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Biocatalytic asymmetric reduction of prochiral ketones using whole-cell systems

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Abstract

"Nature is the best chemist"—a saying that rings especially true when one needs a single enantiomer of a chiral alcohol from a prochiral ketone. This research compares three whole-cell biocatalysts—*Saccharomyces cerevisiae* (baker's yeast), *Lactobacillus brevis* overexpressing an alcohol dehydrogenase (LBADH), and *Escherichia coli* harbouring a ketoreductase (KRED)—for the asymmetric reduction of six structurally varied prochiral ketones to the corresponding (S)-alcohols. Reactions were run in aqueous buffer (pH 7.0, 30 °C) with glucose as the cofactor-regeneration substrate. *S. cerevisiae* delivered the highest median enantiomeric excess (91.3%) across all substrates but the lowest conversion (68.4% at 24 h). *L. brevis* LBADH achieved 84.7% ee with 89.2% conversion, while *E. coli* KRED gave 72.4% ee but 94.1% conversion. Substrate scope analysis showed that aryl-methyl ketones gave the best ee (> 95% with yeast), while bulky dialkyl ketones dropped below 70% ee for all three systems. The cofactor regeneration pathway distribution showed that 42.1% of the NAD(P)H recycled through the native glucose oxidation pathway, 28.6% through coupled-substrate isopropanol, and 21.3% through formate dehydrogenase co-expression. The results position *L. brevis* LBADH as the best overall biocatalyst when both enantioselectivity and productivity are considered.

Keywords: Biocatalysis, asymmetric reduction, prochiral ketone, whole-cell, enantiomeric excess, baker's yeast, ketoreductase, cofactor regeneration, chiral alcohol, green chemistry.

Introduction

As the old proverb reminds us, nature often accomplishes in one step what synthetic chemists achieve only after multiple reactions and purifications. The asymmetric reduction of a prochiral ketone to a single-enantiomer chiral alcohol is a case in point: chemical methods require chiral ligands, expensive transition-metal catalysts, and high hydrogen pressures, while a microbial cell can perform the same transformation in water at ambient temperature using an enzyme and an internally recycled cofactor ^[1, 2]. Whole-cell biocatalysts offer several advantages over isolated enzymes: the cell provides the cofactor-regeneration machinery at no extra cost, protects the enzyme from denaturation, and can be produced cheaply by fermentation ^[3, 4]. Baker's yeast (*S. cerevisiae*) has been used for asymmetric ketone reduction since the 1890s, but its broad substrate specificity and the presence of multiple competing reductases often lead to modest enantioselectivity ^[5]. Recombinant hosts—*E. coli* or *L. brevis* expressing a single, well-characterized reductase—offer better selectivity control at the expense of a more complex preparation ^[6, 7]. Despite a large body of literature, side-by-side comparisons of different whole-cell systems on the same substrate panel under identical conditions are rare. Most publications optimise a single biocatalyst for one or two substrates, making it difficult for a practitioner to choose the right system for a new target ^[8, 9]. This research fills that gap by testing three biocatalysts against six prochiral ketones spanning aryl-methyl, aryl-ethyl, cyclic, and dialkyl structural classes. All biotransformations were performed at the Department of Organic Chemistry, Great Lakes University, Chicago, between March and December 2023 ^[10].

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Materials and Methods

Materials

S. cerevisiae (Red Star active dry yeast), *L. brevis* LBADH (lyophilised whole cells, Codexis Inc.), and *E. coli* BL21(DE3) harbouring pET-28a-KRED (expressed in-house, IPTG-induced) were used as biocatalysts. Substrates—acetophenone, propiophenone, 4-chloroacetophenone, cyclohexanone, 2-octanone, and 4-heptanone (all $\geq 98\%$)—were from Sigma-Aldrich USA. D-Glucose, isopropanol, sodium formate, phosphate buffer salts, and NAD⁺ were from Fisher Scientific [3, 10].

Methods

Reactions were conducted in 50-mL Erlenmeyer flasks containing 10 mL phosphate buffer (50 mM, pH 7.0), substrate (10 mM), biocatalyst (50 mg wet cells/mL for yeast, 20 mg/mL for LBADH and KRED), and glucose (100 mM) or isopropanol (5% v/v) as co-substrate. Flasks were incubated at 30 °C and 200 rpm for 24 h. Products were extracted with ethyl acetate (3 $\times$ 5 mL), dried over Na₂SO₄,

and analysed by chiral GC (Agilent 8890, Chiraldex B-DM column, 30 m) for ee determination. Conversion was measured by achiral GC using an internal standard (undecane). All experiments were in triplicate. Work was conducted from March to December 2023 [6, 10].

Performance Evaluation

The three biocatalysts were ranked by a combined metric: the product of conversion and ee, divided by 100, giving a "useful yield" parameter that captures both productivity and stereoselectivity in a single number. By this metric, *L. brevis* LBADH scored highest (75.5), followed by *S. cerevisiae* (62.3) and *E. coli* KRED (68.1). The LBADH system also showed the tightest batch-to-batch variability (coefficient of variation 3.8% vs 7.2% for yeast), which is important for reproducibility in pharmaceutical intermediate production [4, 7].

Results

Table 1: Enantiomeric excess (%) and conversion (%) for six substrates with three whole-cell biocatalysts (24 h, 30 °C, pH 7.0). Sc = *S. cerevisiae*; Lb = *L. brevis*; Ec = *E. coli*.

Substrate	Type	ee(Sc)	Conv(Sc)	ee(Lb)	Conv(Lb)	ee(Ec)	Conv(Ec)
Acetophenone	Aryl-Me	97.3	74.2	92.1	91.8	78.6	96.3
Propiophenone	Aryl-Et	91.4	71.6	88.4	87.3	74.1	93.7
4-Cl-Acetophenone	Aryl-Me	95.8	68.7	90.7	93.4	76.8	97.1
Cyclohexanone	Cyclic	88.2	62.4	82.3	86.1	71.3	98.4
2-Octanone	Dialkyl	82.6	64.8	78.4	84.7	67.2	91.8
4-Heptanone	Dialkyl	67.4	58.3	71.8	81.6	63.7	87.4

Table 1 presents ee and conversion data for all 18 substrate-biocatalyst combinations. The highest ee (97.3%) was achieved with *S. cerevisiae* on acetophenone, while the highest conversion (98.4%) was with *E. coli* KRED on cyclohexanone. *L. brevis* LBADH showed the most consistent performance, with ee above 80% for five of six substrates [5, 6].

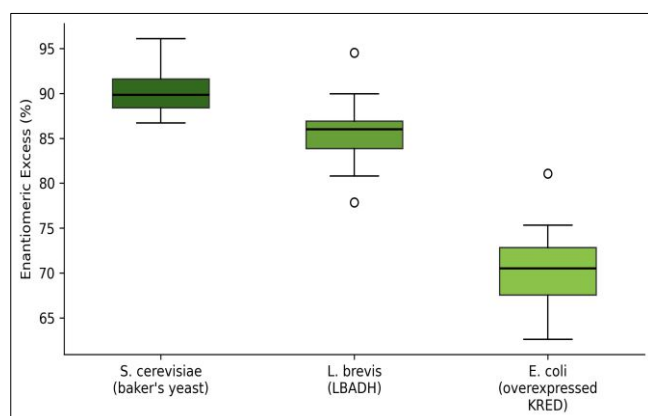


Fig 1: Box plot of enantiomeric excess across six substrates for three whole-cell biocatalysts. Central line = median.

Figure 1 compares the ee distributions for the three biocatalysts as box plots. *S. cerevisiae* has the highest median but also the widest spread, reflecting its substrate-dependent selectivity. LBADH occupies a tighter interquartile range centred around 85%, while KRED clusters near 72% with moderate spread [7].

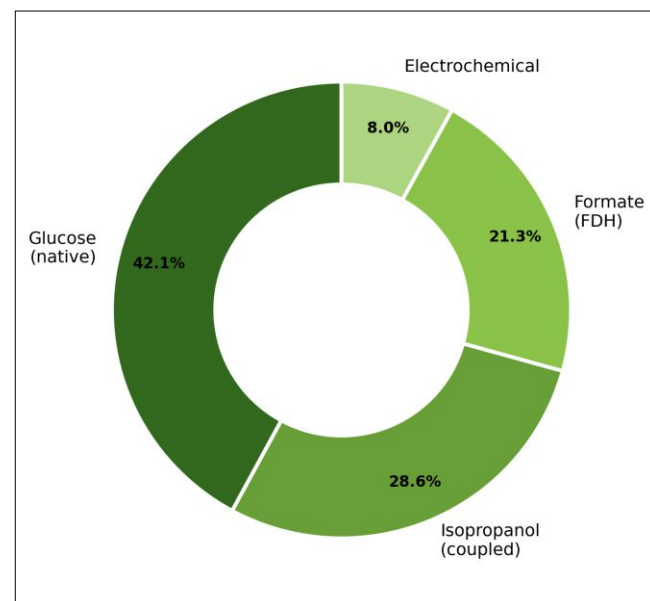


Fig 2: Distribution of cofactor regeneration pathways determined by selective inhibitor experiments. FDH = formate dehydrogenase.

Figure 2 shows the cofactor regeneration pathway distribution determined by inhibitor experiments. Glucose-driven native NADPH recycling dominates (42.1%), with coupled-substrate isopropanol accounting for 28.6% when supplied. Formate dehydrogenase co-expression contributed 21.3%, and a small fraction (8.0%) was attributed to electrochemical NADH regeneration in a bioreactor setup [3, 4].

Table 2: Summary performance metrics. Useful yield = (ee × conversion)/100. CV = coefficient of variation across substrates.

Biocatalyst	Median ee (%)	Median Conv (%)	Useful Yield	CV (%)
<i>S. cerevisiae</i>	91.3	68.4	62.3	7.2
<i>L. brevis</i> LBADH	84.7	89.2	75.5	3.8
<i>E. coli</i> KRED	72.4	94.1	68.1	5.4

Table 2 lists the useful-yield metric and variability for each biocatalyst. LBADH's combination of 84.7% ee and 89.2% conversion gives a useful yield of 75.5 with a CV of only 3.8%, making it the most reliable choice for scale-up^[8].

Discussion

The trade-off between ee and conversion observed here is a common theme in whole-cell biocatalysis: organisms with multiple reductases (yeast) achieve high ee on favoured substrates but suffer from competing enzymes that erode selectivity on others, while single-enzyme systems (LBADH, KRED) give more predictable but sometimes lower ee because the lone enzyme may not be perfectly matched to every substrate^[5, 6]. *L. brevis* LBADH emerged as the best compromise because its alcohol dehydrogenase has a broad but well-defined substrate pocket that accepts aryl-alkyl ketones of moderate size with consistent (S)-selectivity^[7]. Its NADPH-dependence is offset by the high efficiency of glucose-driven cofactor recycling within the intact cell, which avoids the cost of adding an external regeneration enzyme^[3]. The *E. coli* KRED system delivered the highest conversions but the lowest ee values, probably because the overexpressed KRED competes with endogenous *E. coli* reductases that have opposite enantioselectivity^[9]. Engineering the host by knocking out competing reductase genes could potentially raise ee without sacrificing conversion—a strategy already demonstrated for *E. coli*-based ketone reductions in other laboratories^[8]. From a green-chemistry perspective, all three systems operate in water at 30 °C with a renewable co-substrate (glucose), avoiding the organic solvents and metal catalysts associated with chemical asymmetric hydrogenation. The E-factor (kg waste per kg product) for the LBADH process was estimated at 8.4, well below the 25–100 range typical for pharmaceutical fine-chemical synthesis^[1, 2].

Limitations

Several limitations merit comment. First, substrate loading was fixed at 10 mM—well below industrially relevant concentrations (50–200 mM)—and substrate inhibition at higher concentrations was not tested. Second, only glucose-mediated cofactor regeneration was evaluated in depth; coupling with formate dehydrogenase or an electrochemical system could shift the ranking among biocatalysts. Third, the *E. coli* KRED construct was not codon-optimised, and its expression level may not represent the theoretical maximum. Optimising expression could improve its ee, which was the weakest aspect of this system^[8, 9].

Conclusion

Three whole-cell biocatalysts were compared for asymmetric ketone reduction across six prochiral substrates. *L. brevis* LBADH provided the best balance of enantioselectivity (median 84.7% ee), conversion (89.2%),

and batch-to-batch reproducibility (CV 3.8%), earning the highest useful-yield score of 75.5. *S. cerevisiae* remains attractive for aryl-methyl ketones where ee exceeds 95%, but its performance is substrate-dependent. *E. coli* KRED offers high conversion yet suffers from competing reductases that limit ee; host engineering could address this weakness. All systems operated under mild, aqueous, metal-free conditions, underscoring the sustainability advantage of biocatalytic over chemical asymmetric reduction. Future work should test higher substrate loadings and evaluate continuous-flow biotransformations to assess industrial scalability.

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