brevis* CCT 3745 was capable of reducing acetophenone (**2a**), producing (*R*)-**1a** with a 90% yield and 99% ee after 24 hours of reaction. Our objective was to use the immobilized cells of *C. albicans* in alginate beads followed by free cells of *L. brevis* to produce (*R*)-**1a** from *rac*-**1a**. For each 50 mg of *rac*-**1a** a minimum of 0.150 mL of acetone was needed to recycle the NAD⁺ cofactor in the oxidative step (first step), and 0.40 mL of 2-propanol to regenerate the NADH necessary for the reductive step (second step) (**Scheme 1**). These conditions enabled successful deracemization of *rac*-**1a**, resulting in full conversion after 17 hours and (*R*)-**1a** with 99% ee. Changing the microorganisms order using immobilized cells of *L. brevis* in the first step and free cells of *C. albicans* in the second step produced 75% (*S*)-**1a** with 80% ee.

To improve the deracemization efficiency a ketoreductase can be employed instead of a microorganism in the second step of the process. So, ketoreductases from Codex® KRED Screening Kit



Scheme 1. Deracemization process of *rac*-**1a** using a tandem of *C. albicans* and *L. brevis* to obtain both enantiomers separately.

were screened to observe the reduction potential and enantioselectivity toward acetophenone. From the 24 enzymes screened, 14 showed low or no selectivity in reduction of **2a**, while 5 had a Prelog selectivity producing the (*S*)-**1a**, KRED-119 (84% conv. and 98% ee), KRED-130 (100% conv. >99% ee), KRED-P3-H12 (89% conv. 83% ee), KRED-NADH-101 (88% conv. >99% ee) and KRED-P3-B03 (89% conv. and 98% ee) after 24 hours of reaction. Other 5 enzymes showed an anti-Prelog selectivity, giving the (*R*)-**1a**, KRED-NADH-110 (89% conv. 87% ee), KRED-P1-A04 (85% conv. >99% ee), KRED-P1-H10 (88% conv. 89% ee), KRED-P2-C11 (87% conv. 82% ee) and KRED-P2-H07 (88% conv. 87% ee) after 24 hours of reaction.

Conclusion

Both enantiomers of *rac*-**1a** were prepared efficiently, exhibiting good yield and high ee, through deracemization by stereoinversion using resting cells of *C. albicans* and *L. brevis* separately and consecutively. From the screening of the ketoreductases we observed that 10 enzymes can be used in the second step of the process. Five with Prelog selectivity and five with opposite selectivity.

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