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Article

Reductive amination of ketones with ammonium catalyzed by a newly identified *Brevibacterium epidermidis* strain for the synthesis of (*S*)-chiral amines

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ABSTRACT

The asymmetric reductive amination of achiral ketones with ammonia is a particularly attractive reaction for the synthesis of chiral amines. Although several engineered amine dehydrogenases have been developed by protein engineering for the asymmetric reductive amination of ketones, they all display (*R*)-stereoselectivity. To date, there is no report of an (*S*)-stereoselective biocatalyst for this reaction. Herein, a microorganism named *Brevibacterium epidermidis* ECU1015 that catalyzes the (*S*)-selective reductive amination of ketones with ammonium has been successfully isolated from soil. Using *B. epidermidis* ECU1015 as the catalyst, the asymmetric reductive amination of a set of phenylacetone derivatives was successfully carried out, yielding the corresponding (*S*)-chiral amines with moderate conversion and >99% enantiomeric excess.

1. Introduction

Optically pure amines are an important class of chiral intermediates frequently used in the synthesis of active pharmaceutical ingredients, fine chemicals, and agrochemicals [1,2]. For example, approximately 40% of the drugs approved by the FDA contain one or more chiral amine moieties [3,4]. Due to the prevalent use of chiral amines in organic synthesis, efficient synthetic methods such as the asymmetric reductive amination of ketones and asymmetric reduction of ketimines using transition metal catalysts have been extensively developed [1,2]. However, the direct asymmetric reductive amination of prochiral ketones with ammonia using transition metal catalysts, a key reaction for the synthesis of chiral amines, remains a challenge in industry [5,6]. Moreover, these transition metal-catalyzed processes are usually expensive and unsustainable, often requiring harsh reaction conditions with tedious protection and deprotection steps.

With advances in protein engineering technology, biocatalytic processes have been extensively developed as promising alternatives to traditional chemocatalytic routes for the synthesis of chiral amines owing to their green credentials [7–14]. Numerous biocatalytic routes including (dynamic) kinetic resolution of racemic amines by lipases [15–18], transaminases [19–24], amine oxidases [25–32], and reductive aminases [33]; asymmetric reduction of imines by imine reductases (IREDs) [34–42] and artificial IREDs [43–45]; and asymmetric amination of ketones by IREDs [46–50], transaminases [51–56], re-

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ductive aminases [57], and amine dehydrogenases (AmDHs) [58–65] have been successfully developed for the synthesis of chiral amines. Among them, the AmDH-catalyzed asymmetric reductive amination of ketones is a particularly attractive route for the direct synthesis of chiral amines because it uses inexpensive ammonia as the amino donor and generates only water as the by-product.

Recently, a wild-type NADH-dependent AmDH from the thermophile *Petrotoga mobilis* was used for this reductive amination reaction. However, the enzyme only exhibited activity toward aliphatic ketoacids and not aliphatic ketones [66]. Bommarius and co-workers developed two new AmDHs via several rounds of protein engineering using naturally existing amino acid dehydrogenases as scaffolds [58,59]. Subsequently, three other engineered AmDHs, including *Rhodococcus* Phe-AmDH from *Rhodococcus* sp. M4 [63], EsLeu-AmDH from *Exiguobacterium sibiricum* [68], and thermostable Cal-AmDH from *Caldalkalibacillus thermarum* [65], were developed using the same approach. These engineered AmDHs were used to synthesize a broad set of chiral amines with excellent enantioselectivity by reductive amination of ketones with ammonia. More importantly, two of them have been combined with alcohol dehydrogenases for the synthesis of chiral amines from inexpensive racemic alcohols through elegant hydrogen borrowing dual-enzyme cascade reactions [67,68]. Very recently, we successfully expanded the substrate scope of three engineered AmDHs by fine-tuning two key residues surrounding the substrate-binding cavity, thus resulting in steric hindrance for the binding of bulky substrates [69].

However, all these engineered AmDHs display (*R*)-stereoselectivity. Therefore, only *R*-configuration chiral amines can be synthesized from the reductive amination of ketones with ammonia by these enzymes. To date, no (*S*)-stereoselective biocatalyst capable of catalyzing the reductive amination of ketones with ammonia has been reported. Herein, we report the isolation of microbial strains from soil samples able to catalyze the reductive amination of ketones using inexpensive inorganic ammonium as the amine donor for the synthesis of (*S*)-chiral amines.

2. Experimental

2.1. Reagents

Acetophenone (**1a**), 3,4-dihydronaphthalen-1(2H)-one (**1b**), 1-(4-fluorophenyl)propan-2-one (*p*FPA) (**1d**), 1-(4-methoxyphenyl)propan-2-one (**1g**), 1-(3-fluorophenyl)propan-2-one (**1e**), 1-(2-fluorophenyl)propan-2-one (**1f**), (*S*)-*α*-methylbenzylamine, (*R*)-*α*-methylbenzylamine, (*S*,*R*)-4-fluoro-α-methylphenethylamine, (*R*)-1-(4-methoxyphenyl) propan-2-amine, (*S*)-1-(4-methoxyphenyl)propan-2-amine, and (*S*)-1,2,3,4-tetrahydronaphthalen-1-amine were purchased from Sigma-Aldrich (Tianjin, China). All the other chemicals used were of analytical grade and commercially available. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer.

2.2. Screening of microbial strains

Different soil samples were collected from nature and enriched using the following medium at 30 °C for 48 h. Enrichment medium (per liter): (*S*)-*α*-methylbenzylamine, 0.6 g (5 mmol); glycerol, 2.0 g; KH_2PO_4, 3.0 g; NaCl, 1.0 g; $MgSO_4{\cdot}7H_2O$, 0.5 g; and trace elements; pH 7.0. The single strain isolated was initially screened for the deamination of (*S*)-*α*-methylbenzylamine. The reaction mixture for deamination contained 5 mmol/L (*S*)-*α*-methylbenzylamine, wet cells from 4 mL cultured broth, and 0.5 mL glycine-NaOH buffer (0.2 mol/L, pH 10.0). The reaction was carried out at 30 °C and 1000 r/min for 24 h, and the products were analyzed by TLC. The strains producing acetophenone were rapidly identified and further screened for the reductive amination of acetophenone with ammonia. The reaction mixture for amination contained 5 mM acetophenone, wet cells from 4 mL cultured broth, 1 mol/L NH_4Cl, and 0.5 mL Tris–HCl buffer (0.2 mol/L, pH 8.0), which was shaken at 30 °C for 24 h. The products extracted from the reaction mixture were identified by GC.

2.3. Identification of the best strain

For the identification of the microorganism, the 16S rDNA gene was amplified via the polymerase chain reaction (PCR) with the universal primer pair AGAGTTTGATCCTGGCTCAG and GGTTACCTTGTTACGACTT. The sequence was analyzed with the Basic Local Alignment Search Tool (BLAST). In order to identify the organism from the 16S rDNA gene sequence, the phylogenetics and molecular evolutionary genetics were constructed using the Clustal Omega and MEGA version 6.06 software. According to the 16S rDNA sequence of strain Es11, it was identified as *Brevibacterium epidermidis* ECU1015.

2.4. Cultivation of B. epidermidis ECU1015

B. epidermidis ECU1015 was grown aerobically at 30 °C for 24 h in an optimized medium with the following composition (per liter): glycerol, 10.0 g; peptone, 5.0 g; yeast extract, 5.0 g; NaCl, 1.0 g; KH_2PO_4, 0.5 g; and $MgSO_4$, 0.2 g; pH 7.0. After cultivation, the cells were harvested by centrifugation (8000 r/min, 10 min) and washed twice with physiological saline.

2.5. Effects of different parameters on the B. epidermidis ECU1015 activity toward reductive amination

2.5.1. Effect of inorganic ammonium concentration

The effect of the concentration of inorganic ammonium on the reductive amination catalyzed by *B. epidermidis* ECU1015 whole cells was studied at different concentrations of NH_4Cl. The reaction mixture (1 mL) contained 5 mmol/L *p*FPA (**1d**), different concentrations of NH_4Cl ranging from 0 to 2 mol/L, 100 g/L wet cells of *B. epidermidis*, 4% (*v*/*v*) DMSO, and potassium phosphate sulfate (KPB) (0.2 mol/L, pH 7.5). The reactions were performed at 30 °C and 1000 r/min for 24 h. The samples were treated by addition of 100 μL NaOH (10 mol/L) and extracted with dichloromethane (600 μL). The organic