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Microbial stereospecific reduction of 3-quinuclidinone with newly isolated *Nocardia* sp. and *Rhodococcus erythropolis*

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ABSTRACT

Two bacterium strains, *Nocardia* sp. WY1202 and *Rhodococcus erythropolis* WY1406, were isolated from soil samples. They catalyzed the asymmetric reduction of 3-quinuclidinone to give enantiomeric pure (*R*)- and (*S*)-3-quinuclidinol, respectively. The optimal temperatures for the bioreduction by *Nocardia* sp. and *R. erythropolis* were 30 °C and 37 °C, respectively, while both strains showed highest activity at pH 8.0. Without external addition of expensive NADH or NADPH, (*R*)-3-quinuclidinol and (*S*)-3-quinuclidinol were obtained with 93% and 92% isolated yield and >99% enantiomeric excess. As such, microbial reduction by *Nocardia* sp. WY1202 or *R. erythropolis* WY1406 offers a new stereospecific approach to both antipodes of 3-quinuclidinol of pharmaceutical importance.

1. Introduction

Optically pure 3-quinuclidinol is an important intermediate for the synthesis of various anticholinergic drugs [1,2]. For example, (*R*)-(−)-3-quinuclidinol was used to synthesize muscarinic M_1 or M_3 receptor antagonists such as talsaclidine [3], revatropate [4], and solifenacin [5]. (*S*)-(+)-3-Quinuclidinol is a very promising chiral building blocks for synthesis of serotonin receptor antagonist drugs and new anticholinergic drugs [6,7]. The synthesis of optically pure 3-quinuclidinol has been achieved both by chemical [8,9] and biocatalytic methods [10–14]. Most of the reported procedures involved the resolution of a racemic mixture of 3-quinuclidinol derivatives, which are limited by the theoretical yield of 50% and/or low optical purity [9–11,13,14], or an extra recamization step is required for improving the yield [15]. Asymmetric hydrogenation of 3-quinuclidinone with $RuBr_2$[(*S*,*S*)-xylskewphos](pica) in a base containing ethanol has been reported to afford (*R*)-3-quinuclidinol in 88–90% enantiomeric excess (ee) (XylSkewphos = 2,4-bis(di-3,5-xylylphosphino)pentane, PICA = α-picolylamine) [8]. Enzymatic reduction of 3-quinuclidinone required addition of expensive cofactor $NADP^+$, although (*R*)-3-quinuclidinol could be obtained with high enantiomeric purity [12]. In this context, microbial reduction would offer an attractive alternative because of efficient *in situ* supply of cofactors.

In this report, we described the isolation and characterization of two bacterial strains which were able to stereospecifically reduce 3-quinuclidinone to produce (*R*)-3-quinuclidinol and (*S*)-3-quinuclidinol, respectively. The bioreduction conditions using *Nocardia* sp. WY1202 and *Rhodococcus erythropolis* WY1406 as whole-cell biocatalyst were optimized and their application potential in the synthesis of optically pure (*R*)- and (*S*)-3-quinuclidinol was explored.

2. Experimental

2.1. Materials and analytical methods

3-Quinuclidinone hydrochloride (98.0% purity) and (*R*)-3-quinuclidinol (99.0% purity) were purchased from Shanghai Chemical Co. Ltd., China. The other chemicals and the culture media were obtained from commercial sources. Soil samples used for screening were collected from different areas of China.

The following media were used for bacteria isolation, growth and maintenance. Mineral salt medium (MSM) contained 1 g of $K_2HPO_4{\cdot}3H_2O$, 1 g of $Na_2HPO_4{\cdot}3H_2O$, 2 g of $NH_4H_2PO_4$, 2 g of $NaNO_3$, 0.2 g of $MgSO_4{\cdot}7H_2O$, 10 mg of $CaCl_2{\cdot}2H_2O$, 1 mg of $FeSO_4{\cdot}7H_2O$, 0.1 mg of $ZnSO_4{\cdot}7H_2O$ in 1 l of distilled water. Rich medium 1 (RM 1) [16] contained 15 g of glucose, 5 g of peptone, 5 g of yeast extract, 0.5 g of K_2HPO_4, 0.5 g of KH_2PO_4, 0.5 g of $MgSO_4{\cdot}7H_2O$ and 1 g of NaCl in 1 l of distilled water, and was

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adjusted to pH 7.0. Rich medium 2 (RM 2) contained 20 g of glucose, 21 g of peptone, 9 g of yeast extract, 2 g of K_2HPO_4, 0.75 g of KH_2PO_4, 1.8 g of $MgSO_4 \cdot 7H_2O$ and 2 g of NaCl in 1 l of distilled water, and was adjusted to pH 8.0.

Gas chromatography (GC) analysis was performed by Agilent 7890 gas chromatography equipped with FID and Gamma DEX 225 column (SUPELCO, USA, 30 m × 0.25 mm × 0.25 μm) under the following conditions: The injector and detector temperatures were 220 °C, He is used as the carrier gas with a flow rate of 2 ml/min. Column temperature was 120 °C. The retention times were 13.83, 18.02 and 20.13 min for 3-quinuclidinone, (*S*)- and (*R*)-quinuclidinol, respectively. Alternatively, the optical purity of 3-quinuclidinol was also measured by benzoylating the product with benzoyl chloride which was analyzed by high performance liquid chromatography (HPLC) under the following conditions: chiral colum CHIRALPAK AD-H (Daicel Chemical Industries Ltd., 4.6 mm × 250 mm); mobile phase, hexane–isopropanol (90/10) at flow rate of 0.8 ml/min; detection wavelength, 254 nm. The retention times were 12.76 and 21.98 min for (*R*)- and (*S*)-quinuclidinol, respectively.

2.2. Isolation of microorganisms

Soil samples (5 g) were suspended in 50 ml of sterilized water in a 250 ml Erlenmeyer flask with a number of glass beads to scatter the soil sample. The flask was shaken at 30 °C and 200 rpm for 1 h and rested for 20 min, and then 5 ml of the supernatant was transferred into 50 ml of sterilized liquid medium (MSM) in a 250 ml flask. Different concentrations of 3-quinuclidinone-HCl (1–10 g/l, 6.2–62 mM) was mixed with the liquid medium. The enrichment culture was carried out at 30 °C and 200 rpm for 2–5 days, and then 5 ml of the culture liquid was transferred into a fresh medium with the same composition for another round of enrichment culture (2–5 days). 0.2 ml of the culture were sprayed onto the MSM agar plates with 6 g/l (37 mM) of 3-quinuclidinone-HCl. The plates were placed on incubator at 30 °C and monocolonies were individually inoculated into RM 1 agar slopes. The pure isolated strains were inoculated into 20 ml of sterilized RM 1 in a 100 ml flask which were constantly shaken for 24–48 h, and then 3-quinuclidinone-HCl (about 6 g/l, 37 mM) was added for transformation. The reaction mixture was incubated at 30 °C, 200 rpm. Samples were withdrawn after every 12 h up to 96 h and alkalified by the addition of K_2CO_3, then extracted with butyl alcohol (1:1, v/v) and analyzed by thin-layer chromatography (TLC). The samples with an obvious product spot were further analyzed by GC and HPLC for determination of conversion and ee.

2.3. Phenotypic, biochemical characterization and 16S rDNA sequence analysis

Cell morphology of the selected strain was observed *via* a light microscope (Olympus CX21, Japan). Conventional physiological and biochemical characterization tests were carried out using physiological and biochemical kit, as described in Bergey's Manual of Determinative Bacteriology.

Total genomic DNA was extracted using TIANamp Bacteria DNA kit (Tiangen Biotech, Beijing, China). The 16S rDNA was amplified by PCR (96 °C for 5 min, one cycle; 94 °C for 30 s/52 °C for 30 s/72 °C for 90 s, 29 cycles; 72 °C for 10 min, one cycle) using dNTPs mix (0.2 mmol l^{-1}), universal primers (27F: 5′-AGAGTTTGATCCTGGCTCAG-3′, 1494R: 5′-GGTTACCTTGTTACGACTT-3′; 0.5 μmol l^{-1} each), genomic DNA template (100 ng) and Taq polymerase (2.5 U) in a final volume of 50 μl. PCR products were purified using the TIANgel Midi Purification kit (Tiangen Biotech, Beijing, China) and sequenced. The 16S rDNA sequences were submitted to GenBank on NCBI for BLAST search. A phylogenetic tree was constructed by using Clustal X version 2.0, MEGA version 4.0 [17] based on the homologous 16S rDNA sequences.

2.4. Cultivation conditions and preparation of resting cells

Nocardia sp. WY1202 was inoculated into 20 ml of RM 2 for 48 h and 10 ml of the culture liquid (inoculum size 1:50) was transferred into 500 ml of RM 2 which was constantly shaken at 30 °C and 250 rpm for 72 h. *R. erythropolis* WY1406 was inoculated into 20 ml of RM 1 for 24 h and 4 ml of the culture liquid (inoculum size 1:50) was transferred into 200 ml of RM 2 which was constantly shaken at 30 °C and 250 rpm for 48 h. The cells were harvested by centrifugation (7875 × *g*, 20 min) and washed thoroughly with physiological saline (0.85% NaCl) to give the resting cells for use in the reduction.

2.5. Time course of carbonyl reductase production of Nocardia sp. WY1202 and R. erythropolis WY1406

Nocardia sp. WY1202 and *R. erythropolis* WY1406 were cultivated as described above. The cell dry weight (CDW) or the OD value of the cell culture was measured at an interval of 8 h.

Similarly, two strains were cultured under the same conditions to different growth periods. The corresponding resting cells were prepared as described in Section 2.4 and used for determining the carbonyl reductase activity by measuring the conversion for the reduction of 3-quinuclidinone. The reduction of 3-quinuclidinone hydrochloride (6 g/l, 37 mM) in phosphate buffer (100 mM, pH 7.0) containing 10 g/l of glucose by *Nocardia* sp. (80 g/l of wet cells) was carried out at 30 °C for 12 h under aerobic conditions. Similarly, the reduction by *R. erythropolis* (85 g/l of wet cells) was carried out in the presence of 15 g/l of glucose for 24 h. The reaction mixture (400 μl) was alkalified by addition of an equal volume of saturated K_2CO_3 and then the resulting mixture was extracted with 800 μl of butyl alcohol. The extract was dried over anhydrous sodium sulfate and analyzed by GC to determine the conversion and enantioselectivity.

2.6. Effects of added glucose, pH and temperature on the reduction of 3-quinuclidinone by resting cells of Nocardia sp. WY1202 and R. erythropolis WY1406

In the studies on the effect of added glucose, for *Nocardia* sp., the reaction mixture containing 60 mg (0.37 mmol) of 3-quinuclidinone hydrochloride, certain amount of glucose and 0.8 g of wet cells harvested from 55 ml of the culture broth in 10 ml of phosphate buffer (100 mM, pH 7.0) was shaken at 30 °C and 200 rpm. For *R. erythropolis*, 0.85 g of wet cells harvested from 25 ml of the culture broth was used and the reaction was carried out in phosphate buffer (100 mM, pH 8.0) at 37 °C. The work-up and determination of conversion followed the procedures described in Section 2.5.

The reduction reactions were also carried out at 30 °C and various pHs using 100 mM buffers (sodium citrate buffer for pH 3.0–6.0, K_2HPO_4/KH_2PO_4 for pH 6.0–8.0, Tris–HCl for pH 8.0–9.0, $Na_2CO_3/NaHCO_3$ for pH 9.0–10.0), as described above, to evaluate the pH effect. 10 g/l and 20 g/l of glucose was used and the mixtures were shaken for 16 and 40 h for *Nocardia* sp. WY1202 and *R. erythropolis* WY1406, respectively. To determine the temperature effect, the reductions were performed under the same conditions except the pH was kept at 8.0 and the reaction temperature was varied from 20 to 45 °C.

In order to evaluate the effect of substrate concentration on the reduction of 3-quinuclidinone, for *Nocardia* sp. WY1202, 0.8 g of wet cells were added into 10 ml of phosphate buffer solution (100 mM, pH 8.0) containing 40–300 mg (0.25–1.86 mmol)

of 3-quinuclidinone-HCl, 67–500 mg of glucose. The mol ratio between glucose and substrate was about 1.5. The resulting mixture was shaken at 30 °C and 200 rpm for 48 h. For *R. erythropolis* WY1406, the bioreduction was carried out at 37 °C and 200 rpm for 48 h, in which 0.85 g of wet cells were added into 10 ml of phosphate buffer solution (100 mM, pH 8.0) containing 40–200 mg (0.25–1.24 mmol) of 3-quinuclidinone-HCl, 133–667 mg of glucose. The mol ratio between glucose and substrate was about 3.0.

The time courses of the bioreduction of 3-quinuclidinone by *Nocardia* sp. WY1202 and *R. erythropolis* WY1406 were investigated under the optimized reaction conditions. For *Nocardia* sp. WY1202, 0.80 g of wet cells were added into 10 ml of phosphate buffer solution (100 mM, pH 8.0) containing 160 mg (0.99 mmol) of 3-quinuclidinone-HCl, 270 mg of glucose, and the bioreduction was carried out at 30 °C and 200 rpm. While for *R. erythropolis* WY1406, 0.85 g of wet cells, 80 mg (0.495 mmol) of 3-quinuclidinone-HCl and 270 mg of glucose were used and the bioreduction was carried out at 37 °C and 200 rpm. Samples were drawn at the different intervals, worked-up and analyzed as described in Section 2.5.

2.7. Preparation of 3-quinuclidinol

For *Nocardia* sp. WY1202, 3-quinuclidinone hydrochloride (1.6 g, 9.9 mmol) and glucose (2.7 g) were added into suspension of resting cells (8 g, wet weight) in 100 ml of phosphate buffer (100 mM, pH 8.0). The resulting mixture was incubated at 30 °C and 200 rpm for 48 h. For *R. erythropolis* WY1406, 3-quinuclidinone hydrochloride (1.0 g, 6.2 mmol) and glucose (3.3 g) were added into suspension of resting cells (8.5 g, wet weight) in 125 ml of phosphate buffer (100 mM, pH 8.0). The resulting mixture was incubated at 37 °C and 200 rpm for 30 h.

After centrifugation at 7875 × *g* for 20 min, the bacterial cells were washed with water and centrifuged again. The supernatants were combined and alkalified by addition of K_2CO_3 (pH 12). The mixture was evaporated under vacuum, then CH_2Cl_2 was added to the residue. The mixture was stirred and filtrated. The filtrate was concentrated under vacuum to give crude product. Then acetone was added to the crude product, stirred and concentrated under vacuum to yield 3-quinuclidinol as white powder. 1.17 g (9.20 mmol) (*R*)-3-quinuclidinol (93% yield) and 0.73 g (5.74 mmol) (*S*)-3-quinuclidinol (92% yield) were obtained. The ee value were all >99%. ^{1}H NMR: (600 MHz, $CDCl_3$) δ_{ppm}: 1.33 (m, 1H), 1.43 (m, 1H), 1.65 (m, 1H), 1.80 (m, 1H), 1.90 (m, 1H), 2.25 (br, 1H), 2.60 (m, 1H), 2.65 (m, 1H), 2.74 (m, 2H), 2.89 (m, 1H), 3.12 (m, 1H), 3.85 (m, 1H).

3. Results and discussion

3.1. Isolation and identification of strain WY1202 and WY1406

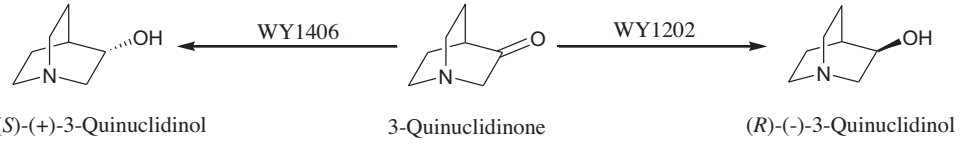
In the screening procedure, 24 strains were able to reduce 3-quinuclidinone to the corresponding chiral alcohol. Among them, two strains reduced 3-quinuclidinone to give (*R*)- or (*S*)-3-quinuclidinol with >95% conversion and >99% ee, respectively, which were marked as WY1202 and WY1406 (Scheme 1).

Various morphological, biochemical and physiological tests, 16S rDNA sequence analysis of the strains WY1202 and WY1406 indicated that they were different in many aspects. Colonies on the plate of strain WY1202 were yellow, rough and wrinkled. The mycelia of cells had partitions and aerial mycelia were broken into long rhabditiform isolates. Colonies on the plate of strain WY1406 were circular, wet and smooth. The color was transformed from soft yellow to orange red after 2–5 days at 30 °C. Cells of the strain were short rhabditiform and each cell was divided into a few coccoid-shaped isolates at the later stage. The physiological characteristics of WY1202 and WY1406 were investigated. Apart from D-glucose and glycerol, various sugars and alcohols like maltose, sucrose, arabinose, mannitol, sorbitol were not utilized by strain WY1202. Liquefaction of gelatin was negative, as well as starch hydrolysis, tyrosine hydrolysis. Strain WY1406 utilized most of the tested sugars except mannitol. Catalase reaction was positive, as well as methyl red test, nitrate reduction reaction. Comparative database analysis of 16S rDNA sequences suggested that strain WY1202 was very similar with strains in the genus of *Nocardia* and strain WY1406 was closely related to several strains in the genus of *Rhodococcus* (see Supplemental Materials). The sequence similarity between stain WY1202 and some *Nocardia* strains was ≥97%, which revealed that stain WY1202 belongs to the genus *Nocardia*. Strain WY1406 was further identified by China General Microbiological Culture Collection Center (CGMCC, Beijing, China) as *R. erythropolis*. Strain WY1202 and WY1406 were deposited at CGMCC under accession number 5095, 5096, respectively.

To the best of our knowledge, only limited microorganisms have been reported for the biocatalytic asymmetric reduction of 3-quinuclidinone to (*R*)- or (*S*)-3-quinuclidinol and the majority of them have been yeasts [12]. Therefore, *Nocardia* sp. WY1202 and *R. erythropolis* WY1406 are among the very few bacteria reported to exhibit catalytic activity for the asymmetric reduction of 3-quinuclidinone [18].

3.2. Course of carbonyl reductase production of Nocardia sp. WY1202 and R. erythropolis WY1406

To further characterize the newly isolated *Nocardia* sp. WY1202 and *R. erythropolis* WY1406, the time-courses of the carbonyl reductase production were monitored by cultivating two strains in the medium and measuring the reductase activity at different cultivation stages. As shown in Fig. 1, the reductase activity of *Nocardia* sp. WY1202 increased in parallel with cell growth during the period of 72 h and the (*R*)-3-quinuclidinol concentration reached the maximum within 72 h. After 72 h of growth, the reductase activity almost kept stable that is different from *Williopsis saturnus* var. *mrakii* [19], in which reductase activity decreased obviously after about 20 h. For *R. erythropolis* WY1406, the reductase activity increased at the early stage of the cell growth and reached the maximum activity after 48 h (Fig. 2). At all stages, the enantioselectivity was unchanged (ee >99%) for both strains. This is in contrast to findings with other reductases from *Trichosporon captitatum* [20] and *Candida magnoliae* [21]. This provides an advantage that highly enantioselective 3-quinuclidinol-producing cells can be easily prepared.



(*S*)-(+)-3-Quinuclidinol 3-Quinuclidinone (*R*)-(-)-3-Quinuclidinol

Scheme 1. Bioreduction of 3-quinuclidinone to (*S*)- or (*R*)-3-quinuclidinol by isolated microorganisms.

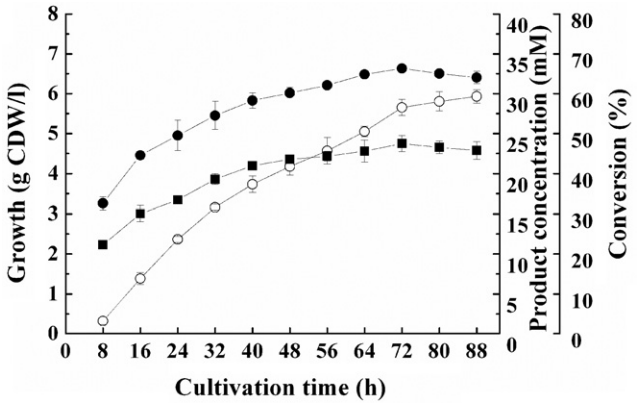


Fig. 1. Time-course of cell growth and enzyme production by *Nocardia* sp. WY1202. (○) Cell growth; (●) conversion of 3-quinuclidinone; (■) concentration of (*R*)-3-quinuclidinol.

3.3. Optimization of the bioreduction conditions of 3-quinuclidinone with Nocardia sp. WY1202 and R. erythropolis WY1406

The optimal reaction conditions were investigated by using resting cells of *Nocardia* sp. WY1202 and *R. erythropolis* WY1406 grown until optimal culture stage. Cofactor recycling is one of the most crucial issues encountered in bioreductions [22] and the cofactor regeneration with glucose as a co-substrate is preferred because of its availability and low cost. For *Nocardia* sp. WY1202, as shown in Fig. 3, the addition of glucose accelerated the reaction and the substrate conversion reached 73% (ee >99%) with 10 g/l glucose in the reaction system. Higher glucose concentration had no effect on reduction, suggesting that 10 g/l glucose is enough for cofactor regeneration. The optimal molar ratio between glucose and substrate is about 1.5.

The effect of glucose on the reduction was found to be significant for *R. erythropolis* WY1406 (Fig. 3). With 5 g/l glucose in the reaction system, the conversion increased 2-fold. The conversion increased as more glucose was added into the reaction system, and the conversion reached 92% with 20 g/l glucose. The results suggest that 20 g/l glucose is enough for cofactor regeneration with the optimal molar ratio between glucose and substrate being about 3.0.

The effects of initial pH and temperature on the reduction of 3-quinuclidinone by *Nocardia* sp. WY1202 and *R. erythropolis* WY1406 were studied, and the results are presented in Figs. 4 and 5. For *Nocardia* sp. WY1202, no product was obtained when pH was below 5.0. The conversion of 3-quinuclidinone improved with the increase

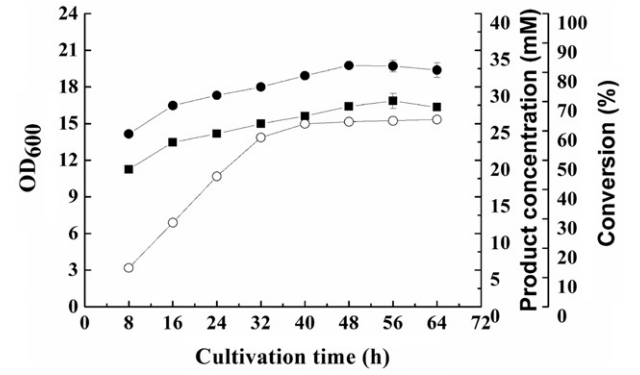


Fig. 2. Time-course of cell growth and enzyme production by *R. erythropolis* WY1406. (○) Cell growth; (●) conversion of 3-quinuclidinone; (■) concentration of (*S*)-3-quinuclidinol.

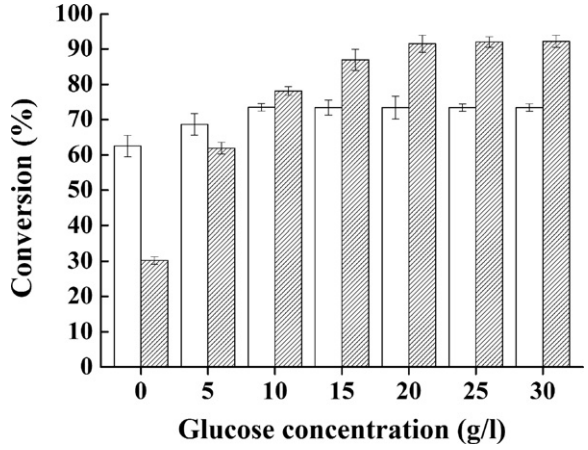


Fig. 3. The effect of glucose addition on the reduction of 3-quinuclidinone by *Nocardia* sp. WY1202 (□) and *R. erythropolis* WY1406 (▨).

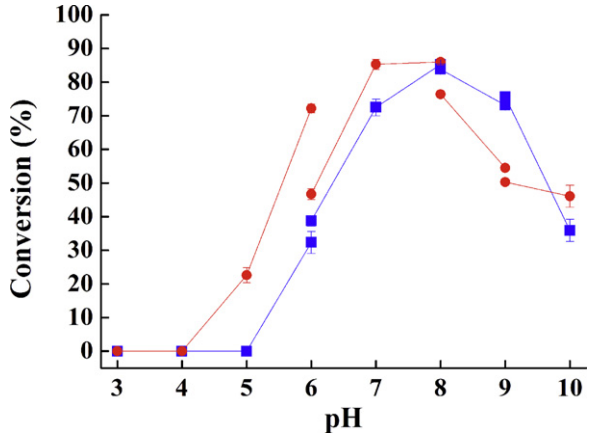


Fig. 4. The effect of pH on the reduction of 3-quinuclidinone by *Nocardia* sp. WY1202 (■) and *R. erythropolis* WY1406 (●).

of buffer pH from 5.0 to 8.0. Obviously, the optimal buffer pH for asymmetric reduction by *Nocardia* sp. was 8.0, with 85% conversion (ee >99%). The reduction of 3-quinuclidinone by *Nocardia* sp. WY1202 reached highest conversion at 30 °C (Fig. 5).

For *R. erythropolis* WY1406, the reaction reached maximum conversion at the pH range of 7.0–8.0 (ee >99%), but no activity was observed with pH below 4.0 (Fig. 4). The optimal reaction

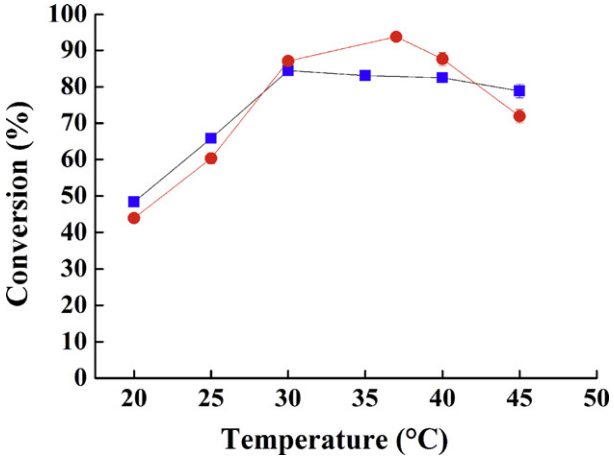


Fig. 5. The effect of reaction temperature on the reduction of 3-quinuclidinone by *Nocardia* sp. WY1202 (■) and *R. erythropolis* WY1406 (●).

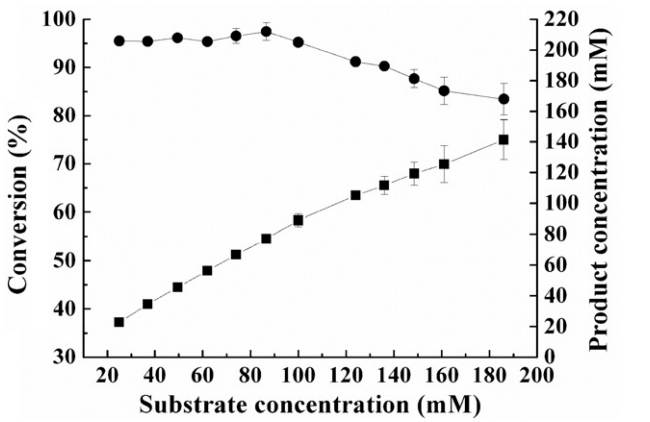


Fig. 6. The effect of substrate concentration on the reduction of 3-quinuclidinone by *Nocardia* sp. WY1202. (●) Conversion of 3-quinuclidinone; (■) concentration of (*R*)-3-quinuclidinol.

temperature for reduction of 3-quinuclidinone by *R. erythropolis* WY1406 was 37 °C (Fig. 5).

Several concentrations of 3-quinuclidinone hydrochloride were tested for the reduction by *Nocardia* sp. WY1202 and *R. erythropolis* WY1406, and the results are presented in Figs. 6 and 7, respectively. When the concentration of 3-quinuclidinone hydrochloride was increased up to 99 mM, the reaction achieved 95.3% conversion (89 mM product alcohol) within 48 h. When the concentration was further increased to 186 mM, the conversion decreased to 85% at 48 h. The product concentration increased almost linearly with the increase of substrate concentration within the range of 25–186 mM, without any effect on the high stereoselectivity of the whole-cell reductase (always >99% ee). This suggests that little substrate inhibition occurred within the substrate concentration.

For *R. erythropolis* WY1406, it is clear from Fig. 7 that, when the concentration of 3-quinuclidinone hydrochloride was below 62 mM, the reaction achieved 95% conversion (56.3 mM product alcohol) in 48 h. Further increased the concentration to 124 mM, the conversion decreased to 70% at 48 h. The product concentration increased with the increase of substrate concentration within the range of 25–99 mM, when the substrate concentration was higher than 99 mM, the product concentration almost did not increase, suggesting that substrate inhibition occurred. The ee value diminished when the substrate concentration was higher than 62 mM.

The time-courses of the bioreduction of 3-quinuclidinone catalyzed by these strains under the optimized reaction conditions

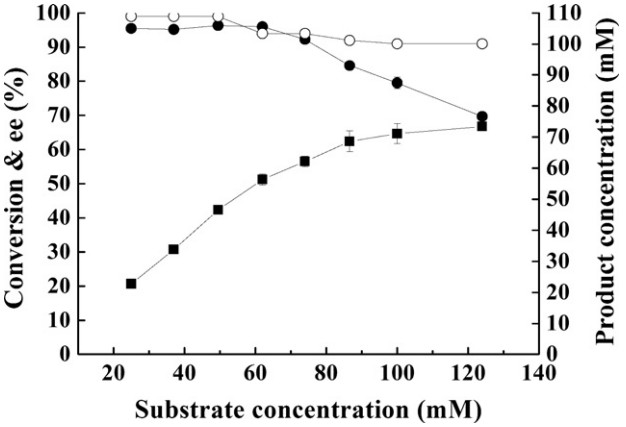


Fig. 7. The effect of substrate concentration on the reduction of 3-quinuclidinone by *R. erythropolis* WY1406. (●) Conversion of 3-quinuclidinone; (■) concentration of (*S*)-3-quinuclidinol; (○) enantiomeric excess of (*S*)-3-quinuclidinol.

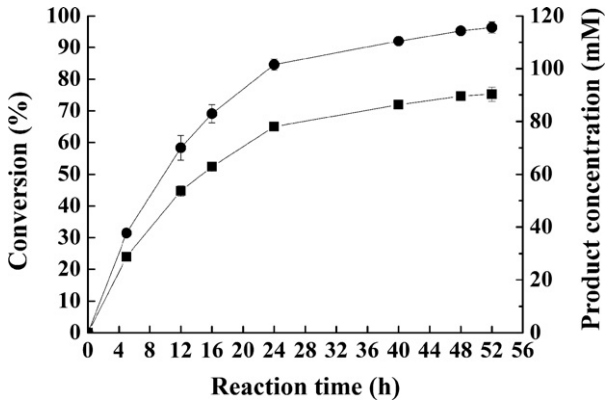


Fig. 8. Time course of the bioreduction of 3-quinuclidinone by *Nocardia* sp. WY1202. (●) Conversion of 3-quinuclidinone; (■) concentration of (*R*)-3-quinuclidinol.

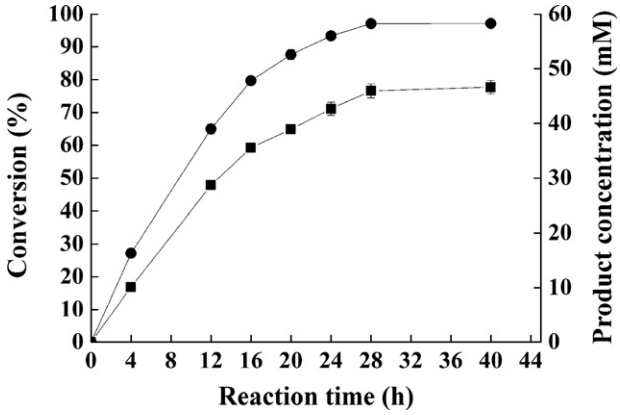


Fig. 9. Time course of the bioreduction of 3-quinuclidinone by *R. erythropolis* WY1406. (●) Conversion of 3-quinuclidinone; (■) concentration of (*S*)-3-quinuclidinol.

were investigated. The results showed that the reaction strain WY1202 was almost completed and 89.6 mM of (*R*)-3-quinuclidinol was produced from 99 mM of 3-quinuclidinone hydrochloride after 48 h (Fig. 8). Similarly, the bioreduction of 3-quinuclidinone catalyzed by strain WY1406 was almost completed in 28 h and 45.9 mM (*S*)-3-quinuclidinol was produced from 49.5 mM of 3-quinuclidinone hydrochloride (Fig. 9). For both reactions, the optical purity of (*R*)- and (*S*)-3-quinuclidinol remained constant throughout the whole process (ee >99%).

3.4. Preparation of (R)- and (S)-3-quinuclidinol

The preparation of (*R*)- and (*S*)-3-quinuclidinol was performed under the optimized condition by resting cells of *Nocardia* sp. WY1202 and *R. erythropolis* WY1406, respectively. At about 100 mM of 3-quinuclidinone, optically pure (*R*)-3-quinuclidinol (ee >99%) was obtained in 93% yield. Similarly, (*S*)-3-quinuclidinol was prepared in 92% yield and >99% ee. These results demonstrated the application potential of *Nocardia* sp. WY1202 and *R. erythropolis* WY1406 in the production of this pharmaceutically important compound.

4. Conclusion

In this study, two 3-quinuclidinone reductase producing bacterial strains were isolated from soil samples and identified as *Nocardia* sp. WY1202, *R. erythropolis* WY1406, based on physiological and biochemical characteristics, and 16S rDNA gene sequence.

Nocardia sp. WY1202 and *R. erythropolis* WY1406 can reduce 3-quinuclidinone to optically pure (*R*)- or (*S*)-3-quinuclidinol, respectively. The bioreduction conditions (pH, temperature and amount of glucose) by these two strains were optimized. Under the optimized conditions, optically pure (*R*)- and (*S*)-3-quinuclidinol were obtained with 93% and 92% isolated yield without addition of expensive cofactor, demonstrating that microbial reduction by *Nocardia* sp. WY1202 and *R. erythropolis* WY1406 offers a new approach to the stereospecific synthesis of (*R*)- or (*S*)-3-quinuclidinol. These results suggest that highly enantiospecific 3-quinuclidinone reductases may exist in *Nocardia* sp. WY1202 and *R. erythropolis* WY1406. Their purification, characterization and application for the synthesis of other optically pure alcohols are in progress in our laboratory.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2012.11.013.

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