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Pseudomonas gessardii growing cells as a new biocatalyst for asymmetric synthesis of α -bromohydrins[☆]

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ABSTRACT

The growing whole cells of bacterial strain, *Pseudomonas gessardii* provides a highly selective biocatalyst for reduction of phenacyl bromides to generate (S)-2-bromo-1-phenylethan-1-ol derivatives in high enantioselectivity (up to 96% ee). The chiral alcohols were isolated with complete conversion (> 99%) employing DMSO as the co-solvent. Effect of various physicochemical parameters on the reduction has been studied. The other α -substituted acetophenones (chloro-, cyano-, and azide-) were reduced their corresponding alcohols with lower enantioselectivity.

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1. Introduction

Asymmetric bioreduction has been designated as a viable greener route for the synthesis of enantiomerically pure alcohols because of its environment friendly mild reaction conditions as well as high selectivity in comparison to its chemical counterpart. The bioreduction employing isolated enzyme (Alanvert et al., 2009) and whole cells (Barbieri et al., 2001) provides an attractive approach to reduce a broad range of carbonyl compound to their corresponding chiral alcohols. An enzymatic bioreduction process catalyzed by isolated dehydrogenase requires the stoichiometric addition of the expensive co-factor or the in vitro regeneration of the co-factor. On the other hand, whole cells offer the advantage of efficient and economical process due to the presence of multiple enzymes which contain necessary cofactor and metabolic pathways for their regeneration. Secondly, the use of whole cells provides high stability to enzyme inside cell wall.

Among chiral alcohols, 2-bromo-1-phenylethan-1-ol derivatives are important intermediates in the synthesis of a variety of biologically active compounds such as β -adrenergic blocking

agents (Mizuno et al., 2005), bronchodilators, anti-depressants, HIV-1 protease inhibitors (Chartrain et al., 1990) and in particular for their transformation to enantiopure epoxides (Haak et al., 2008). However, in literature, the bioreduction of α -chloroacetophenones has been extensively studied (Zhu et al., 2005); while there are few reports on the whole cell bioreduction of α -bromoacetophenones with higher conversions due to contamination of reaction by the formation of by-products (Rocha et al., 2010). The biological reduction of α -bromoacetophenones previously reported employed growing cells of fungi and yeast namely *Geotrichum candidum* (Wei et al., 1998; Fardelone et al., 2011), *Rhodotorula glutinis* (Fardelone et al., 2011), and baker's yeast (Liese et al., 1998; Eckstein et al., 2004). The problem of long growing times needed for the growth of yeast and fungi, handling of large cell mass, complicated extraction process and lower conversions make us to screen various bacterial strains for this reduction. Continuing with our interest in developing biocatalytic methodologies for obtaining chiral synthons (Bala et al., 2012; Chimni et al., 2013) we describe the result of our investigation on of catalytic potential of a bacterial strain *Pseudomonas gessardii* for the enantioselective reduction of phenacyl bromides to corresponding α -bromo alcohol. In the present study, growing bacterial whole cells have been used to obtain α -bromo chiral alcohols from phenacyl bromides in excellent conversion and enantioselectivity and the formation of by-products has been controlled by optimizing the physicochemical conditions.

[☆] Composition of trace element solution (in 1 L): ZnSO₄·7H₂O 10 mg, MnCl₂·4H₂O 3.0 mg, CoCl₂·6H₂O, NiCl₂·6H₂O 2.0 mg, Na₂MoO₄·2H₂O 3.0 mg, H₃BO₃ 30 mg and CuCl₂·2H₂O 1.0 mg.

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2. Experimental

2.1. Materials and methods

Bacterial strain, *P. gessardii* was obtained from the microorganism isolated from petrochemical enriched soil by the Department of Microbiology, Guru Nanak Dev University, Amritsar. NMR spectra were obtained at 300 MHz (JEOL AL-300) using either CDCl₃ or DMSO as solvents with Me₄Si in CDCl₃ as the internal standard. The chemical shifts are given in delta (δ) values and the coupling constants (J) in Hertz (Hz). Spectral patterns are designated as s=singlet; d=doublet; dd=doublet of doublets; q=quartet; t=triplet; br=broad; m=multiplet. Analytical thin layer chromatography (TLC) was performed on either (i) aluminum sheets pre-coated with silica gel GF254 (Merck, India) or (ii) glass plates (7.5 cm \times 2.5 cm) coated with silica gel GF-254 (Spectrochem, India). Visualization of the spots was accomplished by exposing to UV light or iodine vapors. Column chromatography was performed using silica gel 60–120. HPLC analyses were carried out in a Hewlett Packard 1100 chromatograph, UV detector using a SPD-M20A prominence diode array detector (25 cm \times 4.6 mm), Daicel Chiralcel IB (25 cm \times 4.6 mm), Chiralpak AS-H (25 cm \times 4.6 mm), Chiralpak AD-H (25 cm \times 4.6 mm) columns, varying the conditions according to the specific substrate. Measurements of the optical rotation values were done in a Perkin-Elmer 241 polarimeter.

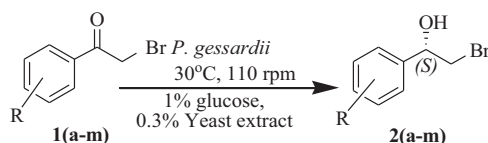
Starting phenacyl bromides were prepared from the corresponding acetophenones by different bromination methods. Alcohols were further prepared by reduction with NaBH₄ in dry methanol as well as solvent free sodium borohydride reduction by grinding depending on the substrates from Sigma-Aldrich or Fluka. All other reagents and solvents were of the highest quality grade available, and purchased from Sigma-Aldrich, Fluka or Acros Organics.

2.2. Determination of enantiomeric excess and absolute configuration

The enantiomeric excess of alcohols was determined by HPLC analysis after the alcohols were purified by column chromatography. The absolute configurations of alcohols were determined by comparison with the signs of the reported specific rotations or with the retention time of authentic samples by HPLC Scheme 1.

2.3. Medium and cultivation

Microorganisms were grown for 48 h on petri plate in the Nutrient agar medium (NAM) of composition (in 1 L): Beef extract 3.0 g, peptones 5.0 g, NaCl 5.0 g and agar 20 g. The liquid medium for the growth of the strains contained (g/L): Na₂HPO₄ 3.6 g, (NH₄)₂SO₄ 1.0 g, KH₂PO₄ 1.0 g, MgSO₄ 1.0 g, ammonium ferric citrate (1%, w/v) 1.0 mL, CaCl₂ · 2H₂O 0.10 g, trace element solution 1.0 mL. The initial pH of this culture medium was adjusted to 7.0 with 1 M HCl and 1 M NaOH and sterilized at 121 °C for 15 min. Yeast extract and glucose were added as required from their respective sterilized stock solutions.



Scheme 1. Enzymatic conditions for bioreduction of various phenacyl bromide derivatives.

2.4. General method for the biocatalyzed reduction of ketones 1a–1m using bacterial strain, *P. gessardii*

2.4.1. Procedure with growing cells

A 250 mL sterilized conical flask containing 50 mL minimal synthetic medium (MSM), 0.3% yeast extract and 1% glucose, was inoculated with a loopful of bacterial isolate, *P. gessardii* from NAM slant. The flask was incubated for 48 h in an orbital shaker at 110 rpm maintained at 30 °C and was used as stock of bacterial broth. Then, 100 mL MSM solution taken in 500 mL sterilized conical flask was fed with 600 μ l of yeast extract and 5% (v/v) of the bacterial broth from the activated bacterial culture and the cells were allowed to grow for 48 h at 30 °C and 110 rpm in an orbital shaker. After 48 h, 50 mg of substrate 3-nitrophenacyl bromide, dissolved in 150 μ l ethanol and 80 μ l DMSO was added to the culture flasks. This flask was further incubated for 48 h at 30 °C and 110 rpm in an orbital shaker. The biotransformation was quenched by the addition of saturated solution of sodium chloride and extracted with chloroform. The chloroform layer was separated and dried over anhydrous sodium sulfate. The distillation of chloroform gave the crude product. The product (2) was confirmed by TLC as well as ¹H NMR. The products purified by column chromatography were analyzed by HPLC in order to determine the conversion and the optical purity of alcohols. Experiments were performed in triplicate.

Alcohols **2a–2m** are already known in the literature. The spectral data (¹H and ¹³C NMR) are in agreement with the reported data.

2.4.2. (*S*)-2-bromo-1-phenylethanol (2a)

Yellow thick oil; [α]_D²⁵ = +36.4° (c 0.50, CHCl₃); 72% ee; ¹H NMR (CDCl₃, 300 MHz), δ [ppm]: 2.69 (s, 1H), 3.67 (m, 2H), 4.94 (m, 1H), 7.40–7.29 (m, 5H); ¹³C NMR (CDCl₃, 75 MHz) δ [ppm]: 40.1, 73.7, 125.9, 128.4, 128.6, 140.3; HPLC analysis: Daicel chiralpak IB, hexane/*i*-PrOH 97:3, 1.0 mL/min, 220 nm, retention times: 11.80 min (S) and 12.63 min (R); IR (KBr) ν_{\max} (cm⁻¹) 3300, 1600; HRMS (EI+) calcd. for C₈H₉BrO: 201.0987, found: 201.0636.

2.4.3. (*S*)-2-bromo-1-(4'-nitrophenyl) ethanol (2b)

Light yellow solid; mp 139 °C; [α]_D²⁵ = +32.6° (c 0.462, CHCl₃); 92% ee; ¹H NMR (CDCl₃, 300 MHz), δ [ppm]: 2.93 (s, 1H), 3.59 (m, 1H), 3.73 (m, 1H), 5.13 (m, 1H), 7.61 (d, J =8.7 Hz, 2H), 8.28 (d, J =8.7 Hz, 2H); ¹³C NMR (CDCl₃, 75 MHz) δ [ppm]: 39.4, 72.4, 121.1, 123.2, 123.3, 129.6, 132.0, 142.3, 148.4; HPLC analysis: Daicel chiralpak AS-H, hexane/*i*-PrOH:90/10, 1.0 mL/min, 264 nm, retention times: 17.79 min (S) and 20.44 min (R); IR (KBr) ν_{\max} (cm⁻¹) 3455, 1601, 1520, 1347; HRMS (EI+): calcd. for C₈H₈ BrNO₃: 244.9667, found: 244.9678.

2.4.4. (*S*)-2-bromo-1-(3'-nitrophenyl) ethanol (2c)

Light yellow solid; mp 120 °C; [α]_D²⁵ = +29.7° (c 0.345, CHCl₃); 84% ee; ¹H NMR (CDCl₃, 300 MHz), δ [ppm]: 2.81 (s, 1H), 3.56 (dd, J =8.3 and 10.6 Hz, 1H), 3.70 (dd, J =3.5 and 10.6 Hz, 1H), 5.06 (dd, J =3.5 Hz and 8.3 Hz, 1H), 7.53 (m, 2H), 8.15 (m, 2H); ¹³C NMR (CDCl₃, 75 MHz) δ [ppm]: 39.3, 72.5, 121.1, 123.2, 123.3, 129.6, 132.0, 142.4, 148.4; HPLC analysis: Daicel Chiralpak AD-H, hexane/ethanol:50/50, 0.5 mL/min, 254 nm, retention times: 9.86 min (S) and 11.93 min (R); IR (KBr) ν_{\max} (cm⁻¹) 3365, 1592, 1517, 1323; HRMS (EI+) calcd. for C₈H₈BrNO₃: 244.9687, found: 244.9688.

2.4.5. (*S*)-2-bromo-1-(2'-nitrophenyl) ethanol (2d)

Oily liquid; [α]_D²⁵ = +28.5° (c 1.00, CHCl₃); 72% ee; ¹H NMR (CDCl₃, 300 MHz), δ [ppm]: 2.80 (s, 1H), 8.21 (m, 2H), 3.55 (dd, J =10.6 and 8.4 Hz, 1H), 3.67 (dd, J =10.6 and 3.3 Hz, 1H), 5.04 (dd, J =8.4 and 3.6 Hz, 1H), 7.54, (m, 1H), 7.73 (m, 1H); ¹³C NMR

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