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Deracemization of 1-phenylethanol via tandem biocatalytic oxidation and reduction

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ABSTRACT

(*R*)-1-Arylethanols and other secondary alcohols were prepared at high *ee* (>90%) by oxidative kinetic resolution using resting cells of the yeast *Candida albicans* CCT 0776. The deracemization process of 1-phenylethanol **1a** catalyzed by the yeast was elucidated by studying each step separately. It was determined that the reaction occurred via cyclic deracemization, to give (*R*)-**1a** in 89% yield and with 98% *ee*. Finally, deracemization by stereoinversion of *rac*-**1a** was studied using a tandem process of *C. albicans* followed by *Lactobacillus brevis* CCT 3745. Inverting the sequence of these microorganisms produced an enantiomerically pure antipode.

1. Introduction

Biocatalytic methods have gained increasing prominence because they meet a number of requirements, including excellent selectivity under mild reaction conditions according to green chemistry principles.[1,2] Enantiopure compounds obtained through biocatalysis has been widely studied, since these compounds are important chiral building blocks for the synthesis of natural products, pharmaceuticals, and agricultural chemicals. In particular, biocatalytic desymmetrization using prochiral derivatives has shown enormous potential and is highly significant in several interesting asymmetric processes.[3] However, racemic substrates are more readily available starting materials than prochiral substrates.[4]

The separation of a racemate into its two enantiomers, commonly known as resolution, is the most common technique used to obtain separate enantiomers in a number of applications. Kinetic resolution is an efficient process involving the conversion of the two enantiomers in racemic mixtures into products at different rates, such that only one of the enantiomers remains. This process is limited in that it yields a maximum return of 50% of the product, since only one of the enantiomers undergoes a reaction.[5] Enzymes such as hydrolases and oxidoreductases are generally used for kinetic resolutions because they can selectively convert one enantiomer from a racemic mixture into a new compound, thus enabling physical separation after the reaction.[6] Alcohol dehydrogenases are most commonly used for enantioselective alcohol oxidations and ketone reductions. These enzymes are NAD^+-dependent or $NADP^+$-dependent and can catalyze the oxidation of secondary alcohols to ketones, and primary alcohols to aldehydes.[7] Oxidative kinetic resolution of secondary alcohols by whole cells or alcohol dehydrogenases is used to oxidase an enantiomer from racemate to its corresponding ketone and obtain a 50% enantiopure product.[8]

Deracemization of a racemate can be achieved by dynamic kinetic resolution, stereoinversion, enantioconvergence or cyclic deracemization. Cyclic deracemization systems traditionally differ from stereoinversion in the use of a non-selective irreversible reaction combined with a second selective transformation that accumulates the enantiomer that is not converted. As such, a typical cyclic deracemization process involves selective enzyme-catalyzed oxidation into a prochiral intermediate, which is then reduced via nonselective chemical reduction.[9–11] Therefore, depending on the selectivity of the enzyme, an enantiopure product can be obtained after a certain number of cycles.[5] Stereoinversion is the selective transformation of one substrate enantiomer into an achiral intermediate, which subsequently reacts to produce the final product, ideally with the opposite configuration to obtain a single enantiomer.[12] Several authors have reported on deracemization via the stereoinversion of one alcohol enantiomer in the presence of growing or resting cells of microorganisms,[12–15] and by a concurrent tandem using a microorganism and an isolated alcohol dehydrogenase.[16] A chemo-biocatalytic deracemization system for secondary alcohols was established in a one-pot reaction: chemical nonselective oxidation and bioreduction with isolated alcohol dehydrogenase occurred sequentially, to yield 1-arylethanols with excellent conversions and enantiomeric

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excess values.[17] Several studies have reported the deracemization of secondary alcohols using a consortium of two microorganisms. Racemic mandelic acid was deracemized in the presence of growing cells of *Pseudomonas polycolor* IFO 3918 and *Micrococcus freudenreichii* FERM-P 13221 after 24 h.[18] Similarly, *Bacillus stearothermophilus* with Prelog specificity was used for the oxidation of (*S*)-sulcatol, while the simultaneous reduction of the corresponding ketone was achieved by *Yarrowia lipolytica*, displaying anti-Prelog activity, to produce the (*R*)-alcohol in 82% yield and with 90% *ee*.[19] Deracemization processes using two microorganisms separately have also been reported: the enantioselective oxidation of (*S*)-mandelic acid from the racemate by *Alcaligenes bronchisepticus*, followed by the reduction of the intermediate benzoyl formate with *Streptococcus faecalis* IFO 12964.[20] For the deracemization of pantoyl lactone, *Nocardia asteroides* and *Candida parapsilosis* were used in tandem.[21]

Herein we report the oxidative potential of *Candida albicans* to catalyze the oxidative kinetic resolution of 1-arylethanols and other secondary alcohols, the mechanism of the deracemization of 1-phenylethanol, and the use a tandem process of *C. albicans* and *L. brevis* to promote efficient deracemization by stereoinversion of 1-arylethanols.

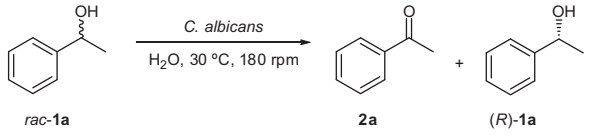
2. Results and discussion

Oxidative kinetic resolution of 1-arylethanols was initially observed using several different biocatalysts to produce the corresponding ketone and one enantiomer of the alcohol. This screening test provides optimum oxidative kinetic resolution by using resting cells of *C. albicans,* as shown in Table 1. Enantioselective oxidation of *rac*-**1a**–**i** was promoted by resting cells of *C. albicans*, which oxidize only the (*S*)-enantiomers of the alcohols to their corresponding ketones with high conversion, leaving the (*R*)-enantiomer intact. For compounds **1a**, **1c**, and **1d** (Table 1, entries 1, 3 and 4), the reaction was complete in only 1 hour, with excellent *ee* values for the remaining (*R*)-**1a**–**i** alcohols. When using different biocatalysts, lower *ee*s than those shown in Table 1 were obtained, thus demonstrating the relevance of *C. albicans*. The reaction time for entries 2 and 6 was nearly 50 h, far slower than for entry 8 (144 h), but the *ee*s obtained were excellent (99%). Silva et al. obtained the (*S*)-enantiomer of substituted (*R,S*)-1-phenylethanols with high *ee* and 50% conversion via oxidative kinetic resolution using cells of *Arthrobacter atrocyaneus* (R1AF57) after 48 h of reaction.[8] The oxidative capacity of *C. albicans* was not restricted to 1-phenylethanol and its derivatives. The yeast *C. albicans* promoted the oxidative kinetic resolution of 1-indanol, to produce the (*R*)-enantiomer with excellent yield (50%) and *ee* (99%). It also oxidized most of the cyclohexanol into its corresponding ketone (84%) after 62 h.

In a detailed study of the action of yeast on 1-phenylethanol *rac*-**1a**, the reaction was monitored for periods exceeding one hour (Table 1, entry 1); the time required to complete the oxidative kinetic resolution is shown in Table 2. The complete deracemization process was slow (Table 2, entries 2–4) and acetophenone concentration decreased from 48 to 36%. After 6 days of incubation, the deracemization process provided (*R*)-1-phenylethanol **1a** with 89% conversion and 97% *ee*, and 11% acetophenone **2a** (Table 2, entry 5). Nakamura et al. conducted deracemization of 1-arylethanols catalyzed by *Geotrichum candidum* IFO 5767, under aerobic conditions after 24 h of reaction. The racemic alcohols were converted into the corresponding (*R*)-alcohols with excellent *ee* and chemical yields.[22]

The reduction of **2a** was studied in order to elucidate the mechanism involved in the deracemization process of **1a** (Fig. 1). The reduction of **2a** was initially (*S*)-selective, and after 1 hour of incubation 61% of (*S*)-**1a** was detected, with 95% *ee*. Thereafter, the *ee* declined rapidly, and after 24 h **1a** was practically racemic. After 120 h, we obtained (*R*)-**1a** in 92% yield and with 92% *ee*. The (*R*)-**1a** formed during the reduction of **2a** remained unchanged in the reaction medium, while (*S*)-**1a** was oxidized back to ketone **2a**. Therefore, after several cycles of (*S*)-partially selective reduction of **2a** and enantioselective oxidation of (*S*)-**1a**, the concentration of (*R*)-**1a** increased. Nakamura et al. reported similar

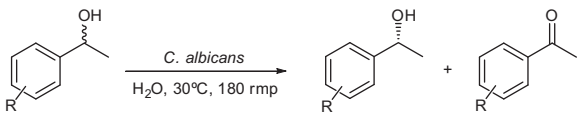
Table 2
Biocatalysis using 200 mg of 1-phenylethanol by resting cells of *C. albicans*



rac-**1a** **2a** (*R*)-**1a**

Entry	Time (h)	**2a** (%)	**1a**	
			(%)	*ee* (%)
1	2	48	52	88 (*R*)
2	12	42	58	66 (*R*)
3	19	41	59	82 (*R*)
4	48	36	64	92 (*R*)
5	144	11	89	98 (*R*)

Table 1
Oxidative kinetic resolution of 1-arylethanols by resting cells of *C. albicans*



rac-**1a-i** (*R*)-**1a-i** 50% **2a-i** 50%

Entry	(*RS*)-1-Arylethanol	R	Time (h)	Ketone (%)	(*R*)-1-Arylethanol	
					Yield (%)	*ee* (%)
1	**1a**	H	1	15	35	93
2	**1b**	4-Cl	50	45	40	87
3	**1c**	4-F	1	41	49	98
4	**1d**	4-Br	1	44	46	99
5	**1e**	4-OH	28	50	47	99
6	**1f**	4-CH_3	44	42	35	99
7	**1g**	4-OCH_3	24	40	33	99
8	**1h**	3-OH	144	42	42	99
9	**1i**	3-OCH_3	3	40	41	90