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ARTICLE

Efficient Synthesis of (*R*)-2-Chloro-1-(3-chlorophenyl)ethanol by Permeabilized Whole Cells of *Candida ontarioensis*

NI Ye*, ZHANG Beihua, SUN Zhihao

The Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, Jiangsu, China

Abstract: (*R*)-2-Chloro-1-(3-chlorophenyl)ethanol is a key pharmaceutical intermediate in the synthesis of β_3-adrenoceptor receptor (β_3-AR) agonists. The asymmetric reduction of 2-chloro-1-(3-chlorophenyl)ethanone to (*R*)-2-chloro-1-(3-chlorophenyl)ethanol catalyzed by resting cells of *Candida ontarioensis* was studied. At a substrate concentration of 10 g/L, the microbial cells showed excellent catalytic activity under the optimum reaction conditions, giving the product in 99.9% ee and 99.0% yield. After cetyltrimethylammonium bromide-pretreatment, the activity of permeabilized *Candida ontarioensis* cells was increased by more than 2-fold and the product could be produced over the significantly shortened reaction period of 24 h in 99.9% ee and 97.5% yield at a substrate concentration of 30 g/L. This work provides a practical approach for the efficient preparation of the important chiral intermediate (*R*)-2-chloro-1-(3-chlorophenyl) ethanol.

Key words: (*R*)-2-chloro-1-(3-chlorophenyl)ethanol; asymmetric reduction; cetyltrimethylammonium bromide; permeability; *Candida ontarioensis*

Optically pure chiral alcohols are valuable building blocks for the synthesis of pharmaceuticals, agrochemicals, and liquid crystals [1]. The α-halohydrins are particularly important as synthons for the preparation of β-adrenergic drugs [2]. Several chemical and biological approaches for the synthesis of optically active α-halohydrins have been developed [3,4] and the asymmetric reduction of the corresponding prochiral aromatic ketones is regarded as the most promising methodology [5]. Owing to its excellent stereoselectivity, mild reaction conditions, and environmental benignancy, biocatalysis has been reported for the production of a variety of optically pure pharmaceutical intermediates [6–8]. In biocatalytic reactions, whole-cell systems are often preferred to isolated enzymes as the tedious enzyme isolation/purification processes required are eliminated and reactions involving multiple enzymes/pathways as well as co-factor regeneration are allowed [9–11].

(*R*)-2-Chloro-1-(3-chlorophenyl)ethanol is a versatile intermediate for the synthesis of β_3-adrenoceptor receptor (β_3-AR) agonists, such as the anti-depressant drug SR58611 and the thermogenic anti-obesity drug BRL 37344 [12]. Several groups have reported the biocatalytic synthesis of (*R*)-2-chloro-1-(3-chlorophenyl)ethanol. Sawa et al. [13] reported the asymmetric synthesis of (*R*)-2-chloro-1-(3-chlorophenyl) ethanol using whole cells of *Rhodotorula glutinis* at a substrate concentration of 3.25 g/L. Lin and co-workers [14] reported the bioreduction of 2-chloro-1-(3-chlorophenyl) ethanone by *Saccharomyces cerevisiae*, providing the product after 48 h in 99% ee and 97% yield at a substrate concentration of 28 g/L. Recombinant *E. coli* cells and cell-extracts from *Nocardia globerula* and *Rhodotorula glutinis* have also been reported for the asymmetric production of (*R*)-2-chloro-1-(3-chlorophenyl) ethanol in over 99% ee. However, the inclusion of expensive additives such as $NADP^+/NAD^+$ and glucose dehydrogenase

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*Corresponding author. Tel/Fax: +86-510-85329265; E-mail: yni@jiangnan.edu.cn
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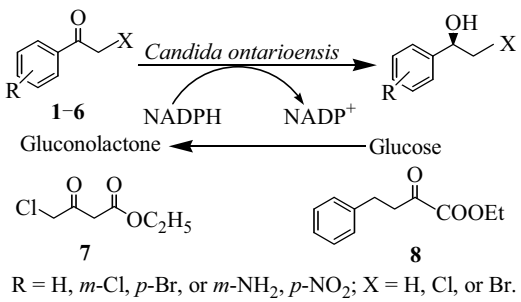

Scheme 1. Bioreduction of compounds **1**–**8** with *Candida ontarioensis* whole cells.

was required for regeneration of the cofactor [15–18].

In this study, *Candida ontarioensis* was selected from a screening panel of 90 strains in our laboratory collection because of its high carbonyl reductase activity towards various halo-acetophenone substrates and 2-chloro-1-(3-chlorophenyl) ethanone was used as a model substrate to investigate the asymmetric reduction catalyzed by whole-cells of *Candida ontarioensis* (Scheme 1). Using cetyltrimethylammonium bromide (CTAB)-permeabilized cells, the reaction time was significantly reduced from 72 to 24 h and the product was obtained in 99.9% ee and 97.5% yield at a 30 g/L substrate concentration.

1 Experimental

1.1 Screening and identification of the target strain

Three strains were screened from the 90 strains in our laboratory using 10 g/L 2-chloro-1-(3-chlorophenyl)ethanone (Porton Co., Ltd, China) as the substrate and *Candida* sp. BC 2A showed the highest carbonyl reductase activity. The 18S rRNA gene sequence of *Candida* sp. BC 2A was determined (GenBank number: JN820129), identifying the strain as *Candida ontarioensis*.

1.2 Cultivation conditions of *Candida ontarioensis*

The fermentation medium was composed of 1% (w/v) glucose, 2% (w/v) corn steep liquor power, and 0.005% (w/v) $CaCl_2$ and was adjusted to pH 6.5. The microorganism was cultured at 30 °C for 20 h with 180 r/min shaking. The cells were collected by centrifugation at 2350 × *g* for 5 min and washed twice with 0.85% saline.

The cell pellet was treated with 4 g/L CTAB at 4 °C for 20 min to improve permeability. The CTAB-treated and untreated *Candida ontarioensis* cells were used as biocatalysts in the bioconversion of 2-chloro-1-(3-chlorophenyl)ethanone.

1.3 Asymmetric bioreduction of 2-chloro-1-(3-chlorophenyl)ethanone in aqueous medium

The reaction system consisted of 1 g of wet cells (collected from 30 ml of culture broth), glucose (5%, w/v), the appropriate amount of 2-chloro-1-(3-chlorophenyl)ethanone, and potassium phosphate buffer (0.2 mol/L, pH 6.5), in a final volume of 10 ml. The reaction was conducted in a 50-ml Erlenmeyer flask with stopper and shaken at 30 °C and 180 r/min for 72 h. The mixture was extracted with an equal volume of ethyl acetate and the organic layer was concentrated under vacuum and diluted with mobile phase for subsequent HPLC analysis.

1.4 Activity assay

The carbonyl reductase activity of *Candida ontarioensis* cells was measured according to the reaction conditions mentioned above by analyzing the reaction during the first 30 min to determine the initial velocity. One unit of the enzyme activity (U) is defined as the amount of enzyme required for generation of 1 μmol (*R*)-2-chloro-1-(3-chlorophenyl)ethanol per minute.

The enantiomeric excess (ee) and analytic yield of (*R*)-2-chloro-1-(3-chlorophenyl)ethanol were determined on an Agilent 1100 HPLC (California, USA) equipped with a 5 μm Chiralcel OB-H (0.46 mm × 250 mm, Tokyo, Japan) column using an UV detector at 220 nm. The HPLC was performed using *n*-hexane/isopropanol (9/1, HPLC grade, Xingke Chemical Reagent Co., Ltd, China) as the mobile phase at a flow rate of 1.0 ml/min and the column temperature was 30 °C.

2 Results and discussion

2.1 Cell growth and carbonyl reductase production by *Candida ontarioensis*

The time course of cell growth and carbonyl reductase production by *Candida ontarioensis* was investigated. As shown in Fig. 1, the cell growth continued throughout the entire fermentation process and the carbonyl reductase activity of whole-cells increased rapidly over the first 20 h. A maximum activity of 25.4 U/L (0.74 U/g_{WCW}) and a cell mass of 34.5 g_{WCW}/L (WCW = wet cell weight) was reached at the 20 h mark. A decrease in enzymatic activity was evident after 20 h because of the degradation of reductase by protease at the end of the fermentation process. Thus, *Candida ontarioensis* cells were collected after 20 h of the fermentation process for further bioreduction studies.

2.2 Optimization of bioreduction conditions

Major parameters in the asymmetric reduction of 2-chloro-1-(3-chlorophenyl)ethanone were investigated, including buffer pH, temperature, cell concentration, and cosubstrate (for