Asymmetric synthesis of chiral cyclic amine from cyclic imine by bacterial whole-cell catalyst of enantioselective imine reductase†

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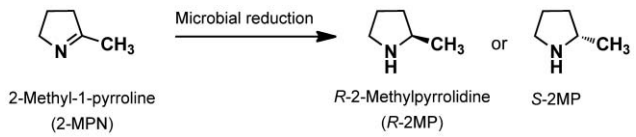
***Streptomyces* sp. GF3587 and 3546 were found to be imine-reducing strains with high *R*- and *S*-selectivity by screening using 2-methyl-1-pyrroline (2-MPN). Their whole-cell catalysts produced 91 mM *R*-2-methylpyrrolidine (*R*-2-MP) with 99.2%e.e. and 27.5 mM *S*-2-MP (92.3%e.e.) from 2-MPN at 91–92% conversion in the presence of glucose, respectively.**

Chiral amines have broad utility for the syntheses of various pharmaceutical and agrochemical intermediates. Numerous syntheses of optically active amines have been studied for many years. Recently, catalytic asymmetric syntheses of amines have attracted attention and have been extensively studied. In the reduction of imine in organic solvent, chiral amine can be synthesized with high enantioselectivity by well-designed transition metal catalysts or organocatalysts.[1]

For enzymatic syntheses of chiral amines, several approaches have been suggested. They include kinetic or dynamic kinetic resolutions with hydrolases, enantioselective desymmetrization with lipase, asymmetric synthesis with amine dehydrogenase, asymmetric synthesis with transaminase and deracemization with monoamine oxidase.[2] In contrast, enzymatic reduction of imines seemed to be difficult because most of them are very unstable in water and rapidly degraded into aldehyde or ketone and ammonia or amine. Stephens *et al.* proposed a novel screening method of imine reductase activity in a biphasic system of water-tetradecane. They found that *Acetobacterium woodii* DSM 1030 catalyzed not only the reduction of C=C double bond of phenylacrylate, such as caffeate,[3,4] but also the reduction of aryl or alkyl imines.[5] Recently, asymmetric reduction of aryl imines synthesized from acetophenone and aniline was examined using whole cells of *Candida parapsilosis* ATCC 7330, having carbonyl reductase activity.[6] Although the imine reduction yielded *R*-amine with moderate conversion and high optical purity, the activity was very low and the addition of large amounts of cells was required to respond to the imine reduction.[7]

We attempted to search for a novel imine-reducing enzyme exhibiting high activity and high enantioselectivity. We used a cyclic imine, 2-methyl-1-pyrroline (2-MPN), which is very stable in water. An optically active 2-methylpyrrolidine (2-MP), *R*-2-MP can be useful for chiral building block of H_3 histamine receptor antagonist, ABT-239.[8] In the present study, we have reported the occurrence of bacterial cyclic imine-reducing activity with high enantioselectivity. We also examined the possibility of the efficient production of *R*- and *S*-2-MP using whole-cell catalyst.

2-MPN, which is barely hydrolyzed at neutral pH, is suitable for the screening of imine reductase activity in microorganisms (Scheme 1). In the screening of imine reductase, we initially expected that yeasts such as *Candida*, *Rhodotorula*, *Saccharomyces*, *Sporobolomyces* and *Pichia* might catalyze the reduction of 2-MPN in a similar way to various carbonyl compounds[9,10] or C=C double bonds.[11,12] However, no 2-MPN-reducing activity was found among 226 yeast strains tested. Furthermore, we attempted to carry out a survey of 2-MPN-reducing activity in our stocked microorganisms (261 strains of bacteria, 117 strains of actinomycetes and 84 strains of fungi). The genera of strains used were shown in the ESI.† Surprisingly, 2-MPN-reducing activity was found in only five filamentous bacteria (Fig. 1). Each of them was identified to be *Streptomyces* sp. based on 16S ribosomal DNA sequence analysis. *Streptomyces* sp. GF3501, 3585, 3587 and 4415 reduced 2-MPN to *R*-2-MP with 72%e.e., 80%e.e. 99.2%e.e. and 90%e.e., respectively. *Streptomyces* sp. GF3546 was the sole strain with high *S*-selectivity (81%e.e.). Among them, *Streptomyces* sp. GF3587 showed the highest conversion and enantioselectivity (Fig. 1).



Scheme 1 Microbial asymmetric reduction of 2-MPN.

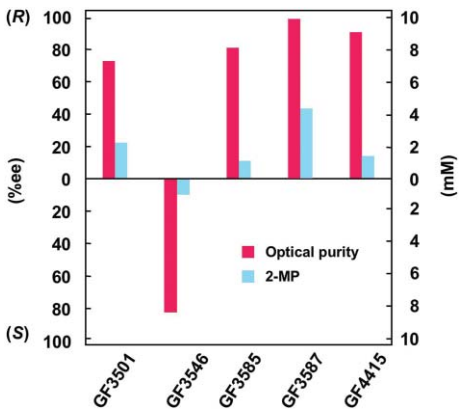


Fig. 1 Screening of imine reductase activity.

We also examined 2-MPN-reducing activity of *Candida parapsilosis* ATCC 7330, which has been reported to catalyze

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† Electronic supplementary information (ESI) available: Experimental details, cultivation, derivatization and HPLC chromatogram, ^1H, ^{13}C NMR and mass spectra for the prepared compounds. See DOI: 10.1039/c0ob00353k

Table 1 Effect of hydroxy compounds on 2-MPN reduction

Compound	Activity/%[a]
None	100
Glucose	130
Glycerol	103
Sorbitol	91
2-Propanal	62

[a] Activity is shown as 100% (354 nmol h^{-1} mg^{-1} dry cell) in the absence of compound. The reaction was carried out at 30 °C for 12 h: 2-MPN (20 mM) and cells (11.1 mg).

the reduction of aryl imines such as (*E*)-*N*-(1-phenylethylidene)benzenamine.[7] Conversion of 2-MPN was conducted using whole cells of *C. parapsilosis* (from 4 mL culture broth) at 25 °C for 72 h in 100 mM phosphate buffer (pH 7.0) supplemented with 2% (w/v) glucose. However, no reductase activity for 2-MPN was detected. These results indicated that the imine reductases found in *Streptomyces* sp. GF3587 and 3546 seemed to be novel and they were promising and most suitable as a whole-cell catalyst for the production of *R*- and *S*-2-MP, respectively. Their culture conditions were also optimized. To recycle an oxidative-reductive coenzyme in the cell, the addition of glycerol, sorbitol or 2-propanol was also examined at 2% (w/v or v/v), as well as glucose. The addition of glucose resulted in 1.3-fold higher activity than in the case without additive, but the others were relatively ineffective or negative effect on the activity (Table 1). The imine reduction by *Streptomyces* sp. GF3587 or 3546 proceeded almost linearly up to 50 mM and 40 mM, respectively. On the basis of these results, we performed an efficient production of optically active 2-MP at 30 or 25 mL scale using whole cells. *Streptomyces* sp. GF3587 produced 91 mM *R*-2-MP (99.2% e.e.) from total 100 mM 2-MPN in the presence of 4% (w/v) glucose after 84-h incubation (Fig. 2). *Streptomyces* sp. GF3546 produced 27.5 mM *S*-2-MP from 30 mM 2-MPN with 92.3% e.e. in the presence of 2% (w/v) glucose after 72-h incubation (Fig. 3). In both cases the optical purity of 2-MP formed remained during the reduction reaction. Their productivities of *R*- and *S*-2-MP were 392 and 21 nmol h^{-1} mg^{-1} dry cell, respectively. *R*- and *S*-2-MP in the reaction solution were

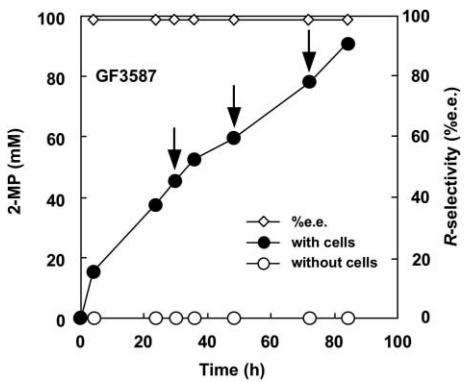

Fig. 2 *R*-2-MP production by whole cells of GF3587. Reduction of 2-MPN using whole cells (83 mg as dry cell weight) was conducted at 25 °C in 30 mL of 100 mM phosphate buffer (pH 7.0) containing 4% (w/v) glucose. Initial concentration of 2-MPN was at 50 mM. With monitoring of 2-MP formation on HPLC, 2-MPN was added to the reaction mixture three times (0.6, 0.6 and 0.3 mmol) during 72-h incubation. Arrows represent the addition of 2-MPN.

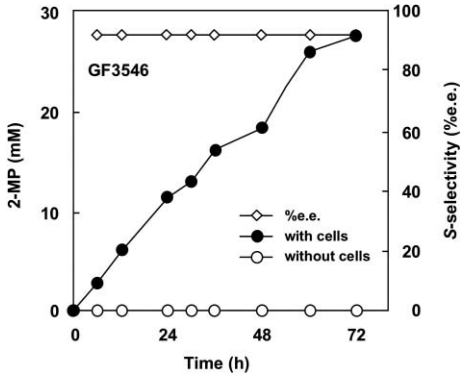

Fig. 3 *S*-2-MP production by whole cells of GF3546. Reduction of 2-MPN (30 mM) using whole cells (455 mg as dry cell weight) was conducted at 25 °C in 25 mL of 100 mM phosphate buffer (pH 7.0) containing 2% (w/v) glucose.

converted to the corresponding amine hydrochloride in 73% and 67% isolated yield *via* both reactions of amino group protection and *N*-Boc group deprotection, respectively.[13]

In conclusion, we elucidated the occurrence of two kinds of novel imine reductase of prochiral 2-MPN in filamentous bacteria for the first time. *Streptomyces* sp. GF3587 and 3546 showed high enantioselectivity for 2-MPN. Under optimized conditions, efficient syntheses of *R*- and *S*-2-MP were achieved using whole-cell catalysts in the presence of glucose. For further application of the microbial imine reduction system to a range of chiral amine production, more detailed information on *R*- or *S*-selective imine reductase is essential. Further studies are in progress.

References

1 (*a*) M. Terada and N. Momiyama, Enantioselective synthesis of amines by chiral Brønsted acid catalysts in *Chiral amine synthesis*, ed. T. C. Nugent, Wiley-VCH, Weinheim, 2010, pp. 109–113; (*b*) Kočovský and S. Stončius, Reduction of imines with trichlorosilane catalyzed by chiral Lewis bases in *Ibid.*, pp 131–156; (*c*) T. L. Church and P. G. Andersson, Chiral amines from transition-metal-mediated hydrogenation and transfer hydrogenation in *Ibid.*, pp. 179–223; (*d*) G. Shang, W. Li and X. Zhang, Transition metal-catalyzed homogeneous asymmetric hydrogenation in *Catalytic asymmetric synthesis*, ed. I. Ojima, John Wiley and Sons, Inc., Hoboken, New Jersey, 2010, pp. 414–436.
2 (*a*) N. J. Turner, *Curr. Opin. Chem. Biol.*, 2010, **14**, 115; (*b*) M. Höhne and U. T. Bornscheuer, *ChemCatChem*, 2009, **1**, 42; (*c*) V. Gotor-Frenández and V. Gotor, *Curr. Opin. Drug Discovery Dev.*, 2009, **12**, 784; (*d*) N. J. Turner and M. D. Truppo, Biocatalytic routes to nonracemic chiral amines in *Chiral amine synthesis*, ed. T. C. Nugent, Wiley-VCH, Weinheim, 2010, pp. 431–459; (*e*) H. Gröger, Enzyme-catalyzed asymmetric synthesis in *Catalytic asymmetric synthesis*, ed. I. Ojima, John Wiley and Sons, Inc., Hoboken, New Jersey, 2010, pp. 269–341.
3 R. Bache and N. Pfennig, *Arch. Microbiol.*, 1981, **130**, 255.
4 A. Tschech and N. Pfennig, *Arch. Microbiol.*, 1984, **137**, 163.
5 H. Li, P. Williams, J. Micklefield, J. M. Gardiner and G. Stephens, *Tetrahedron*, 2004, **60**, 753.
6 B. Basker, N. G. Pandian, K. Priya and A. Chadha, *Tetrahedron: Asymmetry*, 2004, **15**, 3961.
7 T. Vaijayanthi and A. Chadha, *Tetrahedron: Asymmetry*, 2008, **19**, 93.
8 M. Cowart, J. K. Pratt, A. O. Stewart, Y. L. Bennani, T. A. Esbenshade and A. A Hancock, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 689.
9 M. Kataoka, K. Kita, M. Wada, Y. Yasohara, J. Hasegawa and S. Shimizu, *Appl. Microbiol. Biotechnol.*, 2003, **62**, 437.
10 K. Goldberg, K. Schroer, S. Lütz and A. Liese, *Appl. Microbiol. Biotechnol.*, 2007, **76**, 249.
11 M. Kataoka, A. Kotaka, A. Hasegawa, M. Wada, A. Yoshizumi, S. Nakamori and S. Shimizu, *Biosci., Biotechnol., Biochem.*, 2002, **66**, 2651.

12 M. Wada, A. Yoshizumi, Y. Noda, M. Kataoka, S. Shimizu, H. Takagi and S. Nakamori, *Appl. Environ. Microbiol.*, 2003, **69**, 933.

13 After removal of cells from reaction mixture by centrifugation (12,000 *g*, 5 min), di-*tert*-butyl dicarbonate (894 mg, 4.10 mmol), $NaHCO_3$ (0.50 g, 5.95 mmol) and EtOH (20 mL) was added to the reaction solution (30 mL) including 91 mM *R*-2-MP (99.2%e.e.). The reaction was carried out at 25 °C for 15 h. The reaction product was extracted with ethyl acetate (50 mL) thrice, dried over Na_2SO_4, and evaporated. The crude product was treated at 25 °C for 1 h with 4M HCl-ethyl acetate (6 mL) and evaporated. After washing of the residue with ethyl acetate, the corresponding amine hydrochloride was obtained as white solid (243 mg, 2.0 mmol, 99%e.e., 73% yield). In the same manner as *R*-2-MP hydrochloride synthesis, *S*-2-MP hydrochloride was synthesized from the solution (25 mL) including 27.5 mM *S*-2-MP (92%e.e.) to give the corresponding amine hydrochloride as white solid (56 mg, 0.46 mmol, 92%e.e., 67% yield).