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# Using water-miscible ionic liquids to improve the biocatalytic anti-Prelog asymmetric reduction of prochiral ketones with whole cells of *Acetobacter* sp. CCTCC M209061

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## HIGHLIGHTS

- *Acetobacter* sp. was able to efficiently catalyze asymmetric reduction of ketone.
- Use of water-miscible ILs can markedly improve the whole cell-based reductions.
- Various ILs exerted significant but different effects on the reaction.
- The best performance of the microbial cells was observed with $C_2OHMIM \cdot NO_3$.
- The efficient biocatalytic process was feasible on a 400-mL preparative scale.

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## ABSTRACT

The utilization of hydrophilic ionic liquids to improve the synthesis of enantiopure alcohols was successfully performed, via the anti-Prelog asymmetric reduction of ketones with whole cells of *Acetobacter* sp. CCTCC M209061 newly isolated from Chinese kefir. The best results were obtained with $C_2OHMIM \cdot NO_3$, which showed good biocompatibility and also increased moderately cell membrane permeability, thus improving the reaction efficiency. Additionally, the optimal $C_2OHMIM \cdot NO_3$ content, buffer pH, reaction temperature and substrate concentration for 4-(trimethylsilyl)-3-butyn-2-one reduction to (*R*)-4-(trimethylsilyl)-3-butyn-2-ol were 10.0% (v/v), 5.0, 30 °C and 12 mM, respectively. Under the optimized conditions, the initial reaction rate, the maximum yield and the product *e.e.* were 14.0 μmol/min · $g_{cell}$, 91%, and > 99%, respectively, which were much better than the results reported previously. The efficient whole-cell biocatalytic process proved to be feasible on a 400-mL preparative scale, and the immobilized cells still retained above 88.0% of their original activity after successive re-use for 10 batches, showing the good operational stability in the presence of $C_2OHMIM \cdot NO_3$. Furthermore, the established biocatalytic system with *Acetobacter* sp. CCTCC M209061 and $C_2OHMIM \cdot NO_3$ was shown to be highly effective for the anti-Prelog asymmetric reduction of other aryl ketones to the corresponding (*R*)-alcohols.



## 1. Introduction

Biocatalytic process has become an increasingly attractive alternative to conventional chemical approaches owing to its high selectivity, high efficiency, mild reaction conditions and being environment-friendly, and is nowadays widely used for the atom efficient production of many industrially important chemicals (Patel, 2011; Schmid et al., 2001). Furthermore, its applicability increased considerably when it was found that biocatalysts were also able to work in non-aqueous media such as organic solvents (Klibanov, 2001), which can solubilize industrially attractive substrates not soluble in water. Unfortunately, the extensive use of conventional organic solvents in biocatalytic processes may frequently suffer from severe drawbacks such as their toxicity to the environment and the biocatalysts. Ionic liquids (ILs) have recently emerged as a promising new class of relatively biocompatible solvents (van Rantwijk and Sheldon,

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2007; Yang and Pan, 2005). Unlike traditional organic solvents, ILs are nonvolatile, non-flammable, highly stable, and able to dissolve a variety of polar and apolar compounds, thus exhibiting great potential as environment-friendly green solvents. Also, the beauty of ILs is that their properties could be easily altered to match the specific requirements of a particular process by structuring the cations and/or anions. As a result, the catalytic performance of an enzyme or microbial cells may be improved in ILs-containing media. In recent years, the use of ILs, instead of organic solvents, in biocatalysis has been extensively studied and, in many cases, better results have been recorded in terms of the activity, enantioselectivity and stability of the biocatalyst (de Gonzalo et al., 2007; Kaar et al., 2003; Lai et al., 2011; Lou and Zong, 2006; Weuster-Botz, 2007).

Enantiomerically pure chiral alcohols are very useful and valuable chiral building blocks for the synthesis of pharmaceuticals, agrochemicals, liquid crystals and flavors (Gamenara and de Maria, 2009; Nakamura et al., 2003). For instance, (*R*)-4-(trimethylsilyl)-3-butyn-2-ol {(*R*)-TMSBL} is a crucial intermediate for the synthesis of (*R*)-benzyl-hydroxyl-2-pentynoate with the potential therapeutical function (Fu et al., 2002). Currently, enantiopure chiral alcohols can be prepared by biocatalytic asymmetric reduction of prochiral ketones with isolated enzymes as well as whole cells of microorganisms or plants are adopted as biocatalysts. Generally, whole-cell biocatalysts are preferable to isolated enzymes because they are more convenient and stable sources of enzymes, with no need for enzyme purification and coenzyme addition or an additional system for coenzyme regeneration (Patel, 2002) and less enzyme inactivation as the enzymes are kept within the natural environments of living cells. Recently, we isolated a new strain, *Acetobacter* sp. CCTCC M209061, from Chinese kefir grains, which exhibited great potential as the biocatalyst for highly enantioselective anti-Prelog reduction of prochiral ketones (Xiao et al., 2009). In our previous study, *Acetobacter* sp. CCTCC M209061 was found to be capable of catalyzing the reduction of 4-(trimethylsilyl)-3-butyn-2-one to (*R*)-TMSBL with high enantioselectivity in aqueous phase with the product *e.e.* being more than 99%. However, the maximum yield reached only 71% even at a low substrate concentration of 6 mM, due to pronounced substrate and product inhibitions of the reaction. For industrial application, the product yield and the reaction efficiency need to be further increased.

On the other hand, dialkylimidazolium-based ILs are similar in structure to cationic surfactants and may be able to make the cell membrane more permeable (Cornmell et al., 2008; Ranke et al., 2007). Thus, the addition of water-miscible ILs into whole-cell reaction systems may lower product concentration within microbial cells and reduce the inhibitory and toxic effects of product, thus possibly enhancing the efficiency of the bioreduction. To data, there have been some notable successes (Cornmell et al., 2008; Dipeolu et al., 2008; Lou et al., 2009a, 2009b, 2006). For example, the presence of some hydrophilic ILs as additives can facilitate the biocatalytic processes with *Lactobacillus brevis*, *Rhodotorula* sp. AS2.2241 and *Trigonopsis variabilis* AS2.1611 and provide significant increases in product yield and reaction efficiency compared with conventional solvents (Kohlmann et al., 2011; Lou et al., 2009a, 2009b). Moreover, IL was found to have a stabilizing influence on nicotinamide cofactors in the bioreduction.

Herein, we for the first time focused on the evaluation of a variety of hydrophilic dialkylimidazolium-based ILs as the co-solvents for efficient anti-Prelog asymmetric reductions of prochiral ketones catalyzed by a novel bacterium *Acetobacter* sp. CCTCC M209061, and the effects of these ILs on the bioreduction reactions, where 4-(trimethylsilyl)-3-butyn-2-one (TMSBO) was used as an initial model substrate. Also, the applicability of the promising IL 1-(2′-hydroxyl)ethyl-3-methylimidazolium nitrate ($C_2$OHMIM · $NO_3$) in combination with the new strain was examined for prochiral aryl ketones with various substituents, and the efficient biocatalytic process for TMSBO reduction to (*R*)-TMSBL was evaluated on a preparative scale.

## 2. Material and methods

### 2.1. Biological and chemical materials

The strain, *Acetobacter* sp. CCTCC M209061, was isolated from Chinese kefir grains by our research group and conserved in our laboratory. TMSBO (97% purity), TMSBL (97% purity) and *n*-decane ( > 99% purity) were purchased from Sigma-Aldrich (USA). The aryl ketones and their corresponding alcohols were purchased from Aldrich–Fluka and were all of over 96% purity. The used ILs (99% purity), shown in Table 1, were bought from Lanzhou Institute of Chemical Physics (China). All other chemicals were obtained from commercial sources and were of analytical grade.

### 2.2. Cultivation and immobilization of Acetobacter sp. CCTCC M209061 cells

*Acetobacter* sp. CCTCC M209061 was cultivated in medium containing 10 g/L yeast extract and 20% (v/v) tomato juice, and the harvested cells were immobilized in calcium alginate according to our reported method ( Xiao et al., 2009).

### 2.3. General procedure for biocatalytic asymmetric reductions of prochiral ketones

In a typical experiment, 2.0 mL of an IL-containing co-solvent system or aqueous TEA-HCl buffer (100 mM) containing a predetermined amount of various ketone substrates and 2-propanol were pre-incubated in a 20-mL Erlenmeyer flask capped with a septum for 10 min at 180 r/min and 30 °C, and the reactions were initiated by adding 0.3 g/mL the immobilized beads of *Acetobacter* sp. CCTCC M209061 cells to the reaction system. Aliquots (50 μL) were withdrawn at specified time intervals from the co-solvent system and the products were extracted with *n*-hexane (100 μL) containing 5.1 mM *n*-decane (internal standard) prior to GC

**Table 1**
Water-miscible ILs used in this work and their abbreviations.

| Name of ILs | Abbreviation |
|---|---|
| 1-(2′-Hydroxyl)ethyl-3-methylimidazolium nitrate | $C_2$OHMIM · $NO_3$ |
| 1-(2′-Hydroxyl)ethyl-3-methylimidazolium chloride | $C_2$OHMIM · Cl |
| 1-(2′-Hydroxyl)ethyl-3-methylimidazolium trifluoromethanesulfonate | $C_2$OHMIM · TfO |
| 1-(2′-Hydroxyl)ethyl-3-methylimidazolium tetrafluoroborate | $C_2$OHMIM · $BF_4$ |
| 1-Butyl-2, 3-dimethylimidzolum nitrate | $C_4$MMIM · $NO_3$ |
| 1-Ethyl-3-methylimidzolum nitrate | $C_2$MIM · $NO_3$ |
| 1-Butyl-3-methylimidzolum nitrate | $C_4$MIM · $NO_3$ |
| 1-Ethyl-3-methylimidzolum tetrafluoroborate | $C_2$MIM · $BF_4$ |
| 1-Propyl-3-methylimidzolum tetrafluoroborate | $C_3$MIM · $BF_4$ |
| 1-Butyl-3-methylimidzolum tetrafluoroborate | $C_4$MIM · $BF_4$ |
| 1-Pentyl-3-methylimidzolum tetrafluoroborate | $C_5$MIM · $BF_4$ |
| 1-Ethyl-3-methylimidzolum chloride | $C_2$MIM · Cl |
| 1-Butyl-3-methylimidzolum chloride | $C_4$MIM · Cl |
| 1-Ethyl-3-methylimidzolum bromide | $C_2$MIM · Br |
| 1-Butyl-3-methylimidzolum bromide | $C_4$MIM · Br |
| 1-Pentyl-3-methylimidzolum bromide | $C_5$MIM · Br |
| 1-Hexyl-3-methylimidzolum bromide | $C_6$MIM · Br |
| 1-Heptyl-3-methylimidzolum bromide | $C_7$MIM · Br |