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Recent progress in biocatalysis for asymmetric oxidation and reduction

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ABSTRACT

Latest advances for asymmetric synthesis through reduction and oxidation including deracemization by biocatalysts are reviewed. Newly developed methodologies as well as practical applications are covered.

Contents

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1. Introduction

Asymmetric synthesis is increasingly getting important for variety of purposes such as drug synthesis as more than half of drug candidate molecules have more than one chiral centers.[1] To synthesize them in environmentally friendly methods, catalysts play an important role, and both chemical and biological catalysts need to be developed because they are complementary to each other. Now, about 10% of the total drug synthesis depend on the biocatalysts. Biocatalytic processes used in the synthesis of chiral intermediates for pharmaceuticals have been reviewed.[2]

The kind of biocatalytic reaction in majority of reports has been hydrolysis due to the high stability and easiness in handling of hydrolases. Oxidation and reduction have accounted for the second largest portion of the studies of biocatalysis. With recent remarkable progress, the number of commercially available and easy-to-handle oxidoreductases is increasing. For example, 38 of various types of reducing enzymes (ChiralScreen™ OH) are available from Daicel.[3] Codexis also prepares a large number of various types of screening kit for reduction of ketones, diketones, and keto esters as well as for reduction of olefin.[4a,4b] With the increased number of available enzymes, suitable biocatalysts having high enantioselectivities for a variety of substrates are easier to find.

Here, latest advances for asymmetric synthesis through reduction and oxidation including deracemization by biocatalysts are reviewed. Most of the literature reviewed in this report are published after 2003, and other reviews[5a,5b] and books[6a,6b] cover the studies with high originality conducted before.

2. Reaction mechanism

Dehydrogenase, reductase, oxidase, and oxygenase require a coenzyme such as NADH (nicotinamide adenine dinucleotide), NADPH (nicotinamide adenine dinucleotide phosphate), and flavin. Enzyme and coenzyme work together to catalyze reaction. For example, the reduction with NADH proceeds as follows:

(1) Coenzyme and substrate bind to an enzyme.
(2) The substrate is reduced, while the coenzyme is oxidized.
(3) The coenzyme and product dissociate from the enzyme.

Some coenzymes such as flavin are bound to enzyme and do not dissociate from enzyme. Recent studies about mechanism including stereochemistry and recycling of coenzyme are described in this section.

2.1. Mechanism and stereochemistry for NAD(P)H dependent dehydrogenase

The reaction mechanism of NAD(P)H-dependent dehydrogenase has been studied in detail. There are four stereochemical patterns for transfer of a hydride from coenzyme, NAD(P)H, to substrate as shown in Figure 1. The hydride attacks either *si*-face or *re*-face of the carbonyl group depending on the orientation of the binding of the substrate to the enzyme, which results in the formation of (*R*) and (*S*)-alcohols, respectively. On the other hand, enzyme transfers either pro-(*R*)-hydride or pro-(*S*)-hydride of the coenzyme depending on the kind of enzyme.

Crystal structure determined to 1.98 Å resolution of an active site of NADH-dependent enzyme, (*R*)-2-hydroxyglutarate dehydrogenase (HGDH), is shown in Figure 2.[7] HGDH catalyzes the reduction of 2-oxoglutarate to (*R*)-2-hydroxyglutarate. Arg235 interacts with the substrate's α-carboxylate and carbonyl groups, having a dual role in both substrate binding and activation, and the γ-carboxylate group can dock at an arginine cluster composed of Arg76, Arg52, and Arg9. The proton-relay system built up by Glu264 and His297 permits His297 to act as acid–base catalyst, and the pro-(*R*)-hydrogen from NADH is transferred as hydride to

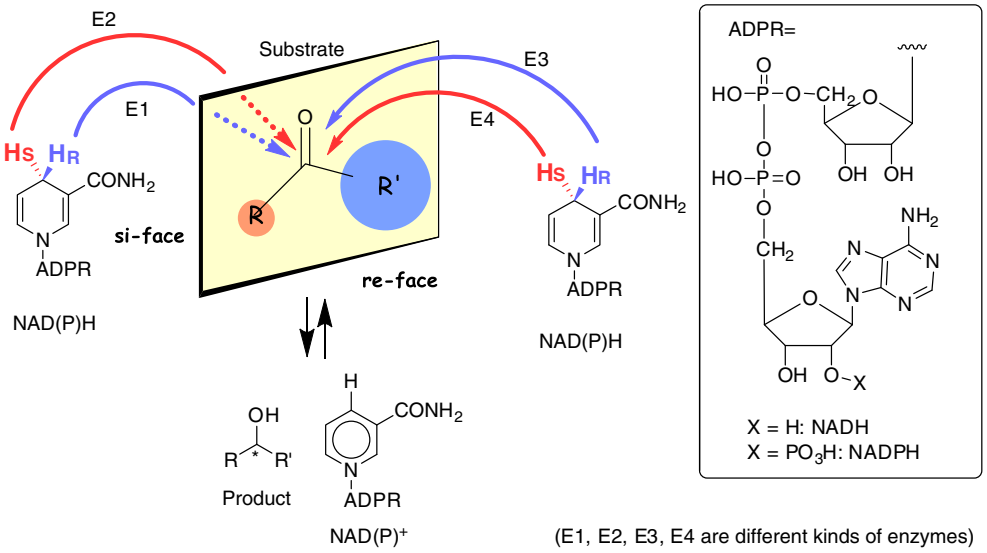


Figure 1. Hydride transfer from coenzyme, NAD(P)H, to carbonyl compound.

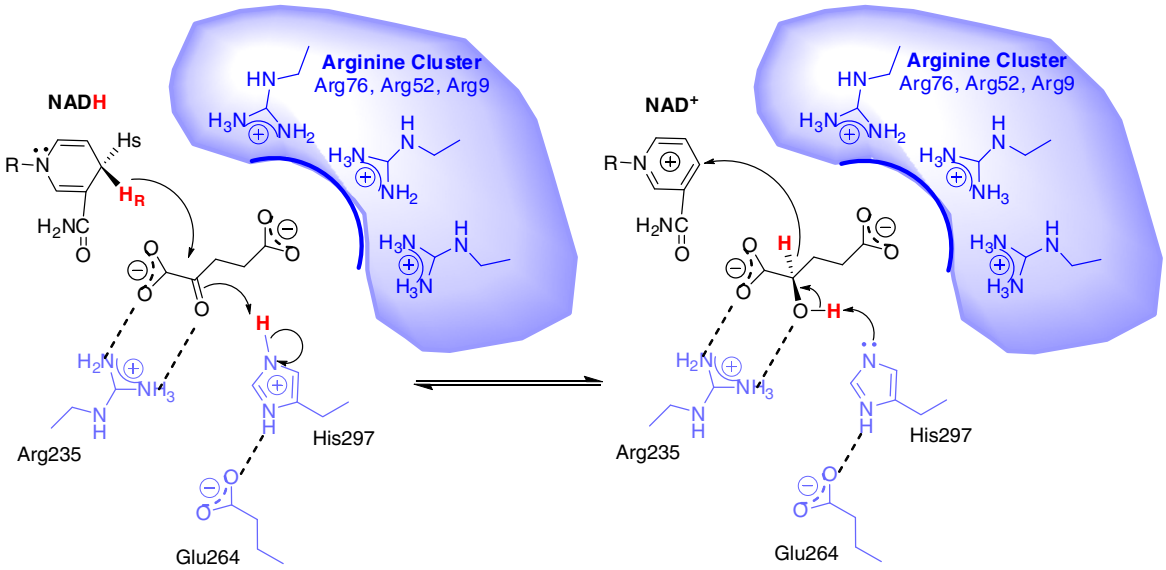


Figure 2. Active site of an NADH-dependent dehydrogenase transferring a hydride between the substrate and coenzyme.

the carbonyl group *si*-face leading to the formation of (*R*)-2-hydroxyglutarate.

2.2. Mechanism for flavin-dependent enzyme

Reaction mechanisms of Baeyer–Villiger oxidation of ketones by phenylacetone monooxygenase from *Thermobifida fusca* is described here as an example for the flavin-dependent enzyme. The enzyme has flavin as the cofactor and also requires NADPH as the second cofactor.[8] As shown in Figure 3, first step is the reduction of flavine fixed to the enzyme with NADPH. Then, the addition of oxygen to the reduced form of flavin occurs giving hydroperoxide anion, which reacts with the ketone substrate to form a Criegee intermediate. At last, the ester product is formed, and H_2O, product, and $NADP^+$ dissociate from the enzyme, sequentially. Overall, molecular oxygen and NADPH are necessary. Only one oxygen atom of an oxygen molecule is used for the oxidation of the substrate, and another is emitted as a water molecule.

2.3. Hydrogen source for regeneration of reduced form of coenzyme

Enzymes which catalyze reduction of carbonyl groups require a coenzyme from which a hydride is transferred to the carbonyl carbon. Since a coenzyme is too expensive as a throwaway reagent, the oxidized form of the coenzymes has to be transformed to the reduced form for the next cycle of the reaction. Hydrogen sources are necessary to perform this reduction reaction. Alcohols such as ethanol and 2-propanol, sugars such as glucose, glucose-6-phosphate(G6P), and glucose-6-sulfate, formic acid, amino acids such as glutamic acid and dihydrogen can be used for this purpose. Power sources such as electric and light power are also possible to use. Examples are shown in this section.

2.3.1. Alcohols

Alcohols such as ethanol and 2-propanol have been widely used to recycle a coenzyme for reduction catalyzed by alcohol

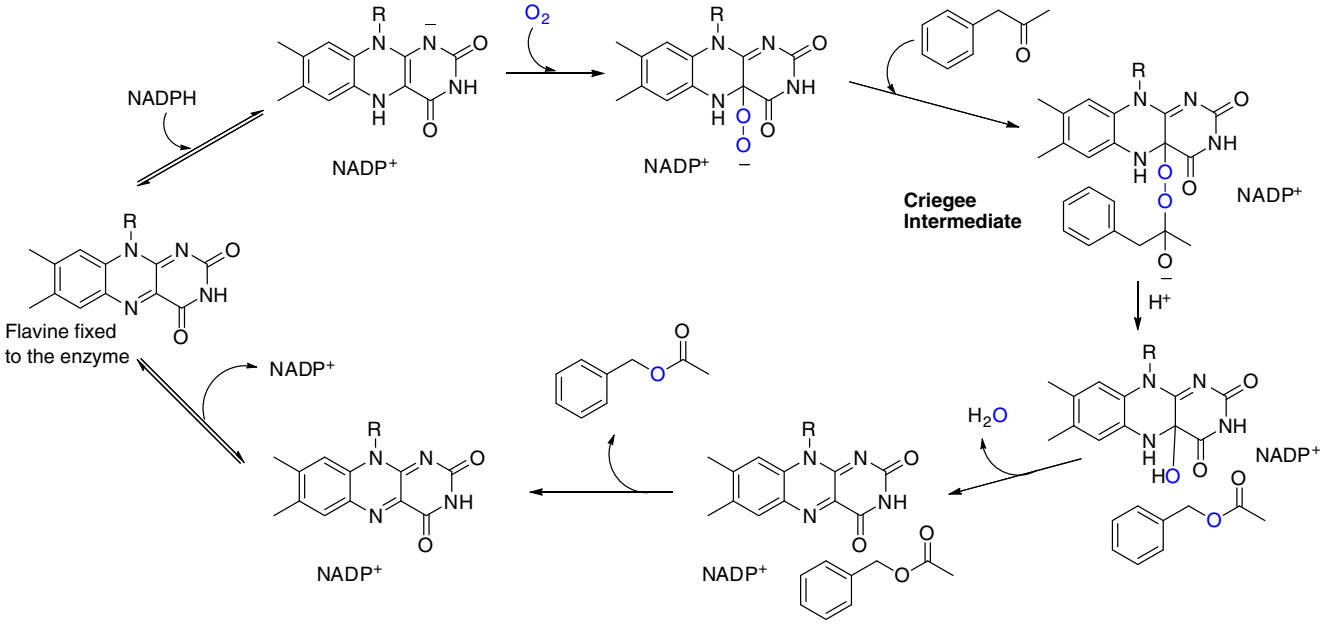


Figure 3. Reaction mechanism of Baeyer–Villiger oxidation of ketones by monooxygenase with flavine and NADPH.

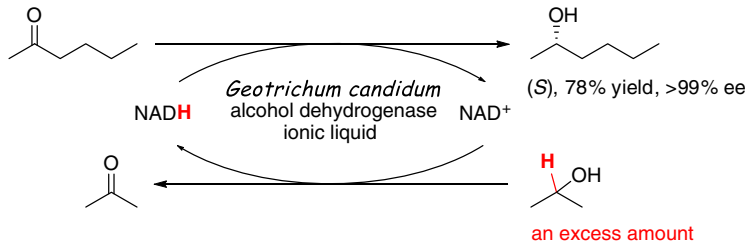


Figure 4. Recycling of NADH using alcohol for reduction of a ketone.

dehydrogenase since the enzyme catalyzes both reduction and oxidation. Usually, an excess amount of the hydrogen source is used to push the equilibrium to the formation of product alcohols. For example, 2-propanol was used to reduce hexanone to (*S*)-2-hexanole in 72% yield with >99% ee by alcohol dehydrogenase of *Geotrichum candidum* with NAD^+ in an ionic liquid as shown in Figure 4.[9]

2.3.2. Sugars

Glucose and glucose-6-phosphate (G6P) have been widely used to recycle coenzymes. For example, reduction of ketones by reductase from *Candida magnolia* with anti-Prelog enantioselectivity was proceeded with the aid of glucose and glucose dehydrogenase to regenerate the reduced form of the coenzyme as shown in Figure 5a.[10]

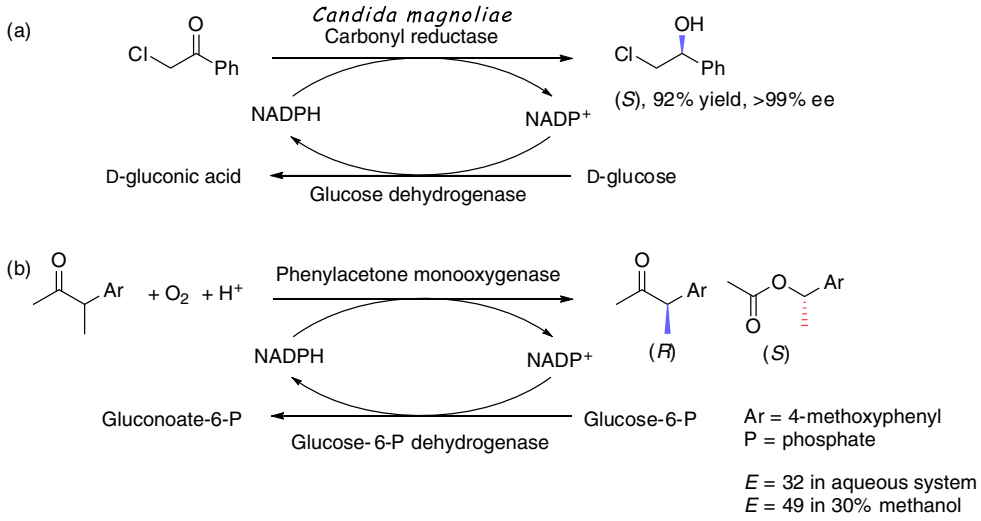


Figure 5. Recycling of NADPH using sugar (a) for reduction of a ketone and (b) for Baeyer–Villiger oxidation.

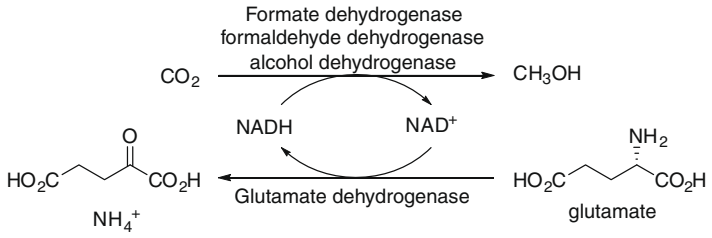


Figure 6. Recycling of NADH using amino acid.

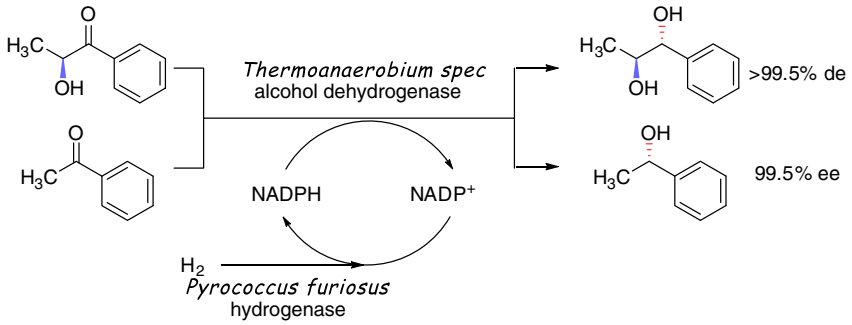


Figure 7. Recycling of NADPH using molecular hydrogen.

Enzymatic Baeyer–Villiger oxidation with molecular oxygen also needs reduced form of the coenzyme, NADPH (Fig. 3). In the oxidation of 3-(4-methoxyphenyl)butan-2-one, NADPH was converted to $NADP^+$. Thus produced oxidized form of the coenzyme was recycled to the reduced form by glucose-6-phospate (G6P) and glucose-6-phosphate dehydrogenase (G6PDH) as shown in Figure 5b.[11]

2.3.3. Amino acid

Glutamate dehydrogenase has been frequently used for recycling of NADH. For example, in the reduction of carbon dioxide to methanol through formic acid and formaldehyde by multi-enzymatic system, the oxidized form of the cofactor (NAD^+) was recycled to the reduced form (NADH) by glutamate dehydrogenase and glutamic acid as shown in Figure 6.[12]

2.3.4. Hydrogen

Molecular hydrogen has been used for the recycling of coenzymes.[13] For example, a soluble hydrogenase (EC 1.18.99.1) from the marine hyperthermophilic strain of the archaeon *Pyrococcus furiosus* (PF H_2ase I) has been used for the regeneration of NADPH with molecular hydrogen. Utilizing the thermophilic NADPH-dependent alcohol dehydrogenase from *Thermoanaerobium* sp. with PF H_2ase I, (2*S*)-hydroxy-1-phenyl-propanone was quantitatively reduced to the corresponding (1*R*,2*S*)-diol in >98% de with total turnover numbers (mol product/mol consumed cofactor $NADP^+$) of 160 as shown in Figure 7.[13] Similarly, acetophenone was also reduced by using the same reduction system.

2.3.5. Electrochemical regeneration

Electrochemical regeneration of cofactors is expected to be a promising, clean, and sustainable technology since the 1980s. However, most concepts for the coupling of this technology to enzymatic reaction have suffered from low productivities, insufficient stabilities, or difficulty to scale up. Here, an efficient method for electrochemical regeneration of NADH for enzymatic reaction is reported.[14] A rhodium complex was used for regeneration of NADH, and octane was used as the second organic phase to avoid product inhibition. Thus, the reduction of 3-methylcyclohexanone with the thermophilic alcohol dehydrogenase from *Thermus* sp. gave (1*S*,3*S*)-3-methylcyclohexanol with diastereomeric excess of 96% from the corresponding racemic ketone with a productivity of 0.13 g L^{-1} h^{-1} and a current efficiency of 85% as shown in Figure 8.

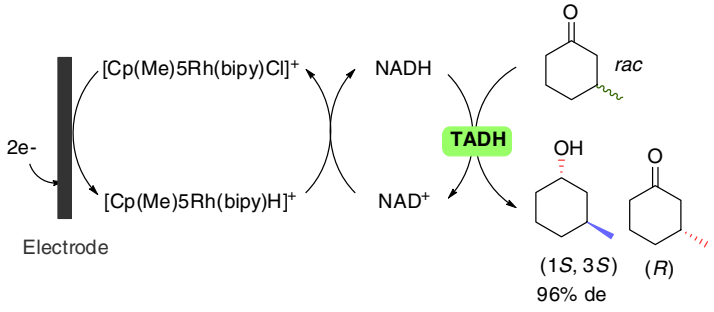


Figure 8. Recycling of NADH by electric power.

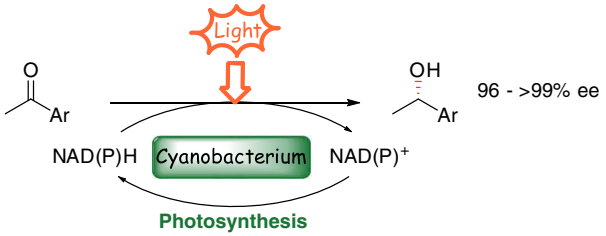


Figure 9. Recycling of NAD(P)H by light energy using a photosynthetic microorganism.

2.3.6. Light-driven regeneration

Photochemical methods have been developed to provide an environmentally friendly system, that employ light energy to regenerate NAD(P)H or flavin. In the example shown in Figure 9, the reduction of acetophenone derivatives using a cyanobacterium, a photosynthetic microorganism, occurred more effectively under illumination than in the dark.[15,16] The light energy harvested by the cyanobacterium was converted into chemical energy in the form of NADPH through an electron transfer system, and, consequently, the chemical energy (NADPH) was used to reduce the substrate to chiral alcohol (96–>99% ee). The light energy, which is usually utilized to reduce CO_2 to synthesize organic compounds in the natural environment, was used to reduce the artificial substrate in this case.

Reduced form of flavin can also be regenerated using light energy. For example, light-driven reduction of ketoisophoron was reported (Fig. 10a).[17] Using old yellow enzyme homologue, YqiM, from *Bacillus subtilis*[18] to reduce substrate and using FAD and EDTA to recycle enzyme-bound flavine with light energy, ketoisophoron was reduced to the corresponding (*R*)-product in quantitative yield with 88% ee. As shown in Figure 10b, the same regeneration system was used for Baeyer–Villiger oxidation.[17] Oxidation of 2-phenylcyclohexanone by monooxygenase PAMO-P3 with the system gave the corresponding chiral lactone.

2.4. Oxygen source for oxidation

For oxidation, oxidized form of coenzymes is necessary to be regenerated. Here, examples using molecular oxygen and electric power are shown.

2.4.1. Molecular oxygen

NADH oxidase from *Lactobacillus brevis* is a useful enzyme for oxidation of NADH using molecular oxygen to perform an oxidation of alcohols in mild conditions. The system was used for oxidation of primary alcohols to the corresponding acids using *Brevibacterium* alcohol dehydrogenase and aldehyde dehydrogenase as shown in Figure 11.[19]

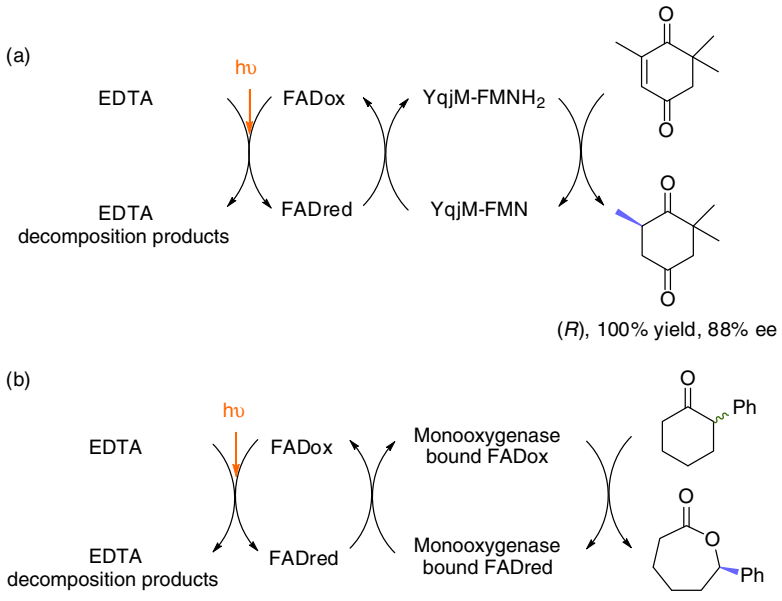
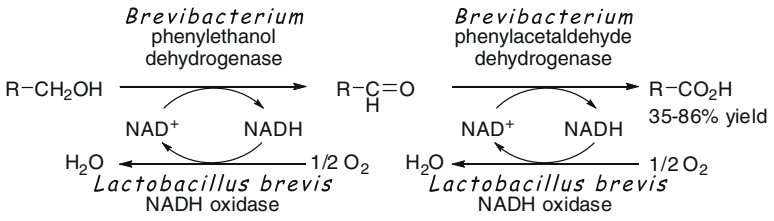


Figure 10. Recycling of reduced form of flavin by light energy using EDTA for (a) reduction and (b) Baeyer–Villiger oxidation.



R=Ph, CH_2Ph, $(CH_2)_2Ph$, CH_2OPh, C_5H_{11}, C_7H_{15}

Figure 11. Recycling of NAD^+ by molecular oxygen.

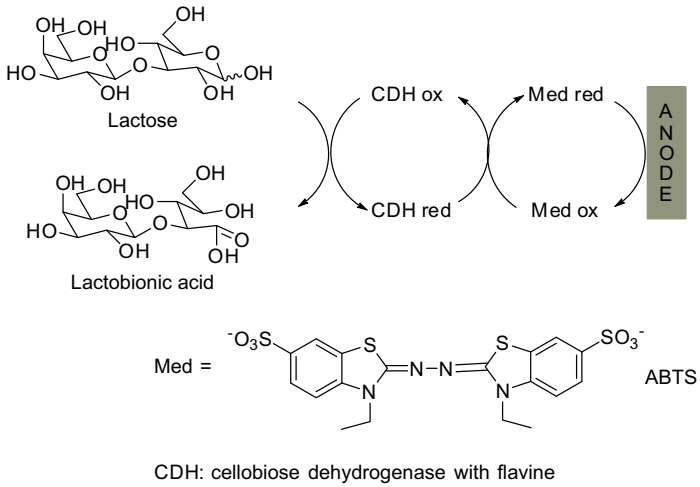


Figure 12. Recycling of oxidized form of mediator by electric power for oxidation of lactose.

2.4.2. Electric regeneration

Oxidized form of coenzyme can be also regenerated using electric power. Lactose was oxidized to lactobionic acid by cellobiose dehydrogenase (CDH), hemoflavoprotein, as shown in Figure 12.[20] Mediator, ABTS (2,2′-azinobis-3-ethylbenzothazoline-6-sulfonate), was oxidized at the anode, and oxidized mediator was used to oxidize the enzyme, followed by the oxidation of the substrate.

3. Preparation of biocatalysts and investigation of reaction conditions

Methods to find, mutate, and immobilize enzyme as well as to examine reaction conditions are described. Screening sources from which to search new biocatalysts became diverse with the recent progress in molecular biology. The following sources are available.

(1) Enzymes expressed in microorganisms were found in environment and cultivated in laboratory.
(2) Enzymes expressed using DNA were extracted from environment (metagenome).
(3) Enzymes expressed using information from databank.

Regarding mutation of oxidoreductase, rational and random mutagenesis has been conducted and is explained here. Concerning the reaction conditions for oxidoreductase, examples for the reaction in organic solvents as well as in supercritical CO_2 and ionic liquids are introduced.

3.1. Screening of microorganism

Screening of enzymes expressed in microorganism, which is found in environment and cultivated in laboratory, has a merit of finding a novel enzyme because the method does not use the protein sequence information of known enzymes. However, enzymes in the microorganisms unable to be cultivated or enzymes unable to be expressed in the original microorganisms cannot be found.

A novel screening method using multi-well plates has been reported.[21] Microbial cultures of about 300 microbes, demonstrating utility in reducing model ketones, were arrayed in multi-well plates, stored until use at −80 °C and used to rapidly identify specific organisms capable of producing chiral alcohols used as intermediates for several drug candidates as shown in Figure 13. Approximately 60 cultures were shown to selectively reduce a ser-

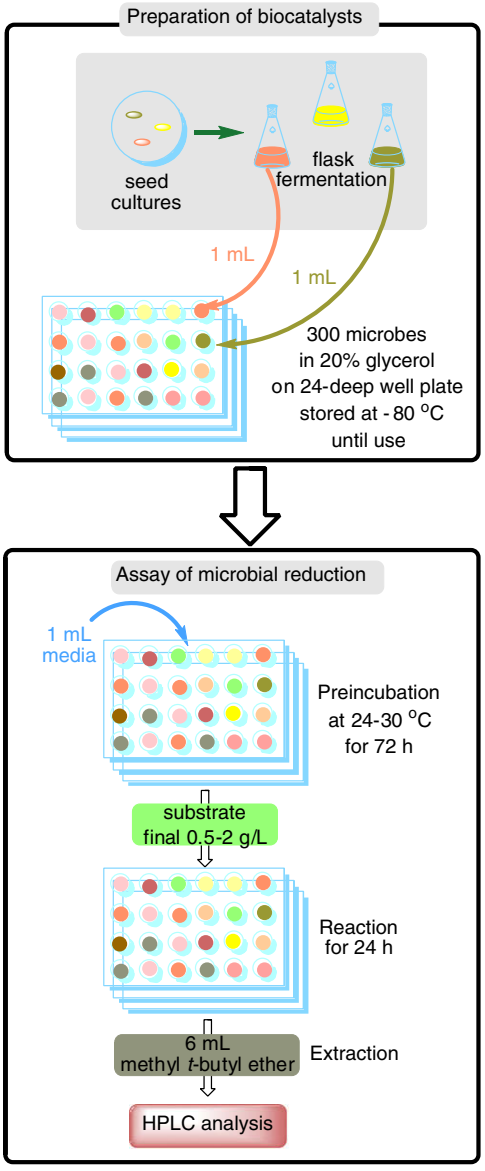


Figure 13. Screening of microorganisms using multi-well plates.

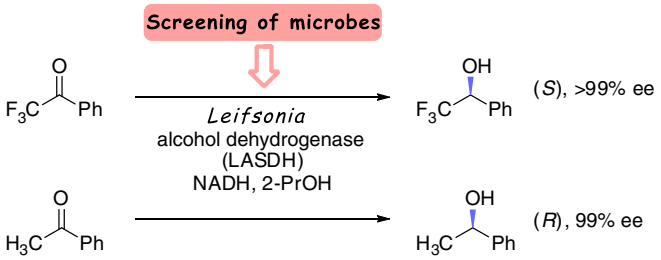


Figure 14. Screening of styrene-assimilating bacteria for reduction of trifluoroacetophenone.

ies of alkyl aryl ketones, providing both the *R* and *S* enantiomers of the corresponding alcohols in 92–99% ee with yields up to 95% at 1–4 g/L.

Another example of the screening is to find biocatalysts to convert trifluoroacetophenone to the corresponding (*S*)-alcohol in the presence of 2-propanol as a hydrogen donor.[22] By screening of styrene-assimilating bacteria (ca. 900 strains) isolated from soil samples, *Leifsonia* sp. *strain* S749 was found to convert the desired reaction. The enzyme catalyzing the reaction was isolated and characterized. The enzyme could reduce trifluoroacetophenone to (*S*)-1-phenyltrifluoroethanol and could also reduce acetophenone to (*R*)-1-phenylethanol both in >99% and 99% ee, respectively, as shown in Figure 14.

Next example shows the screening for a catalyst for enantioselective reduction of bicyclo[2.2.2]-octane-2,6-dione.[23,24] Yeast strains (327 strains) from more than 31 different genera were screened, and reducing activity was found in 80% of the screened yeasts (262 strains) containing 229 strains which gave (−)-*endo* isomer and 83 strains which gave *exo* isomers. As shown in Figure 15, *Candida wickerhamii* UOFS Y-0652 afforded mainly (+)-*exo*-isomer, and *Cryptococcus albidus* UOFS Y-2127 afforded (1*R*,4*S*,6*S*)-6-hydroxybicyclo[2.2.2]octane-2-one((−)-*endo*-isomer) in 79% yield with >98% ee.[23] The reduction by baker's yeast also afforded the (1*R*,4*S*,6*S*)-isomer. Glucose was added as the co-substrate. The co-substrate yield (ketoalcohol/glucose consumed (1.7–2.5%)) was enhanced by using genetically engineered strains with reduced phosphoglucose isomerase activity by 2.3-fold and with deleted alcohol dehydrogenase gene by 2.4-fold.[24]

3.2. Screening of enzymes using metagenome

Metagenome, collection of DNA extracted directly from environmental samples, can be screened to find an enzyme to catalyze a desired reaction. This screening method enables to find enzymes in organisms that are not easily cultured in laboratory. In fact, by using this method, alcohol oxidoreductase for formation of carbonyls from short-chain polyols was found.[25] As shown in Figure 16, metagenomic DNA libraries from three different soil samples (meadow, sugar beet field, cropland) were constructed. The libraries, comprising approximately 1,267,000 independent clones (approximately 4.05 Gbp of DNA), were screened for the production of carbonyls from short-chain (C2 to C4) polyols such as 1,2-ethanediol, 2,3-butanediol, and a mixture of glycerol and 1,2-propanediol on indicator agar. Through initial screening and sequencing of the positive clone, 26 complete and 14 incomplete predicted protein-encoding genes were found, and most of these genes were similar to genes with unknown functions from other microorganisms or unrelated to any other known gene. The further analysis was focused on the seven plasmids recovered from the positive clones, which exhibited an NAD(H)-dependent alcohol oxidoreductase activity with polyols or the correlating carbonyls as substrates in crude extracts.

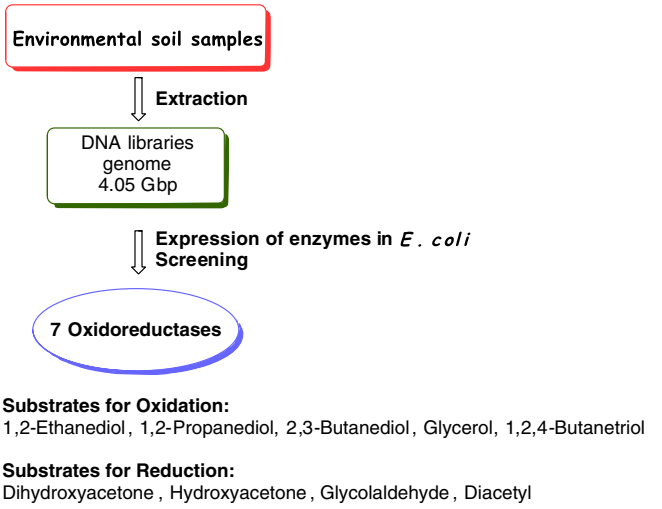


Figure 16. Screening of metagenomes of complex microbial consortia derived from soils for oxidation and reduction.

3.3. Screening of enzymes of microorganisms of known genome data

Screening of a library of over-expressed enzymes from microorganisms with known genome data is useful to search a catalyst for a desired reaction.[26] For example, enantio- and diastereoselective reduction of ethyl 2-chloro-3-oxoalkanoates was investigated using a reductase library (18 known and putative enzymes) from baker's yeast expressed in *Escherichia coli*. As shown in Figure 17, in nearly all cases, it was possible to produce at least two of the four possible 3-chloro-2-hydroxy ester diastereomers with high optical purities. four enzymes (YJR096w, YDL124w, YGL185c, and YNL274c) also reduced 2-chloro-3-phenylpropanoate to the corresponding *syn*-(3*R*)-hydroxy ester. 2-Chloro-3-oxopentanoate was reduced by YGL157w to the *anti*-(3*S*)-hydroxy ester selectively, and 2-chlorobutanoate was reduced by YOR120w to the *syn*-(3*S*)-product. The utility of this approach was further demonstrated by reducing ethyl 2-chloroacetoacetate to the corresponding *syn*-(2*R*,3*S*)-alcohol on a multigram scale using whole cells of *E. coli* strain overexpressing a single yeast reductase.

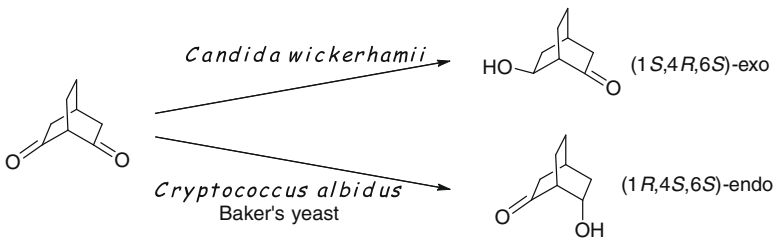


Figure 15. Screening of microorganisms for reduction of bicyclo[2.2.2]-octane-2,6-dione.

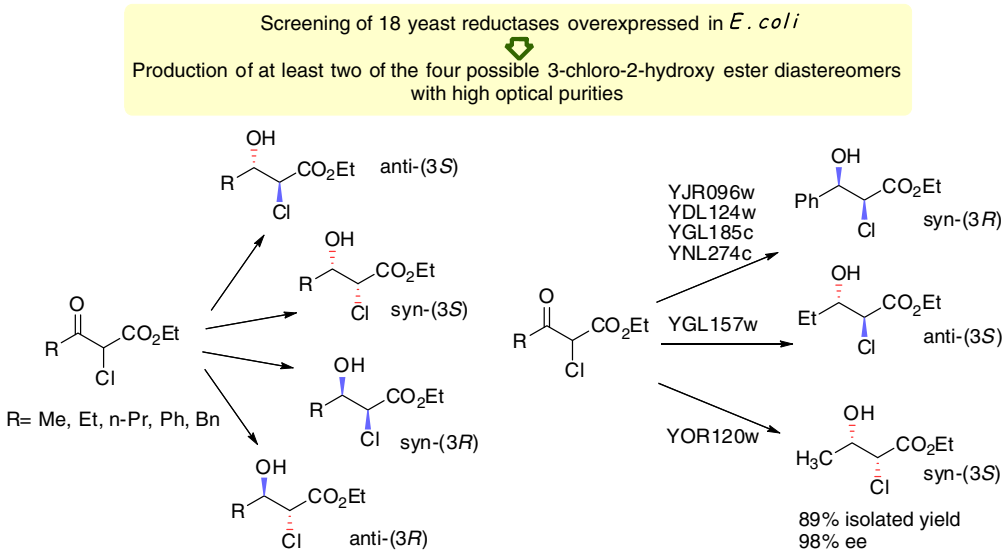


Figure 17. Screening of reductase library (18 known and putative enzymes) from baker's yeast expressed in *E. coli* for reduction of ethyl 2-chloro-3-oxoalkanoates.

A library of 20 baker's yeast reductases over-expressed in *E. coli* was also screened against reduction of 3-oxo-3-phenylpropanenitrile for the synthesis of precursors for both antipodes of fluoxetine, atomoxetine, and nisoxetine, popular serotonin/norepinephrine reuptake inhibitors.[27] Four enzymes were found to reduce this substrate, and by changing the enzyme both enantiomers of 3-hydroxy-3-phenylpropanitrile could be prepared with a high ee as shown in Figure 18. In addition, the *E. coli* whole-cell system was optimized (a nitrogen-deficient media was used) to decrease the competing alkylating product (2Et-PPN) to 4–6% while the reduction with baker's yeast gave 90% yield of 2Et-PPN.

Next example shows the screening of yeast overexpressing reductases and dehydrogenases for reduction of bicyclo[2,2,2]octane-2,6-dione (BCO2,6D).[28] From a set of recombinant yeast cells expressing aldo-keto reductases (six clones), short-chain alcohol dehydrogenases (SADH 12 clones) and dihydroflavonol reductases (four clones), two strains with aldo-keto reductases, and one strain with SADH were selected for the next stage, and, at last, YMR226cp was selected to reduce BCO2,6D with the aid of glucose at the rate of six times faster than the original host yeast as shown in Figure 19.

3.4. Mutation of enzymes

Improvement of enzyme performance by mutation can be classified into two categories; (1) rational mutation such as point

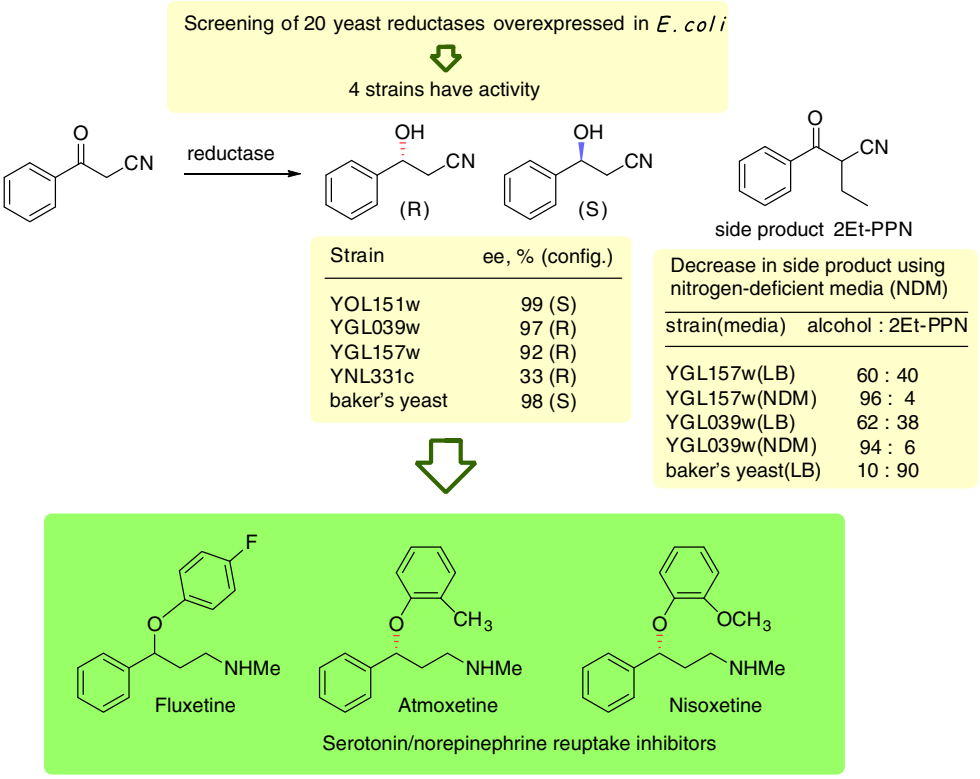
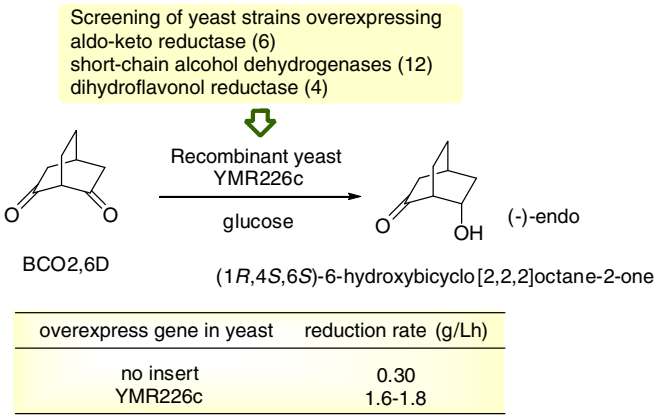


Figure 18. Screening of reductase library from baker's yeast expressed in *E. coli* for reduction of 3-oxo-3-phenylpropanenitrile.



overexpress gene in yeast	reduction rate (g/Lh)
no insert	0.30
YMR226c	1.6-1.8

Figure 19. Screening of reductases and dehydrogenase library expressed in yeast for reduction of bicycle[2,2,2]octane-2,6-dione.

mutation and site-directed saturation mutagenesis (change of one amino acid to all 20 naturally occurring amino acids at a specific site within a protein) and (2) random mutation such as directed evolution (change of amino acids at random to create a large library of mutants proteins followed by the selection of the proteins possessing the desired property. The process is repeated as in the nature). When the structure of the enzyme is not known or cannot be predicted, only random mutation can be performed. In some cases, an enzyme performance has been improved by a random mutation at unpredictable position from the structural-based information. Examples for the mutations are as follows.

3.4.1. Rational mutation to widen substrate specificity

Rational design in modification of enzyme by point mutation has been reported.[29] The secondary alcohol dehydrogenase from *Thermoanerobacter ethanolicus* 39E (TeSADH) is highly thermostable and solvent-stable, and it is active on a broad range of substrates. These properties make TeSADH an excellent template. However, TeSADH has no detectable activity on (*S*)-1-phenyl-2-propanol, a precursor to major pharmaceuticals containing secondary alcohol group, but it is highly active on 2-butanol. From the structural model research, tryptophan-110 obstructs the proper fitting of (*S*)-1-phenyl-2-propanol while the residue does not interfere the fitting of 2-butanol. Then, tryptophan-110 (W110) was replaced with alanine (A). The W110A mutant could use (*S*)-1-phenyl-2-propanol, (*S*)-4-phenyl-2-butanol, and the corresponding ketones as substrates and produce (*S*)-4-phenyl-2-butanol from benzylacetone with >99% ee as shown in Figure 20.

The reduction by W110A–TESADH in Tris buffer was conducted using 2-propanol (30%, v/v) as co-solvent and co-substrate. This concentration of 2-propanol was crucial not only to enhance the solubility of hydrophobic phenyl ring-containing substrates in the aqueous reaction medium, but also to shift the equilibrium in the reduction direction. A series of phenyl ring-containing ketones, such as 4-phenyl-2-butanone and 1-phenyl-1,3-butadione, were reduced with good to excellent yields and high enantioselectivities and regioselectivity (for the latter substrate). On the other hand, 1-phenyl-2-propanone was reduced with lower ee (37% ee) than 2-butanone derivatives. The reduction of 3-chloro-4-(4-chlorophenyl)-2-butanone afforded (2*S*,3*R*)-anti-alcohol in 83% conversion and >99% ee with 84% de. Since the recovered ketone is a racemic mixture, some dynamic kinetic resolution proceeded during the reduction.[30]

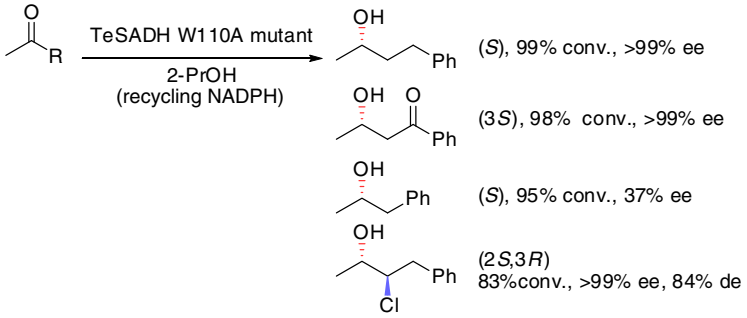
3.4.2. Rational followed by random mutations to widen substrate specificity

Galactose oxidase (GOase), which oxidizes 6-OH of galactose (primary alcohol) to the corresponding aldehyde, was subjected to saturation mutagenesis and directed evolution to widen substrate specificity. The mutation of GOase to introduce 6-OH oxidase activity of glucose was investigated.[31] Combinatorial library by saturation mutagenesis of the Trp290, Arg330, and Glu406 residues were constructed, and the mutant M-RQW (Trp290Phe, Arg330Lys,

(a) [29]

	TeSADH W110A mutant (U/mg protein)	TeSADH wild type (U/mg protein)
2-butanol	3.65	45.27
phenylacetone	37.61	1.02
(*S*)-1-phenyl-2-propanol	14.62	
(*R*)-1-phenyl-2-propanol	0.51	
benzylacetone	34.58	5.94
(*S*)-1-phenyl-2-butanol	26.83	
(*R*)-1-phenyl-2-butanol	0.26	

(b) [30]



W110A: mutant where tryptophan-110 (W110) was replaced with alanine (A)
TeSADH: *Thermoanaerobacter ethanolicus* secondary alcohol dehydrogenase

Figure 20. Rational mutation of *Thermoanerobacter ethanolicus* 39E secondary alcohol dehydrogenase to widen substrate specificity (a) comparison of activities of wild type and mutant, W110A (b) synthesis using W110A mutant.

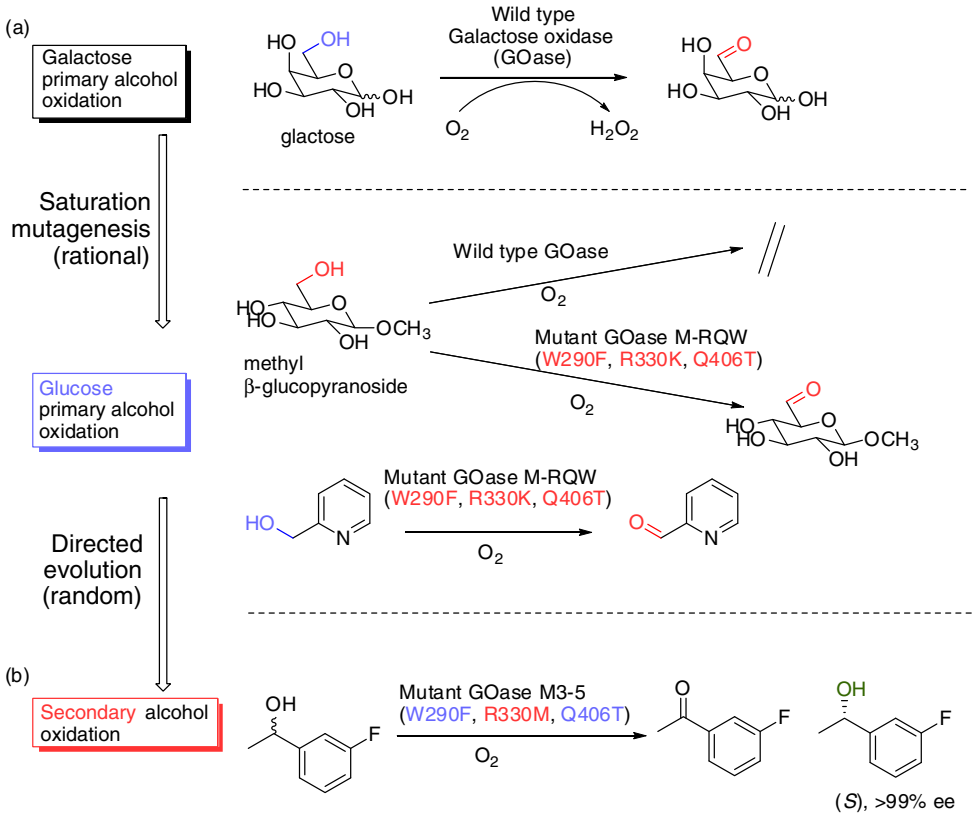


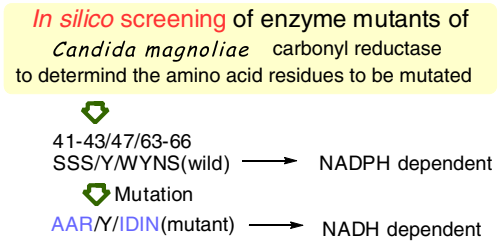
Figure 21. Mutation of galactose oxidase to widen substrate specificity (a) saturation mutagenesis to introduce glucose oxidation activity and (b) directed evolution to introduce enantioselective secondary alcohol oxidation activity.

and Gln406Try) showed activity for oxidation of glucose as shown in Figure 21a. The mutant M-RQW catalyzed the reaction of glucose (4.8% of the activity with galactose), methyl β-glucopyranoside (3% of the activity with galactose), and 2-pyridine methanol (1,80,000% of the activity with galactose).

Further modification was done by directed evolution of the M-RQW mutant to gain secondary alcohol oxidative ability.[32] Although M-RQW displays activity scarcely toward secondary alcohols, M3-5 variant (Lys330Met) exhibit high activity toward oxidation of secondary alcohols such as 3-fluoro-1-phenylethanol. As a result, (*S*)-alcohol was obtained in 99% ee as shown in Figure 21b.

3.4.3. In silico screening of enzyme followed by site-directed mutation to change coenzyme dependency from NADPH to NADH

Coenzyme dependency of *C. magnoliae* carbonyl reductase was changed from expensive NADPH to cheaper NADH by point muta-



Enzyme	Activity for COBE (U/mg)	K_M for coenzyme (μM) NADPH	NADH	K_M for COBE (μM)	k_{cat} for coenzyme (sec^{-1})
Wild	27.8 (NADPH)	16.7	-	3.0	870 (NADPH)
Mutant	9.9 (NADH)	-	62	2.6	120 (NADH)

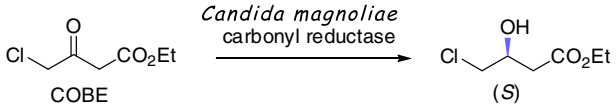


Figure 22. Mutation to change co-enzyme from NADPH to NADH with computer-aided screening of mutant and site-directed mutagenesis.

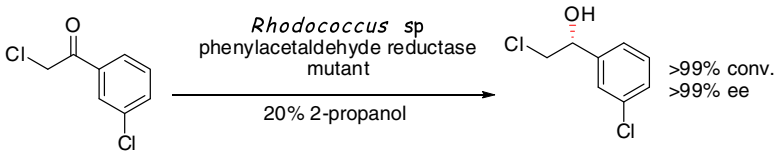


Figure 23. Mutation to improve catalytic efficiency in concentrated 2-propanol and substrate.

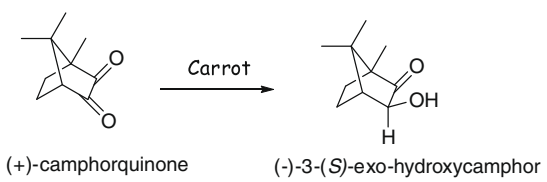


Figure 24. Reduction of a diketone by carrot.

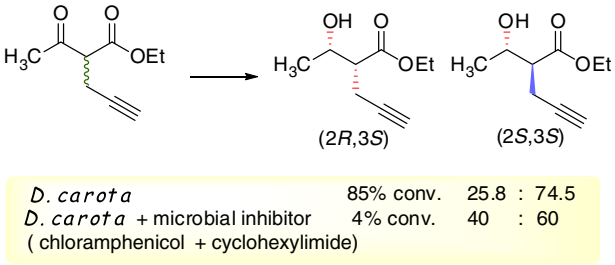


Figure 25. Effect of bacterial inhibitor on reduction of an α-substituted β-keto ester by carrot.

tion. The design for the mutation was done by in silico screening of enzyme mutants.[33] By comparing amino acid residues of both NADH and NADPH-dependent dehydrogenases with computer-aided calculation methods, including three-dimensional structure modeling and in silico screening of enzyme mutants, eight amino acid residues were selected to be modified. Then, seven or eight amino acid residues on the adenosine-binding pocket of the enzyme were substituted, systematically. The resulting 41–43/47/63–66 mutants (AAR/Y/IDIN from SSS/Y/WYNS of wild type) showed NADH dependency and lost their ability to utilize NADPH as a coenzyme, but retained those catalytic activities. Kinetic parameter V_{max} and K_m values are shown in Figure 22. As a model system for industrial production of optically active alcohols, the mutant was applied to an asymmetric reduction of ethyl 4-chloro-3-oxobutanoate, cooperating with a coenzyme-regeneration system that uses an NAD-dependent formate dehydrogenase in a water-organic two phase system; 162 g/l of the substrate was reduced to the corresponding hydroxyl ester (>99% ee) in 163 g/l where the turnover number of NAD^+ to the product was calculated to be 1000.

3.4.4. Random followed by rational mutations to improve catalytic efficiency in concentrated 2-propanol and substrate

Phenylacetaldehyde reductase (PAR) from *Rhodococcus* sp. was mutated to improve the conversion efficiency in high concentrations of substrate and 2-propanol.[34] Here, 2-propanol acts as a solvent and hydrogen donor of coupled cofactor regeneration during the conversion of substrates. First, the PAR library was generated by mutagenic PCR. With only a single selection round of a PAR mutant library followed by combination of advantageous mutations, PAR was successfully adapted with six amino acid replacements for the conversion of high concentrations of substrate with concentrated 2-propanol. *m*-Chlorophenacylchloride was reduced by the mutant in 20% (V/V) 2-propanol to the corresponding (*R*)-alcohol in >99% conversion with >99% ee as shown in Figure 23.

3.5. Hyperthermophilic enzyme as a biocatalyst

As a source for the biocatalyst, thermophilic organism is important because thermo stability of the enzyme is very critical parameter to evaluate the biocatalyst. So far, there are many hydrolytic enzymes with high thermo stability, but there are only a few dehydrogenases with such properties. Here is an interesting example where dehydrogenase shows a high resistance to thermal inactivation. Asymmetric reduction of simple aromatic ketones and keto esters has been investigated by an alcohol dehydrogenase from the hyperthermophilic archaeon *P. furiosus* (PFADH) obtained by overexpression in *E. coli*.[35] The half-life at 100 °C was 130 min. The increase in reaction temperature raised the enzyme activity, but exerted no effect on the enantioselectivity. This enzyme also showed a high tolerance to organic solvents such as DMSO, *iso*-propanol, methyl *tert*-butyl ether, and hexane, which is a particularly important and useful feature for the reduction of ketones with a low solubility in aqueous buffers.

3.6. Plant cell culture as a biocatalyst

Many microorganisms have been screened for the use as biocatalysts, but enzymes derived from plant have not yet been fully explored, so they may have unknown and unique enzymes. Some examples for the use of the plant cell culture are described in Section 2.3. 'Light-driven regeneration (of coenzyme)'. This section also shows some examples for the use of plant cell as a biocatalyst.

Vegetables such as carrot root are frequently utilized as biocatalysts for asymmetric reduction. Carrot-mediated reduction of prochiral ketones has been reviewed.[36] Here, one of the examples for the reduction of α-diketones such as camphorquinone by carrot is shown in Figure 24.[37]

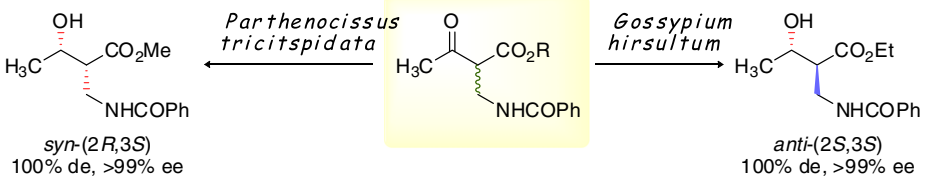


Figure 26. Diastereo- and enantioselective reduction of a β-ketoester by plant cell cultures.

A serious problem in the use of vegetable from market for biocatalysts is the effect of microbial contaminations.[38] Actually, 360 endophytic microorganism strains were isolated from carrot.[39] Four strains of endophytic microorganisms isolated from carrot root were reported to be able to carry out the reduction of the carbonyl group with diverse degree of enantio- and diasteroselectivity. Furthermore, biotransformation in the presence of bacterial inhibitor affects the stereochemical outcome of the reduction of α-substituted β-keto ester; although *D. carota* gave the (3*S*)-hydroxy ester in 85% conversion with 48.7% de (*anti*), the addition of yeast and bacterial inhibitors (cyclohexylimide and chloramphenicol) results in a large decrease in the conversion and de (4% conversion with 20% de (*anti*)) as shown in Figure 25. These results indicate that endophytic microorganisms might be involved in the enantioselective reduction of ketones and keto esters with fresh carrot root pieces.Figure 26 shows diastereo- and enantioselective reduction of a β-keto ester.[40] The reduction of methyl 2-benzamidomethyl-3-oxobutanoate by *Parthenocissus tricitspidata* gave *syn*-(2*R*,3*S*)-hydroxyester in 100% de with >99% ee. The anti-product, (2*S*,3*S*)-isomer was obtained by the reduction of the corresponding ethyl ester with *Gossypium hirsultum* in 100% de with >99% ee.

Figure 27 shows the reduction of a natural bioactive diketone 1-(5-acetyl-2-hydroxyphenyl)-3-methylbut-2-en-1-one to 6-(1(*S*)-hydroxyethyl)-2,2-dimethyl-2,3-dihydro-4*H*-chromen-4-one in >97% ee with >78% yield by *Brassica napus* hairy roots.[41] The diketone was firstly changed to the corresponding chromanone derivative and then reduced to the product.

Reduction of acetophenone derivatives and oxidation of (*R*/*S*)-1-phenylethanol derivatives have been evaluated using edible plants as biocatalysts.[42] Chiral (*R*)- and (*S*)-alcohols were prepared by different plants in up to 98% ee. Ketones can also be prepared from alcohol by oxidation using this clean protocol. In other examples, Manihot roots were used for reduction of aldehydes and ketones.[43]

3.7. Treatment of cell for improvement in selectivity

This section describes methods to treat cell for improvement in selectivity. This methodology is effective for reduction by cell containing several competing enzymes with different selectivities. Figure 28 shows that lyophilization and rehydration improved enantioselectivity for reduction of a ketone with *Debaryomyces hansenjii*.[44] The enantioselectivity increased up to 95% ee from 80% ee for the reduction by untreated cells. The improvement in ee was explained by selective deactivation of enzyme(s) during lyophilizaton.

Figure 29 shows that mild detergent treatment of *Candida tropicalis* improved selectivity of reduction of a bicyclic diketone, bicyclo[2.2.2]octane-2,6-dione.[45] Cytosolic *endo*-alcohol reductase could be separated from the membrane-bound *exo*-alcohol reductase by treatment with detergent, Y-Per (yeast extraction protein reagent, non-ionic detergent mainly consists of *n*-octyl-β-D-thioglucopyranoside). As a result, the reduction gave pure *exo*-alcohol with the aid of NADPH, glucose-6-phosphate dehydrogenase, and glucose-6-phosphate. In this case, cultivation conditions also affects the enzyme activities. When *C. tropicalis* was grown on D-sorbitol, a twofold increase in the *exo*-reductase activity was observed as compared to that grown on glucose.

3.8. Immobilization of enzymes

Immobilization of enzyme is one of the most effective methods to improve stability and easiness in handling and recycling of biocatalysts, so various methods have been reported. For example, a method using calcium alginate was used to immobilize baker's yeast.[46–48] As shown in Figure 30a, cells were immobilized in calcium alginate fibers with double gel layers. The immobilization procedure prevents cell leakage from alginate fibers into the medium because the alginate fibers cells were restricted to the inner layer, whereas the outer layer helped to prevent leakage and contact with potential inhibitors.

Figure 30b shows the use of the immobilized cell for asymmetric reduction of 3-oxobutanoate.[46] Particle size and cell concentration in the particle largely affected the reaction rate and enantioselectivity. At larger particle sizes, the specific reduction rate decreased while maintaining the enantioselectivity at ⩾98% ee. However, use of higher cell concentrations reduced the rate by 22% and ee to 83–90%. Calculation of the concentration profiles in alginate beads showed that among the reacting species, severe diffusion limitation is expected only for oxygen. This may have led to the lower reduction rates of the immobilized cells at larger particle sizes and at high cell concentrations and in addition, to the change in enantioselectivity.

Figure 30c and d also shows the utilization of immobilized baker's yeast for asymmetric reduction of ketones in a continuous process.[47,48] The reduction of ethyl benzoylformate gave (*R*)-ethyl

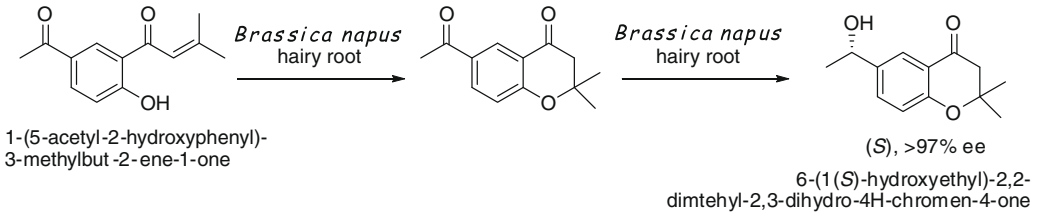


Figure 27. Transformation of a natural bioactive diketone by plant.

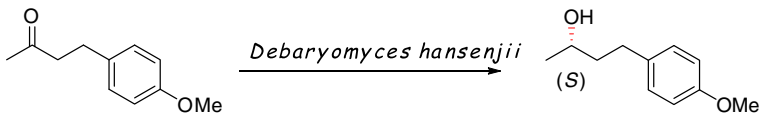


Figure 28. Lyophilization and rehydration of cell to improve enantioselectivity of reduction.

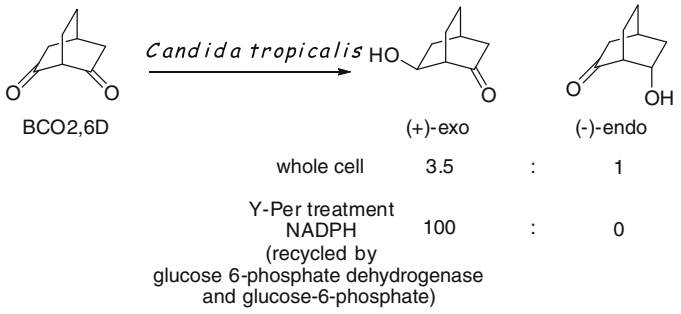


Figure 29. Treatment of cell with detergent to improve selectivity of reduction of diketone.

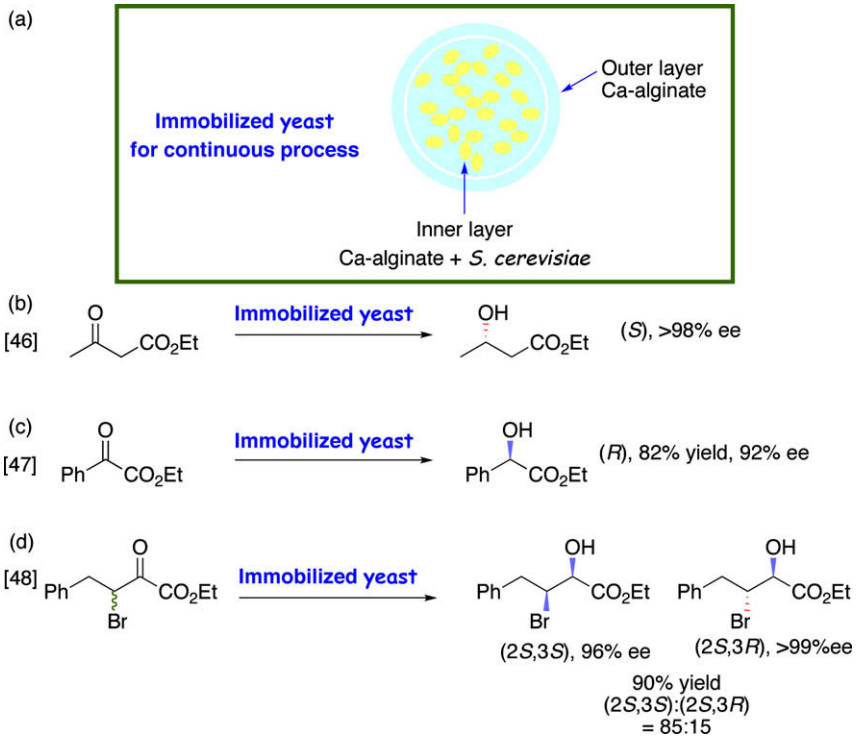


Figure 30. Immobilization of baker's yeast in alginate fibers with double-layer for asymmetric reduction of ketones in a continuous process: (a) immobilized yeast; (b) reduction of ethyl 3-oxobutanoate; (c) reduction of ethyl benzoylformate; (d) reduction of ethyl 2-oxo-3-bromo-4-phenylbutanoate.

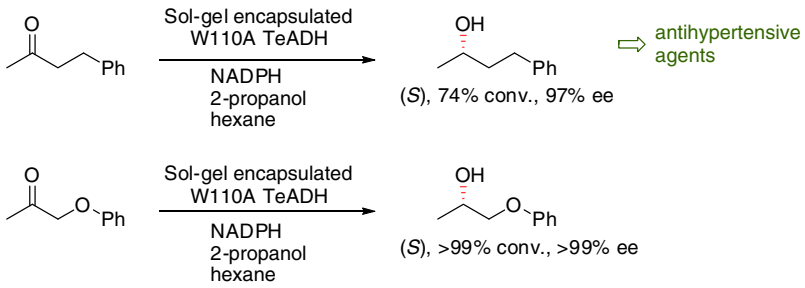


Figure 31. Encapsulation of W110A mutant of *Thermoanerobacter ethanolicus* alcohol dehydrogenase in sol–gel for asymmetric reduction of ketones.

mandelate in 82% yield with 92% ee, and the reduction of ethyl 2-oxo-3-bromo-4-phenylbutanoate gave *syn*-(2*S*)-isomer in 70% de with 96% ee.

Figure 31 shows that the use of sol–gel-encapsulated alcohol dehydrogenase (W110A mutant of *T. ethanolicus* alcohol dehydrogenase (TeADH)) for asymmetric reduction of 4-phenyl-2-butanone to (*S*)-4-phenyl-2-butanol, a precursor for the synthesis of bufeniode and labetalol (antipertensive agents).[49] Immobilization makes it possible to use the biocatalyst in organic media and increase the substrate concentrations to 140 mM from 35 mM in

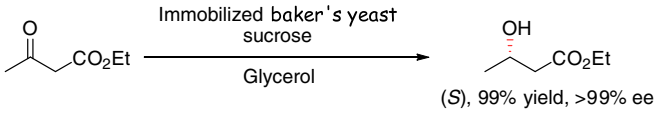


Figure 32. Glycerol as a solvent for reduction of a ketone by baker's yeast.

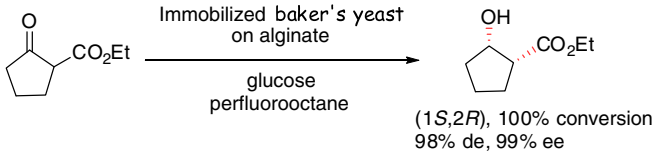


Figure 33. Perfluorooctane as a solvent for reduction of diketone by baker's yeast.

the buffer system. The immobilized biocatalyst could be reused three times. 1-Phenoxy-2-propanone was also reduced with a very high yield and enantioselectivity to the corresponding (*S*)-alcohol.

3.9. Solvent engineering

Non-aqueous solvent has been used for reaction with hydrolytic enzyme such as lipase frequently because lipase works at the surface of water and oil in nature. Oxidoreductase also works in non-aqueous solvent, but immobilization is necessary for some cases to stabilize the enzyme. Examples to use oxidoreductases in non-aqueous solvents are shown in this section.

3.9.1. Organic solvent

Organic solvents have been used for the oxidoreductase-catalyzed reaction to solubilize hydrophobic substrates, to construct two-layer system using hydrophobic solvents to reduce concentration of toxic substrate and product around enzymes in aqueous layer, and to simplify work-up procedure. Water miscible and immiscible solvents have been used. For example, sol–gel-encapsulated alcohol dehydrogenase (W110A mutant of *T. ethanolicus*) was used for asymmetric reduction of 4-phenyl-2-butanone to (*S*)-4-phenyl-2-butanol in hexane as shown in Figure 31.[49]

3.9.2. Glycerol

Glycerol is a non-toxic, biodegradable, and recyclable liquid manufactured as a by-product of transesterification of a triglyceride in the production of natural fatty acid derivatives and bio diesel. Use of glycerol allowed easy separation of the product by simple extraction with diethyl ether. As shown in Figure 32, free and immobilized baker's yeast was successfully employed in the asymmetric reduction of β-keto esters and ketones in glycerol.[50] The activities with immobilized cells were always higher than that with free cells while the enantioselectivity was very high (99%) with both catalysts.

3.9.3. Fluorous solvent

The merit of using fluorous media is simple work-up procedure, which contribute to the development of green chemistry. As shown in Figure 33, the reduction of ethyl 2-oxocyclopentanecarboxylate with immobilized baker's yeast (alginate) in perfluorooctane was investigated.[51] The reaction proceeded smoothly and gave the corresponding *syn*-(1*S*,2*R*)-hydroxyester in 98% de with 99% ee.

3.9.4. Supercritical CO_2

The concentration of atmospheric CO_2 has increased by about 35% since the beginning of the age of industrialization due to human activities such as combustion of fossil fuels and deforestation.[52] Organic synthesis also needs a large amount of organic solvents, and its use should be minimized. Therefore, supercritical CO_2 should be used instead of organic solvents, and enzymatic systems using supercritical CO_2 are important to be developed. Here is an example to use supercritical CO_2 for the reduction by alcohol dehydrogenase. Asymmetric reduction in supercritical CO_2 was realized by using immobilized *G. candidum* in 10 MPa supercritical CO_2; high activities and excellent enantioselectivities were observed for the asymmetric reduction of aromatic and cyclic ke-

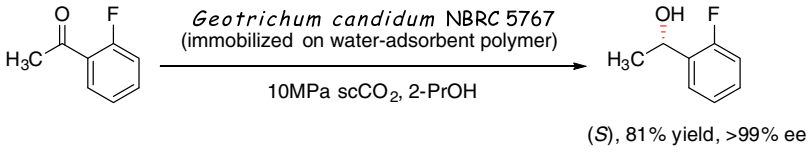


Figure 34. Supercritical CO_2 as a solvent for reduction of ketone by *Geotrichum candidum*.

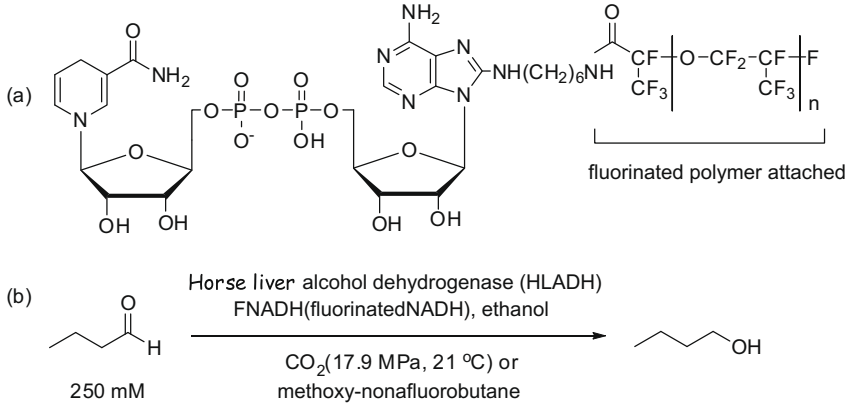


Figure 35. Supercritical CO_2 as a solvent for horse liver alcohol dehydrogenase (HLADH)-catalyzed reaction: (a) fluorinated coenzyme soluble in CO_2 and fluorous solvent; (b) reduction of aldehyde.

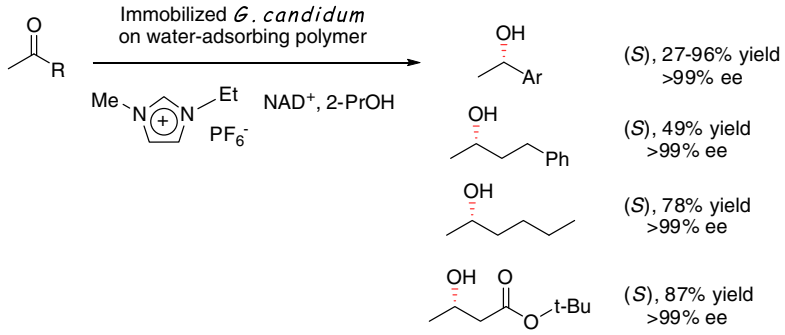


Figure 36. Ionic liquid as a solvent for reduction of ketone by immobilized *Geotrichum candidum*.

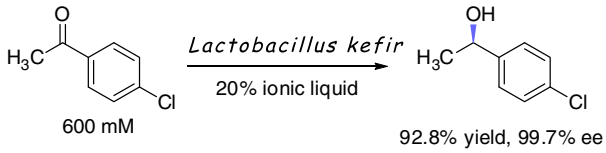


Figure 37. Water immiscible ionic liquid as a co-solvent for reduction of ketone by *Lactobacillus kefir*.

tones. For example, 2-fluoroacetophenone was reduced to the (*S*)-alcohol in 81% yield with >99% ee as shown in Figure 34.[53–55]

3.9.5. Fluorinated coenzyme for the reaction in supercritical CO_2 and fluorous solvent

The drawback of the use of supercritical CO_2 and fluorous solvent for isolated alcohol dehydrogenase-catalyzed reaction is that the coenzyme NAD(P)H as well as enzyme does not dissolve in supercritical CO_2 and fluorous solvent. This problem was solved by preparing fluorinated NADH (FNADH) by attaching a perfluoropolyether to NADH as shown in Figure 35a. Using FNADH, horse liver alcohol dehydrogenase (HLADH) was active in liquid carbon dioxide and a fluorous solvent, methoxy-nonafluorobutane (Fig. 35b). In both solvents, the activity of HLADH using FNADH was greater than the same molar amount of unmodified (insoluble) NADH.[56]

3.9.6. Ionic liquids

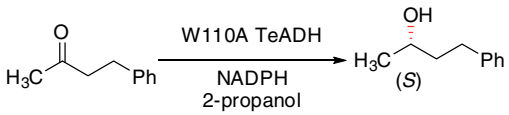
Ionic liquids, recyclable green solvent with no vapor pressure, have been used for oxidoreductase-catalyzed reaction. For example, Figure 36 shows the asymmetric reduction by a crude alcohol dehydrogenase, dried cells of *G. candidum*, in hydrophobic ionic liquid ([bmin]PF_6).[9]

When the cell was immobilized on water-absorbing polymer containing water, the reaction proceeded smoothly with excellent enantioselectivity, while the reaction without the immobilization on polymer did not proceed.

In the next example, to decrease substrate toxicity toward microbes, biphasic ionic liquid-water system was investigated for the reduction of 4-chloroacetophenone by *Lactobacillus kefir* (Fig. 37).[57] At 600 mM of the substrate concentrations, the substrate was highly toxic toward the cells, and the reduction did not proceed in aqueous system. Although no reduction occurred in the addition of 20% of decane, and only 4.2% yield resulted by addition of *tert*-butyl methyl ether, the addition of 20% BMIM[Tf_2N](1-*n*-butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide) enabled the reduction to proceed smoothly giving the (*R*)-alcohol in 92.8% yield with 99.7%. The result was explainable that the ionic liquid does not damage the cell membrane and reduce the substrate concentration in aqueous layer around the cell.

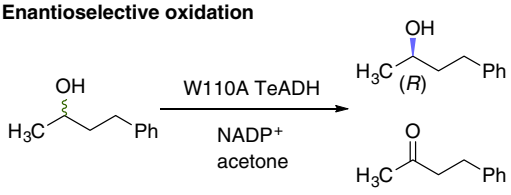
In the case using *T. ethanolicus* dehydrogenase mutant, W110A–TeSADH, water-miscible ionic liquid ([bmim][BF_4]) and conventional organic solvents (DMF, acetonitrile, and *tert*-butanol) were used as monophasic reaction media, and water-insoluble media such as ionic liquid ([bmim][NTf_2]) and organic solvents (diisopropyl ether, toluene, hexane, etc.) were used as biphasic systems (Fig. 38).[58] The reduction in high substrate concentrations (100 mM) proceeded in both systems and gave (*S*)-alcohols in high ee. The enantioselective oxidation of racemic alcohols in biphasic media also proceeded smoothly, and (*R*)-alcohols were obtained in high ee.

Asymmetric reduction



Solvent	conv.(%)	ee(%)
[bmim][BF_4] (monophasic)	86	98
DMF (monophasic)	95	95
acetonitrile (monophasic)	94	97
[bmim][NTf_2] (biphasic)	65	97
di*iso*propyl ether (biphasic)	94	99
toluene (biphasic)	36	97

Enantioselective oxidation



Solvent	conv.(%)	ee(%)	E-val
[bmim][NTf_2] (biphasic)	44	80	>100
tert-butyl methyl ether (biphasic)	31	38	17
toluene (biphasic)	18	22	>100

Figure 38. Water miscible and immiscible ionic liquid as a solvent for reduction and oxidation by *Thermoanerobacter ethanolicus* dehydrogenase mutant, W110A.

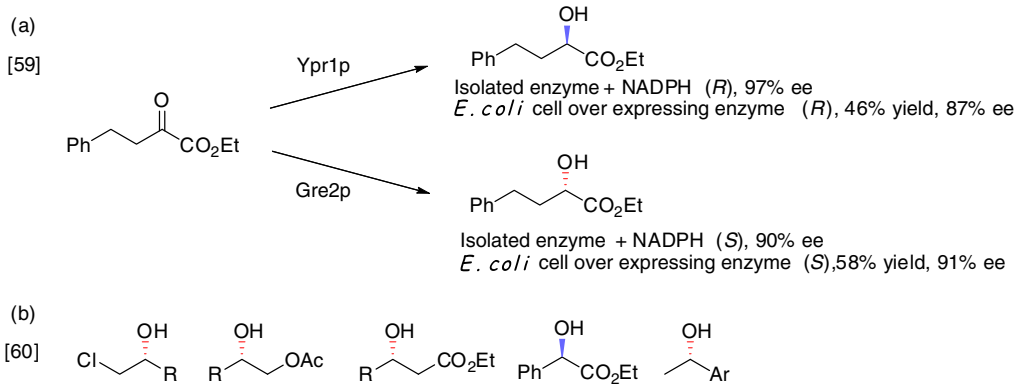


Figure 39. Reduction of ketones by enzymes from baker's yeast: (a) comparison of reaction system with isolated enzyme and coenzyme and system with only *E. coli* whole cell over-expressing the enzyme; (b) products by reduction with recombinant enzyme.

4. Examples for asymmetric reductions

Various examples for asymmetric reduction are described in this section. Reduction of carbonyl compounds as well as reduction of olefines and nitro group are described.

4.1. Reduction of ketones

Examples of organisms with efficient enzymes for reduction of ketones are shown in this section. In particular, reaction using baker's yeast, *C. magnoliae*, and *Sporobolomyces salmonicolor* are de-

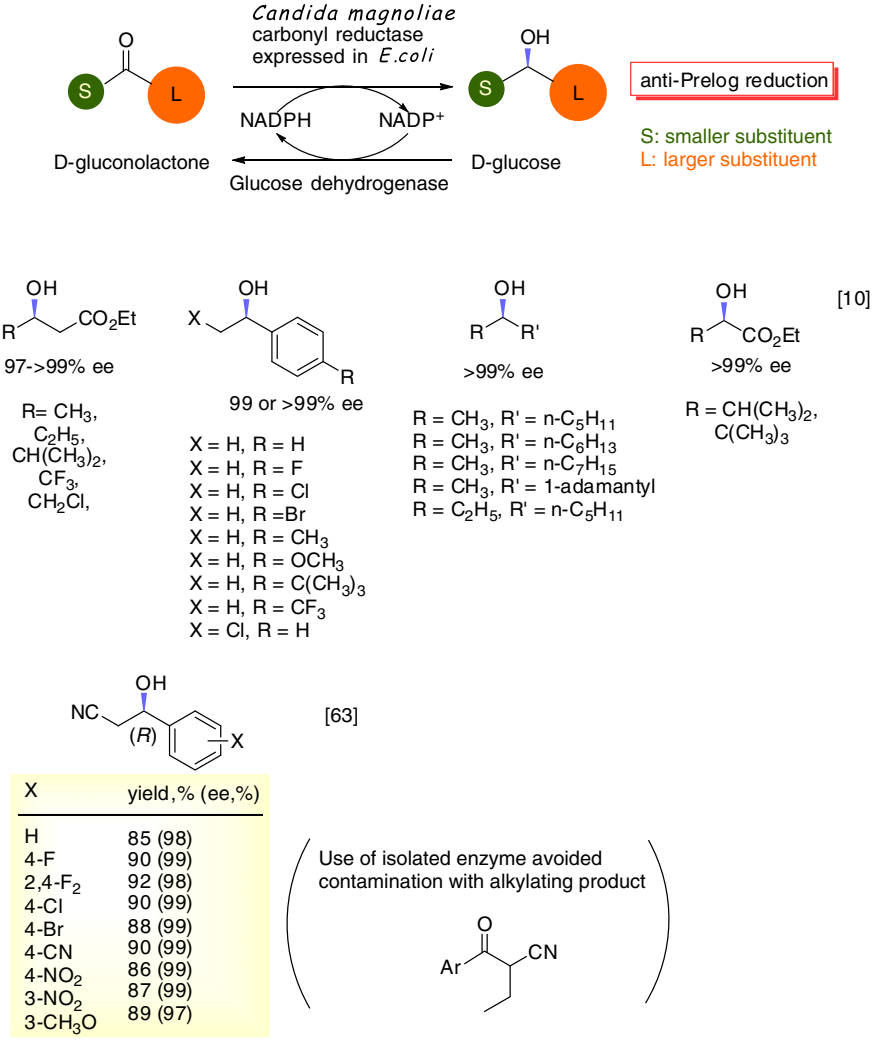


Figure 40. Anti-prelog reduction of ketones by over-expressed enzymes of *Candida magnoliae* (CMCR).

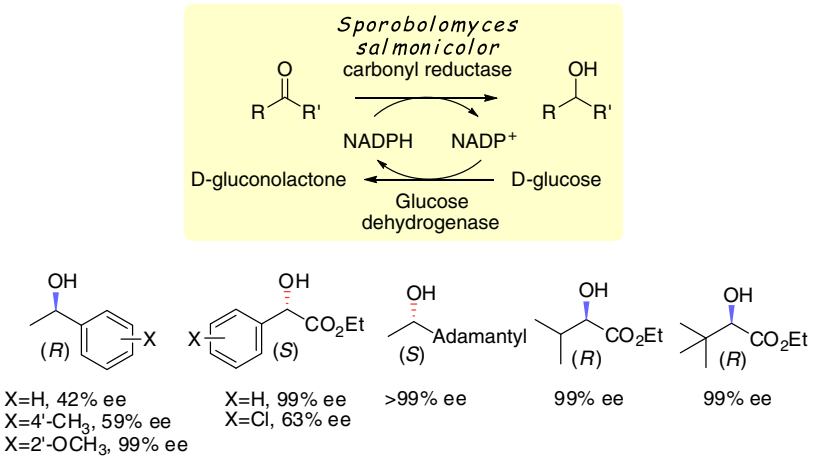


Figure 41. Reduction of ketones by over-expressed enzymes of *Sporobolomyces salmonicolor*.

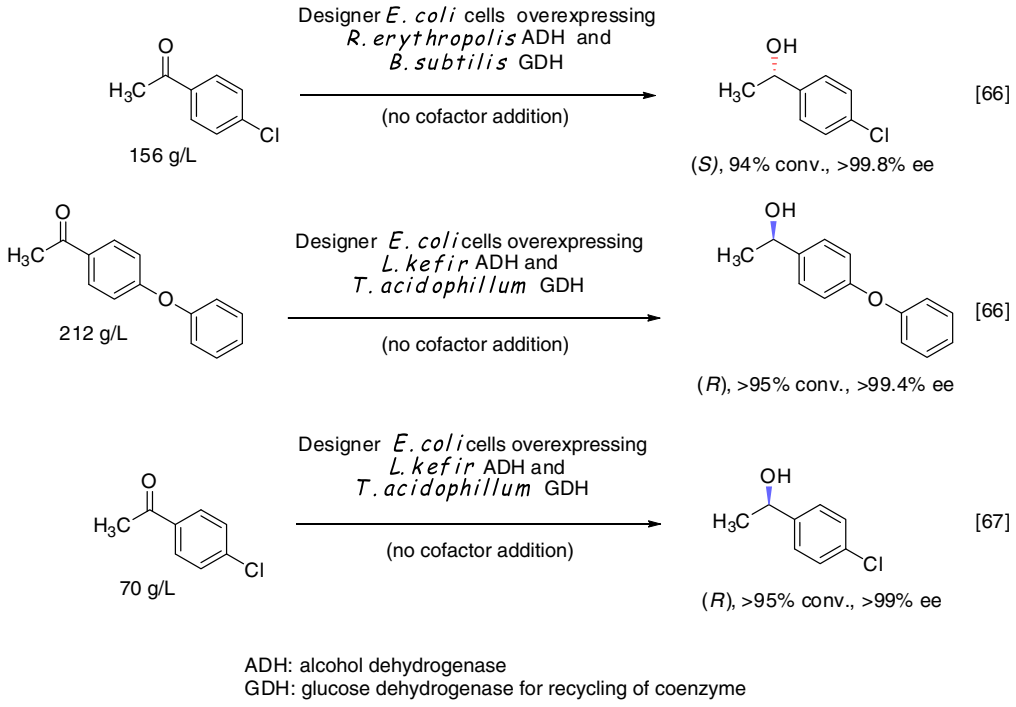


Figure 42. Large-scale reduction of ketones by designer *E. coli* expressing both alcohol dehydrogenase for the reduction of substrate and glucose dehydrogenase for the recycling of coenzyme.

scribed in detail. Then, examples for practical synthesis of valuable compounds are also described. At last, extraneous and natural substrates of some enzymes are compared.

4.1.1. Baker's yeast

Baker's yeast (*Saccharomyces cerevisiae*) has been widely used as a catalyst for asymmetric reductions. Now many genes including putative gene of dehydrogenases have been expressed in *E. coli*, and the recombinant *E. coli* cells or the isolated enzymes have been used for asymmetric reductions as shown in Section 3.3. 'Screening of enzymes of microorganisms of known genome data'. Other examples for the reactions by enzyme from baker's yeast are shown in Figure 39.[59,60]

4.1.2. *Candida magnoliae*

NADPH-dependent carbonyl reductase from *C. magnoliae* (CMCR) was selected by screening for asymmetric reduction of ethyl 4-chloro-3-oxobutanoate and overexpressed in *E. coli*.[61,62] The application of reduction by CMCR to other ketones has been reported (Fig. 40).[10,63] 3-Oxoalkanoates, alkyl ketones, and aryl ketones were reduced enantioselectively to *anti*-Prelog configurated alcohols in excellent ee.[10] For the reduction of aromatic β-ketonitriles catalyzed by whole-cell biocatalysts over-expressing CMCR (yeast or *E. coli* whole cell), α-Ethylation concomitantly occurred.[27] Use of isolated CMCR with a catalytic amount of NADPH recycled by D-glucose and glucose dehydrogenase has completely eliminated this competing reaction, and (*R*)-β-hydroxy nitriles (97–99% ee) were successfully obtained. The products were further converted to (*R*)-β-hydroxy carboxylic acids via a nitrilase-catalyzed hydrolysis.[63]

4.1.3. *Sporobolomyces salmonicolor*

NADPH-dependent carbonyl reductase from *S. salmonicolor* was firstly studied for the reduction of ethyl 4-chloro-3-oxobutanoate to afford the corresponding (*S*)-alcohol.[64] Substrate specificities

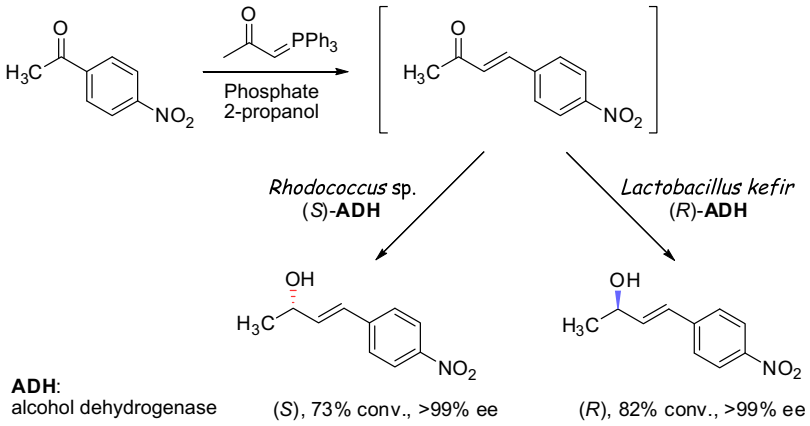


Figure 43. Chemo-enzymatic synthesis.

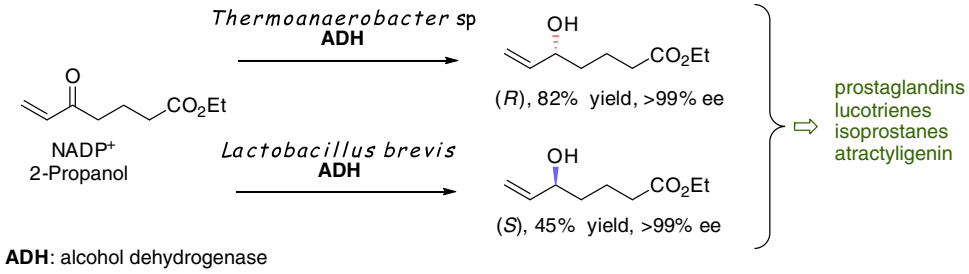


Figure 44. Synthesis of an intermediate for arachidonic acid metabolites by reduction of ketones by enzymes from *Thermoanerobacter* sp. and *Lactobacillus brevis*.

and enantioselectivities have been investigated (Fig. 41).[65] The enzyme reduced substituted acetophenones to the (*R*)-alcohol with moderate to high ee. The reduction of aliphatic ketones were not selective with exception of methyl adamantyl ketone (>99% ee (*S*)). The reduction of substituted benzoyl formats was relatively high.

4.1.4. Tailor-made whole-cell biocatalyst

Tailor-made whole-cell biocatalyst 'designer cells' was used for large-scale asymmetric reduction of ketones (Fig. 42). For example, (*S*)-*p*-chlorophenyl-1-ethanol was obtained from the reduction of the corresponding ketone (156 g/L) by the recombinant *E. coli* expressing alcohol dehydrogenase from *Rhodococcus erhtyropois* and NADH-dependent glucose dehydrogenase (NAD^+) from *B. subtilis* in 94% conversion with >99.8% ee. (*R*)-*p*-Phenoxyphenyl-1-ethanol (>95% conversion with >99.4% ee) was yielded by the cells expressing alcohol dehydrogenase from *L. kefir* and NADPH-dependent glucose dehydrogenase from *Thermoplasma acidophillum* under the conditions of 212 g/L substrate concentrations.[66] (*R*)-4-Fluoropheny-1-ethanol (>95% conversion, >99% ee) was also obtained with the same method from 70 g/L of the corresponding ketone.[67]

4.1.5. Chemo-enzymatic synthesis

Dehydrogenase can be used in one-pot with a chemical reagent. For example, optically active allyl alcohols were prepared by combination of Wittig reaction and asymmetric reduction in one-pot as shown in Figure 43.[68] 4-Nitrobenzaldehyde was reacted with Wittig reagent in phosphate buffer-2-propanol (3:1 v/v) solvent for 2 h. Then, alcohol dehydrogenase (ADH) from *Rhodococcus* sp. and NADH were added and asymmetric reduction proceeded. The (*S*)-allyl alcohol was obtained in 75% yield with >99% ee. Similarly, ADH from *L. kefir* was used for the production of (*R*)-alcohol.

4.1.6. Synthesis of an intermediate for arachidonic acid metabolites

Useful intermediates for the synthesis of a variety of arachidonic acid metabolites were prepared (Fig. 44). (*R*)-5-Hydroxyhept-6-enonoate was obtained from the reduction of ethyl 5-oxo-6-heptenoate with NADPH and 2-propanol catalyzed by *Thermoanerobacter* sp. alcohol dehydrogenase (ADH) expressed in *E. coli*.[69] When the biocatalyst was changed to *L. brevis* ADH, the opposite enantiomer, (*S*)-5-hydroxyhept-6-enonoate, yielded. These chiral alcohols are important intermediates for prostaglandins, leukotrienes, isoprostanes, and atractyligenin.

4.1.7. Synthesis of an intermediate for antagonists

Asymmetric reduction of 1-[3,5-bis(trifluoromethyl)phenyl]-ethanone is investigated using various enzymes because optically active 1-[3,5-bis(trifluoromethyl)phenyl]ethanols are intermediates for antagonist. The reduction with *L. kefir* gave the corresponding (*R*)-alcohol in >99% ee, an intermediate for tachykinin NK1 receptor (Fig. 45a).[70] On the other hand, the reduction by an alcohol dehydrogenase from *Rhodococccus erythropolis* gave the corresponding (*S*)-alcohol, an intermediate for antagonists currently under clinical evaluation (Fig. 45b).[71] A pilot scale reaction in pH 6.5 phosphate buffer at 45 °C using NADH, glucose, and glucose dehydrogenase gave the product alcohol in 96% yield with 99% ee(*S*). The substrate concentrations could be increased to 580 mM with a space-time yield of 260 g/L d.

4.1.8. Synthesis of an intermediate for a new generation antibacterial quinolone

For the synthesis of new generation antibacterial quinolone carboxylic acids, reduction of azaspiroketones by *Phaeocrepsis* sp. JCM 1880 was investigated and gave the corresponding (*R*)-alcohols in high ee as shown in Figure 46.[72,73]

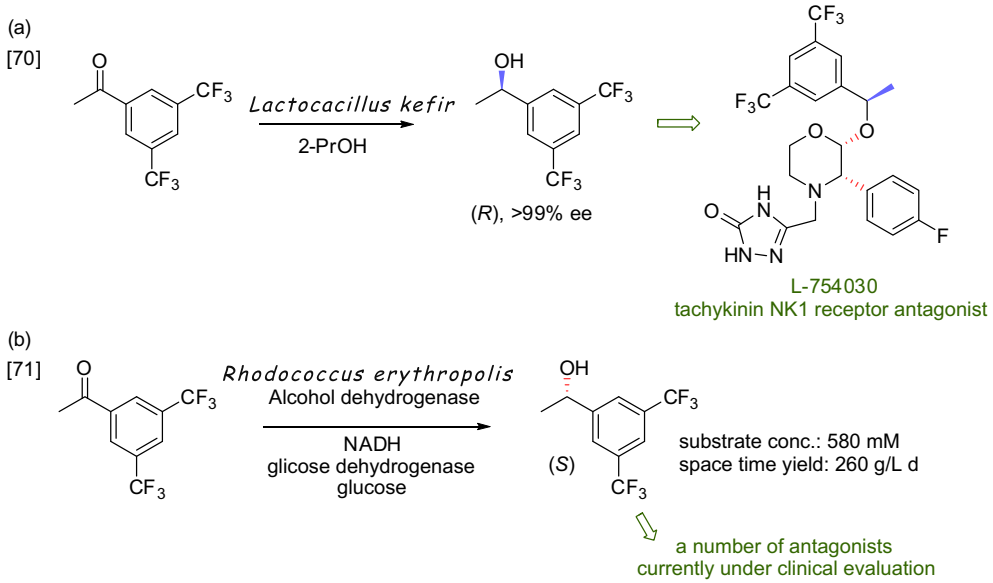


Figure 45. Synthesis of an intermediate for (a) NK-1 receptor antagonists by *Lactobacillus kefir* and (b) antagonists currently under clinical evaluation by *Rhodococccus erythropolis*.

4.1.9. Total synthesis of modiolide A

Modiolide A, a 10-membered ring lactone from a marine-origin with antibacterial and antifungal activities, was synthesized using two microbial reductions as shown in Figure 47. An important chiral building block for constructing the chirality at C-4, (*S*)-6-[(4-methoxybenzyl)oxy]-1-trimethylsilyl-1-hexyn-3-ol, was obtained by asymmetric reduction of the corresponding ketone with *Pichia minuta* IAM 12215 in 96.1% ee. Another chiral center, C-9, was synthesized from the (*R*)-acetal alcohol obtained by asymmetric reduction of the corresponding acetal ketone with *Yamadazyma farinose* NBRC 10896 in 76% yield with 92.6% ee.[74]

4.1.10. Reduction of sterically hindered ketone

Asymmetric reduction of sterically hindered ketones is one of the most challenging subjects in biocatalysis. Despite the difficulty, microbes that can reduce sterically hindered ketones, isopropyl phenylsulfonylmethyl ketone, were found by screening. As a result, *Trichosporon cutaneum* IAM 12206 and *P. minuta* IAM 12215 were found to reduce the ketone to the corresponding (*R*)-alcohol (60% yield and 94.0% ee) and (*S*)-alcohol (92% yield and 96.8% ee), respectively (Fig. 48).[75]

4.1.11. Comparison of extraneous and natural substrates

When an enzyme to catalyze a desired reaction is searched for, structural-based approach by comparing the natural substrate of the enzyme and substrate for the desired reaction (extraneous substrate) is not usually taken. Therefore, the structure of natural substrate differs largely from that of extraneous substrate as shown in Figures 49 and 50. For example, the enzyme that reduces acetylpyridine to the corresponding (*S*)-alcohols isolated from rat liver was found to be the rat liver 3α-hydoxysteroid dehydrogenase (3α-HSD) from amino acid sequences.[76,77] The natural substrate of 3α-HSD is steroids and is known to catalyze the conversion of androsterone and naloxone.[78]

Moreover, the kinetics of 3α-HSD was fully investigated with extraneous substrates, which gives the information useful for drug design to exploit this reduction pathway. Kinetic studies of reduction of *p*-substituted acetophenones revealed that a plot of $\log\{(V_{max}/K_m)_X/(V_{max}/K_m)_H\}$ (X = halogens, etc., H = hydrogen) versus the substituent parameter (π, σ *para*, Es) showed an increasing rate mainly for electron-withdrawing substituents with a correlation coefficient (r^2) of 0.97. With this in mind, new drugs can be designed that exploit this reduction pathway by introducing an elec-

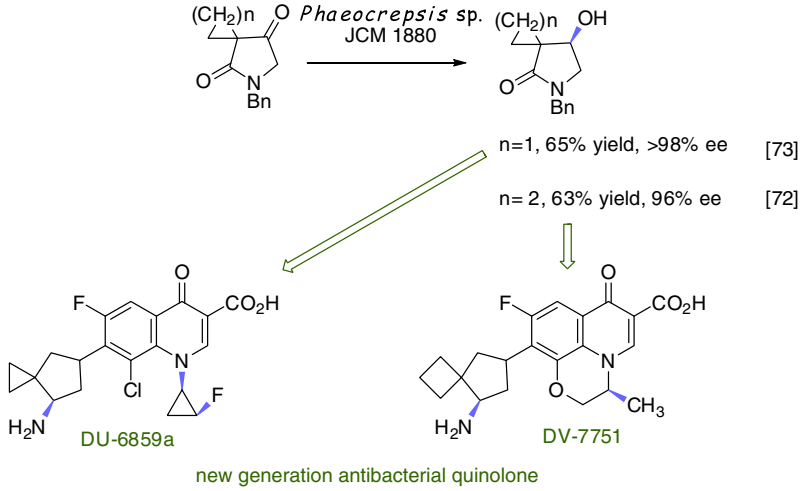


Figure 46. Synthesis of intermediates for antibacterial quinolone by *Phaeocrepsis* sp. JCM 1880.

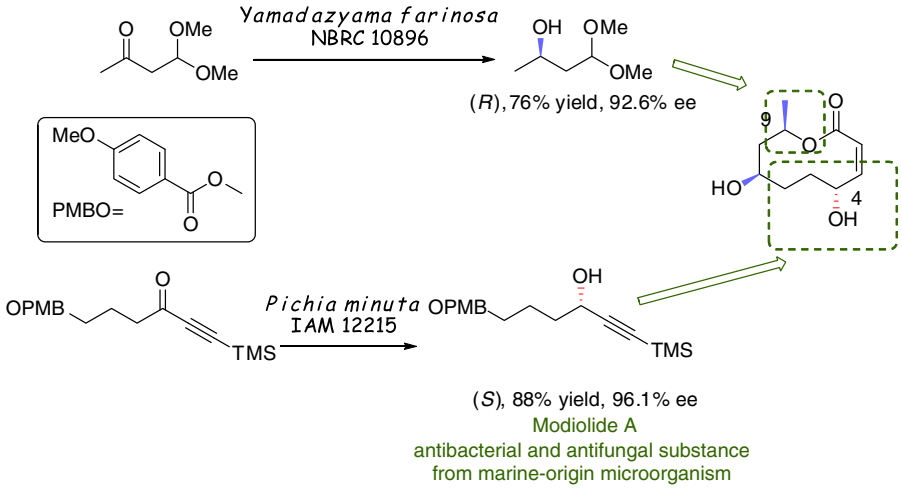


Figure 47. Synthesis of modiolide A using *Pichia minuta* IAM 12215 and *Yamadazyma farinose* NBRC 10896.

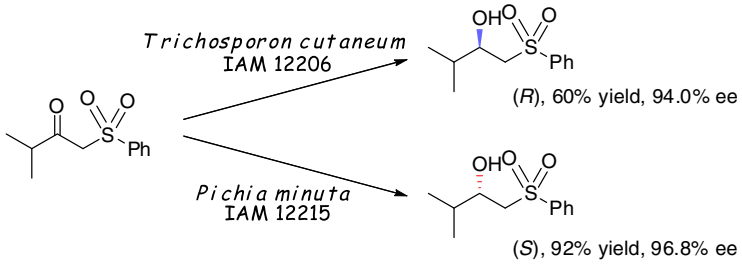


Figure 48. Reduction of sterically hindered ketone.

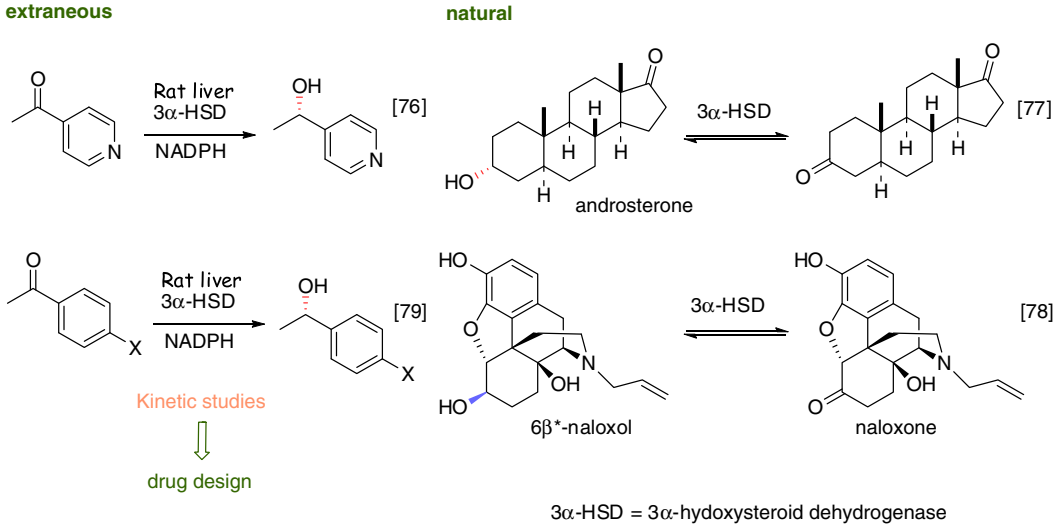


Figure 49. Comparison of extraneous and natural substrates of rat liver 3α-hydoxysteroid dehydrogenase (3α-HSD).

tron-withdrawing group when the reaction is desired, or by introducing an electron-donating group when the reaction is undesired.[79]

Examples in Figure 50 show comparison of extraneous and natural substrates of enzyme from baker's yeast. The enzyme that reduces bicycle[2,2,2]octane-2,6-dione to the corresponding (−)-*endo*-alcohol was isolated from baker's yeast, and the gene was found to be YMR226c[28] (Fig. 50a). The gene is known as L-allothreonine dehydrogenase, and the enzyme oxidize L- and D-serine, L-allothreonine, D-threonine, L-3-hydroxyisobutyrate, and D-3-hydroxyisobutyrate in k_{cat}/K_m of 116, 110, 14600, 7540, 558, and 151 M^{-1} s^{-1}, respectively.[80] As shown in Figure 50b, the natural substrate of an another yeast reductase that converts ketovaline ethyl ester to the corresponding (*R*)-hydroxy ester is also known; it was found to be methylglyoxal reductase.[81,82]

4.1.12. Various examples

The list of the reduction of ketones to (*R*)-alcohols, ketones to (*S*)-alcohols, ketoesters to (*R*)-hydroxy esters, and ketoesters to (*S*)-hydroxy esters are summarized in Tables 1–4, respectively.

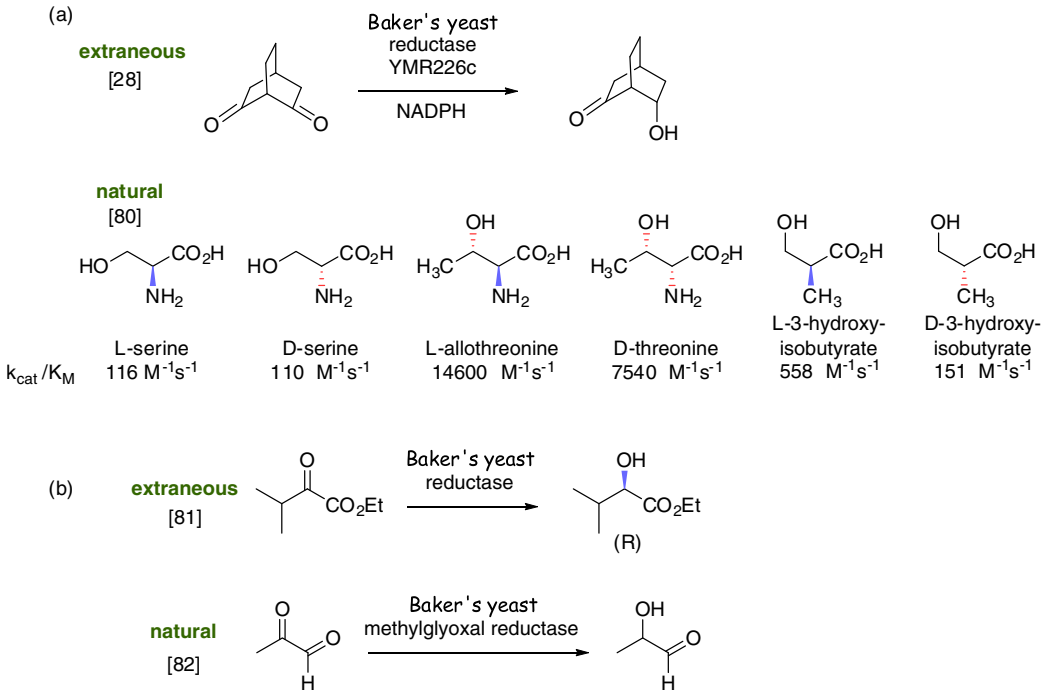


Figure 50. Comparison of extraneous and natural substrates of enzyme from baker's yeast: (a) reduction of bicycle[2,2,2]octane-2,6-dione; (b) reduction of ketovaline ethyl ester.

Table 1
Reduction of ketones to (*R*)-alcohols

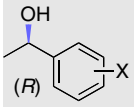
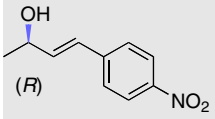
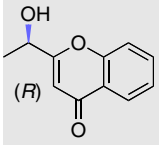
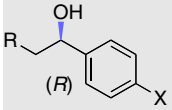
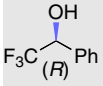
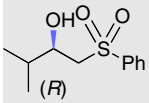
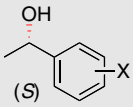
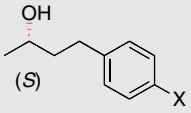
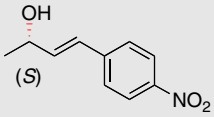
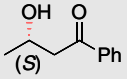
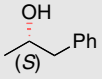
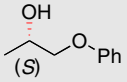
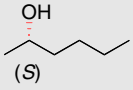
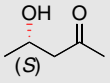
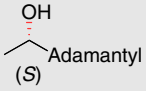
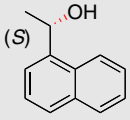
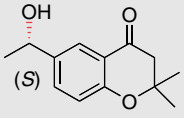
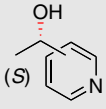
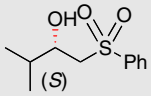
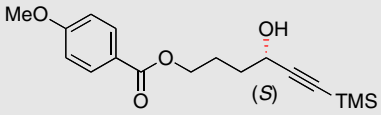
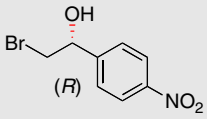
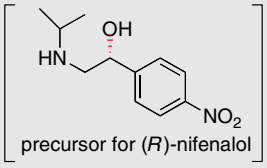
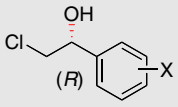
	Product	Biocatalyst	Ref.
	X = 4′-F, 87% yield, >99% ee, 4′-phenoxy, >99.4% ee	*Lactobacillus kefir* (designer cells + GDH in *E. coli*)	66,67
	X = 4′-Cl, 92.8% yield, 99.7% ee	*Lactobacillus kefir* 20% ionic liquid	57
	X = 3′,5′-CF_3, >99% ee	*Lactocacillus kefir*	70
	X = H, 42% ee,	*Sporobolomyces salmonicolor*	65
	X = 4′-CH_3, 59% ee, X = 2′-OCH_3, 99% ee		
	X = H, 99% ee	*Leifsonia* alcohol dehydrogenase (LASDH)	22
	X = H, 4′-F, Cl, Br, CH_3, OCH_3, CF_3, *t*-Bu >99% ee	*Candida magnolie*	10
	82% conv. >99% ee	*Lactobacillus kefir* (*R*)-ADH	68
	68% yield, 90% ee	*Lactobacillus kefir* ADH NADPH recycled by 2-PrOH	83
	R = Br; X = Br, 96% yield, 97% ee	*Lactobacillus kefir* (designer cells + GDH in *E. coli*)	66
	R = Cl, X = H 92% yield, >99% ee	*Candida magnoliae* Carbonyl reductase	10
	>99% ee	*Leifsonia* alcohol dehydrogenase (LASDH)	22
	60% yield, 94.0% ee	*Trichosporon cutaneum* IAM 12206	75

Table 2
Reduction of ketones to (*S*)-alcohols

Product	Biocatalyst	Ref.
		
X = H, halogen, etc., ee >99%	Archaeon *Pyrococcus furiosus* ADH (hyperthermophilic, half-life of 130 min at 100 °C) NADH, recycle with glucose	35
X = *o*- or *m*-NO_2 98% yield, >98% ee	*Aspergillus terreus* CCT4083	84
X = H, 2′-Cl, 3′-Cl, 4′-Cl, 4′-Br, 4′-CH_3, 4′-CF_3, 4′-CH_3O >99% ee	Yeast SADH (YMR226c, recombinant)	85
X = 2′-Cl, >99% ee, X = 2′-Br, 96% ee	*Aspergillus niger* EBK-9	86
X = H, 2′-F, 2′-Cl, 2′-Br, 3′-F, 3′-Cl, 3′-Br >99% ee	*Alternaria alternata* EBK-4	87
X = 2′-Cl, >99.4% ee 4′-Cl, >99.8% ee	*Rodococcus erythropolis* (designer cells + GDH in *E. coli*)	66
X = 2′-F, >99% ee	*Geotrichum candidum* MBRC 5767 (immobilized on water-adsorbent polymer) 10 MPa $scCO_2$ 2-PrOH	33
X = H, 2′-F, 2′-OMe, 4′-F >99% ee	*Geotrichum candidum* MBRC 5767 in ionic liquid [emim]PF_6	9
X = 3′,5′-CF_3	*Rhodococcus erythropolis* substrate concd: 580 mM	71
X = H, 2′-F, 3′-F, 4′-F, 4′-Cl, 4′-Br, 4′-I 67–99% yield, >97% ee	*Candida tropicalis* PBR-2MTCC5158	88
		
X = H, >99% ee	W110A TeSADH	30,49
X = 4′-OMe	*Debaryomyces hansenjii*	44
X = H	*Geotrichum candidum* MBRC 5767 in ionic liquid [emim]PF_6	9
		
73% conv., >99% ee	(*S*)-ADH *Rhodococcus* sp.	68
		
98% conv., >99% ee	W110A TeSADH	30
		
95% conv., 37% ee	W110A TeSADH	30
		
>99% conv., >99% ee	W110A TeSADH	49
		
>99% ee	*Geotrichum candidum* MBRC 5767 in ionic liquid [emim]PF_6	9
		
40% yield, 79% ee	Baker's yeast in ionic liquid ([bmim]PF_6)	51
22% yield, 95% ee	Baker's yeast in ionic liquid ([bmim]PF_6)	51
		
>99% ee	*Sporobolomyces salmonicolor* carbonyl reductase	65
		
97% yield, >99% ee	*Candida viswanathii* MTCC 5158	89
		
>97% ee	*Brassica napus* hairy root	41
		
2-: 27% yield, 93% ee, 3-: 9% yield, 84% ee, 4-: 66% yield, 94% ee	Rat liver S-9 NADPH	76

(*continued on next page*)

Table 2 (*continued*)

	Product	Biocatalyst	Ref.
	R = C_2H_5–C_7H_{15}, 45–75% yield, 37–80%	*Cyanidium caldarium*	90
	92% yield, 96.8% ee	*Pichia minuta* IAM 12215	75
	88% yield 96.1% ee	*Pichia minuta* IAM 12215	74
	97% ee  precursor for (*R*)-nifenalol	*Rhodotorula* sp. AS.2241	91
	X = H, 98% ee, X = 4′-F, 4′-Cl, 99% ee, X = 3′-Cl, 99% ee	Yeast SADH (YMR226c, recombinant)	91
	X = 3′-Cl, >99% conv., >99% ee	*Rhodococcus* sp. ST-10., Phenylacetaldehyde reductase 20%, 2-propanol	34

4.2. Reduction of diketones

Regio- and enantioselective reduction of diketones have been conducted successfully by biocatalysis. For example, methyl (−)-1,4a-dimethyl-5-oxodecahydronaphthalene-1-carboxylate, a key intermediate for the synthesis of terpenoids, was synthesized using a baker's yeast-catalyzed asymmetric reduction of a σ-cyclohexanedione (Fig. 51). DMSO (10%) was used to solubilize the substrate.[93]

For the reduction of (±)-bicyclo[2,2,2]oct-7-ene-2,5-dione, an genetically engineered yeast TMB4100 overexpressing YMR226c, reductase gene from baker's yeast, was shown to be an efficient catalyst (Fig. 52). The reaction resulted in affording (+)-*endo*-hydroxyketone (>99% ee) and (−)-*exo*-hydroxyketone (84–98% ee) in 45:55 ratio at the rate of more than 40-fold faster than that by the original control strain.[94] The (+)-substrate was reduced to the *endo* compound, and (−)-diketone was reduced to the *exo* hydroxyketone. Using the same enzyme, the reduction of the bicyclooctane compound ((±)-bicyclo[2,2,2]octane-2,5-dione) produced hydroxyketones with higher ee (>99% ee).

Figure 53 shows regioselective reduction of 1,3-diketone with an alcohol dehydrogenase from *L. brevis* (recombinant) to monohydroxy compounds in 99.5% ee.[95] The monoalcohol, which is an advanced building block in the synthesis of a mevinic acid type HMG-CoA reductase inhibitor, was reduced chemically to afford the *syn*- and *anti*-diols diastereoselectively.

Microbes which reduced 3,5-dioxo-6-(benzyloxy)hexanoic acid ethyl ester were screened, and *Acinetobacter* sp. SC13874 was found to reduce the diketone to the corresponding *syn*-(3*R*,5*S*)-diol, potential intermediates for the synthesis of HMG-CoA reductase inhibitors, in 99.4% ee with 52–74% de depending on substrate concentrations (74% de in 2 g/L and 52% de in 10 g/L) (Fig. 54).[96] After the reaction, XAD-16 resin was added to facilitate the recovery process by adsorbing the product. Three reductases were isolated from the microbe, and the mechanism of the reduction was disclosed; the reductase I catalyzed the reduction to both mono-hydroxy products ((5*S*)-product and (3*R*)-product), the reductase II catalyzed the conversion of the two monohydroxy ketones to *syn*-(3*R*,5*S*)-diol, and the reductase III catalyzed reduction of the diketone directory to *syn*-(3*R*,5*S*)-diol.

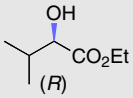
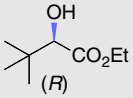
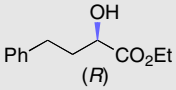
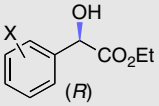
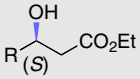
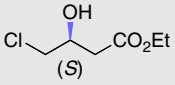
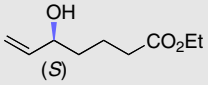
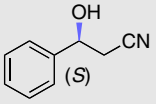
The list of the reduction of diketones to hydroxyl ketones and diols is in Table 5.

4.3. Reduction of C=C bonds

Reduction of C=C bonds has been conducted with catalysis by plant and microbial reductase. For example, the stereoselective reduction of 2-butenolides by two reductases, p51 and p83, from cultured plant cells of *Glycine max* was investigated (Fig. 55). The reduction of 2,3-dimethyl-2-butenolide by p51 reductase produced (2*R*,3*R*)-2,3-dimethylbutanolide, whereas the reduction by p83 reductase gave (2*S*,3*R*)-product. The p51 reductase-catalyzed *trans* addition of hydrogen and p83 reductase gave *cis* product.[99]

Table 6 summarizes the enantioselective reduction of olefins by plant and microalgae. Reduction of enones to saturated ketones has been reported using reductases from tobacco[99–101] and cyanobacteria.[102,103] *exo* and *endo* Olefins in cyclohexenones were reduced to the corresponding (*S*)-ketones by *Synechococcus* PCC7942, which was previously described in Section 2.3. 'Light-driven regeneration'.[15,16,104] Tobacco reductase p74[100,105] and p90[100] also reduced the enones to the corresponding (*S*)-ketones while the antipode was obtained by the reduction with tobacco p44 reductase.[100] When the ring size is reduced to cyclopentenone, *Synechococcus* also reduced the C=C bond to *S* enantiomer. Both enantiomers of carvone were reduced to the 1-(*S*)-ketone ((1*S*,4*R*)- and (1*S*,4*S*)-dihydrocarvone) by cyanobacteria.[102,103] Both the tobacco reductase p44 and p90 reduced *N*-phenyl-2-methylmaleimide to the corresponding (*R*)-succimide.[101]

Table 3
Reduction of keto esters to (*R*)-alcohols

Product	Biocatalyst	Ref.
 99% ee	*Sporobolomyces salmonicolor* carbonyl reductase	65
 99% ee		
 Ypr1p + NADPH 97% ee, *E. coli* cell (Ypr1p), 46% yield, 87% ee	*Saccharomyces cerevisiae* putative dehydrogenase (recombinant)	59
 X = H, 4′-halogen, etc. >99% ee X = 4′-Cl, 4′-Me, 99% ee, X = 4′-F, 94% ee X = H, 82% yield, 92% ee	Archaeon *Pyrococcus furiosus* ADH (hyperthermophilic, half-life of 130 min at 100 °C), NADH, recycle with glucose Yeast SADH (YMR226c, recombinant), NADPH Immobilized Baker's yeast	35 85 47
 R = *iso*-C_3H_7, 87% ee R = CF_3 ee, 58% ee	Yeast SADH (YMR226c, recombinant), NADPH	85
 85% yield, 100% ee, $NADP^+$ turnover >21,000	*Candida magnolie* carbonyl reductase (recombinant), NADPH recycled by *Bacillus megaterium* glucose dehydrogenase aqueous-butyl acetate	92
 45% yield, >99% ee	*Lactobacillus brevis*, ADH, $NADP^+$ 2-propanol	69
 99% ee	Yeast reductase, YOL151w	27

Enzymes from baker's yeast also catalyzes reduction of olefin. As shown in Figure 56, old yellow enzyme from *Saccharomyces carlsbergensis* expressed in *E. coli* reduced 2- and 3-alkyl-substituted 2-cyclohexenones to the corresponding 3(*S*)- and 2(*R*)-substituted cyclohexanones in 89–96% ee.[106]

4.4. Reduction of CO_2

The production of methanol by the reduction of CO_2 was reported.[12] As shown in Figure 57, four enzymes including formate, formaldehyde, alcohol, and glutamate dehydrogenases were co-immobilized using the same particles as that used for cofactor immobilization (enzymes and cofactor were immobilized separately). Reactions were performed by bubbling CO_2 in a suspension solution of the immobilized enzymes and the immobilized cofactor, NADH, with in situ cofactor regeneration. The immobilized system showed fairly good stabilities in reusing.

4.5. Reduction of nitro group

Reduction of nitro group to amino group was performed with biocatalysis. *Peptostreptococcus productus* U-1 was used for reduction of (*E*)-2-nitro-1-phenyl-1-propene and 2-nitro-1-phenyl-1-propane in a two-liquid phase reaction system, and the aminoalkanes were formed in 47% and 45% yield, respectively (Fig. 58).[107]

5. Oxidations

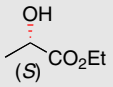
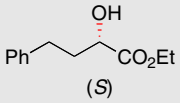
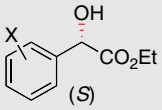
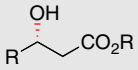
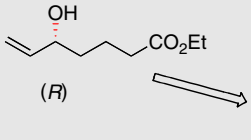
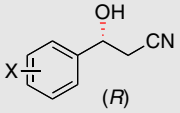
Enantioselective oxidation of racemic alcohols, oxidation of C–H bonds, Baeyer–Villiger oxidation and oxidative polymerization are described in this section.

5.1. Oxidation of alcohols

Chiral intermediates for pharmaceuticals have been synthesized through enantioselective oxidation of racemic alcohols. For example, Daicel group has reported the synthesis of (*R*)-1,3-butandiol by enantioselective oxidation of the racemate (Fig. 59).[108] By screening over 1000 strains, *Candida parapsilosis* IFO 1369 was selected as the best strain for the purpose. Although the enzyme required NAD^+ as the coenzyme, *E. coli* cell overexpressing the dehydrogenase catalyzed the oxidation without any co-substrate to regenerate NAD^+ from NADH. As a result, (*R*)-1,3-butandiol of 95% ee was obtained in 48.4% yield at the substrate concentration of 15%. The chiral alcohol was used for synthesis of penem and carbapenem drugs.

Figure 60 shows the synthesis of both enantiomers of nonactate using *Rhodoccocus erythropolis*. Oxidation of the racemic methyl nonactate in aerobic conditions gave (−)-ketone, remaining (+)-isomer in 48.5% yield with >98% ee. The resulting ketone was reduced with the same microbe under anaerobic conditions affording

Table 4
Reduction of keto esters to (*S*)-alcohols

	Product	Biocatalyst	Ref.
	60% yield, 76% ee	Baker's yeast in ionic liquid [bmin]PF_6	51
	Gre2p + NADPH, 90% ee, *E. coli* cell (Gre2p) 58% yield, 91% ee	*Saccharomyces cerevisiae* putative dehydrogenase (recombinant)	59
	93% yield, 96% ee	*Rodococcus erythropolis* (designer cells + GDH in *E. coli*)	66
	X = H, 99% ee	Yeast SADH (YMR226c, recombinant), NADPH	85
	X = H, 99% ee, X = Cl, 63% ee	*Sporobolomyces salmonicolor* carbonyl reductase	65
	R = CH_3, R′ = Et, 95% ee	Archaeon *Pyrococcus furiosus* ADH (hyperthermophilic, half-life of 130 min at 100 °C), NADH, recycle with glucose	35
	R = CH_3, R′ = Et, 84% ee, R = Ph, R′ = Et, 85% ee	Yeast SADH (YMR226c, recombinant), NADPH	85
	R = CH_3, R′ = CH_3, >99% ee R = CH_3, R′ = Et, >99% ee	Baker's yeast in glycerol	50
	R = CH_3, R′ = Et 70% yield, 95% ee	Baker's yeast in ionic liquid [bmin]PF_6	51
	R = CH_3, R′ = *t*-Bu 87% yield, >99% ee	*Geotrichum candidum* MBRC 5767 in ionic liquid [emin]PF_6	9
	82% yield, >99% ee prostaglandins lucotrienes isoprostanes atractyligenin	*Thermoanerobacter* sp., ADH, $NADP^+$ 2-propanol	69
	X = H, 4-F, 2,4-F, 2, 4-Cl, 4-Br 4-CN, 4-NO_2, 3-NO_2, 3-CH_3O 97–99% ee	CMCR	63
	X = H, 97% ee 92% ee	Yeast reductase, YGL039w, YGL157w	27

(−)-isomer in 57% yield with >98% ee. Both the isomers are the important units for the synthesis of nonactin, a macrotetrolide natural product.[109]

Figure 61 shows the use of plant cells, *Cucurbita maxia* (pumpkin), for chemoselective oxidation of prenyl alcohol to the corresponding acid. Remote and allylic olefinic moieties were inert to

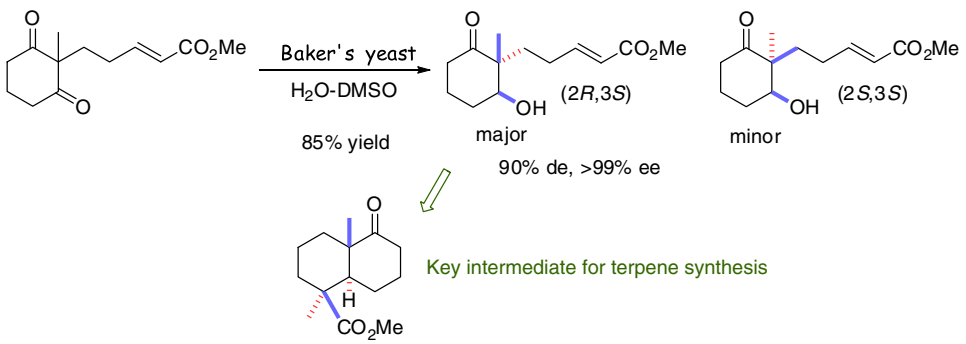


Figure 51. Regio- and enantioselective reduction of diketones by baker's yeast for the synthesis of key intermediate for terpenoids.

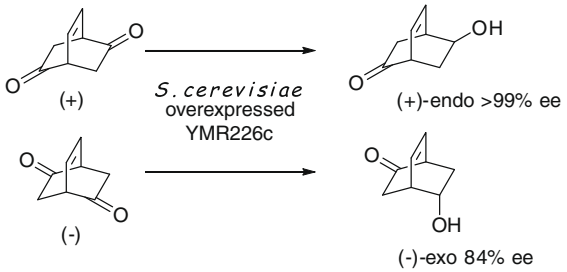


Figure 52. Regio- and enantioselective reduction of diketones by engineered yeast TMB4100 overexpressing YMR226c.

the oxidation.[110] The product, geranylgeranoic acid, is a useful inducer for apoptosis of mammalian cells.[111]

A clean and effective alcohol oxidizing system using three enzymes and oxygen has been developed as shown in Figure 11.[19] *Brevibacterium* alcohol dehydrogenase and aldehyde dehydrogenase were used for conversion of primary alcohols to the corresponding acids. Regeneration of NAD^+ to NADH was done by molecular oxygen and NADH oxidase from *L. brevis*.[19]

Figure 62 shows oxidative decarboxylation of α-methyltropate by *Rhodococcus* sp. KU1314. Optically active α-phenylpropionate was obtained in 53% yield with 65% ee.[112] The reaction proceeded through non-selective oxidation of tropate, decarboxylation of semialdehyde, enantioface-differentiating protonation, and oxidation of phenylpropionaldehyde to phenylpropionate.

5.2. Oxidation of C–H bonds

Oxidation of C–H bonds to C–OH (and to C=O) is described in this section. Figure 63 shows oxidation of C–H to carbonyl group for the synthesis of Nootkatone, the most important and expensive aromatic of grapefruit. It decreases the somatic fat ratio, and thus its demand is increasing in the cosmetic and fiber sectors. A sesquiterpene hydrocarbon, (+)-valencene, which is cheaply obtained from Valencia orange, was transformed by microbes such as microalgae and fungi. Among them, the green algae, *Chlorella* species, afforded nootkatone in high yield.[113,114]

A series of 2-, 3-, and 4-substituted pyridines were metabolized using the mutant soil bacterium, *Pseudomonas putida* UV4, which contains a toluene dioxygenase (Fig. 64).[115] The regioselectivity depended on the position of the substituent. 4-Alkylpyridines were hydroxylated exclusively on the ring to give the corresponding 4-substituted 3-hydroxypyridines, while 3-alkylpyridines were hydroxylated stereoselectively on C-1 of the alkyl group (90% ee (*R*)) with no evidence of ring hydroxylation. 2-Alkylpyridines gave both ring and side-chain hydroxylation products (83% ee (*R*)).

5.3. Baeyer–Villiger oxidation

Enzymatic Baeyer–Villiger oxidation is widely used for synthetic organic chemistry.[116] The reaction mechanisms are described in Figure 3. As a biocatalyst for Baeyer–Villiger oxidations, monooxygenase from *Acinetobacter* has been widely used since 1990s.[117,118] Now various enzymes used for Baeyer–Villiger oxidation were expressed in *E. coli* or *S. cerevisiae* (baker's yeast). For example, monooxygenase from *Xanthobacter* sp. ZL5 was used for Baeyer–Villiger oxidation of cyclohexanones, cyclobutanones, and polycyclic ketones as shown in Figure 65.[119]

Whole cell of *E. coli* overexpressing *Acinetobacter* sp. NCIB 9871 cyclohexanone monooxygenase has been used for the Baeyer–Villiger oxidations of a variety of 4-mono- and 4,4-disubstituted cyclohexanones as shown in Figure 66.[120] The efficient production of cyclohexanone monooxygenase in the *E. coli* expression system (ca. 30% of total soluble protein) allowed these oxidations to reach

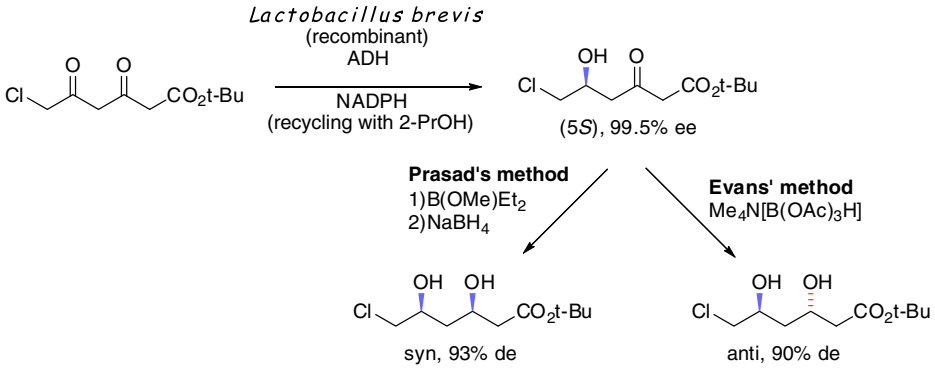


Figure 53. Regio- and enantioselective reduction of 1,3-diketone by *Lactobacillus brevis* alcohol dehydrogenase.

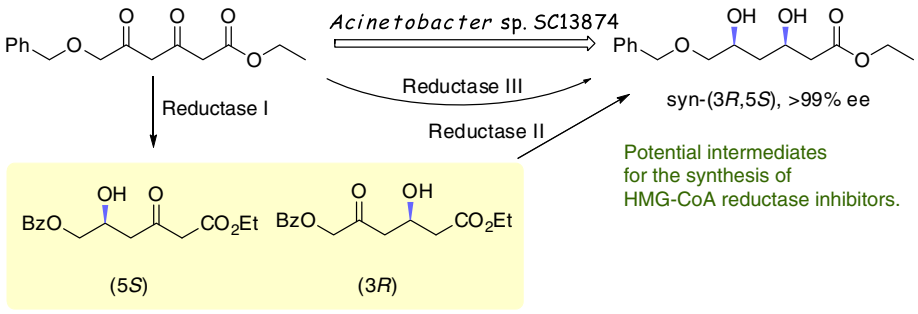
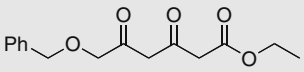
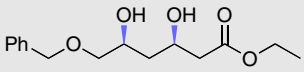
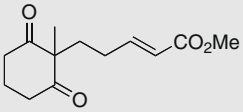
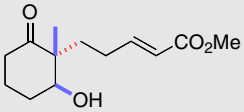
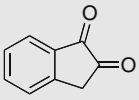
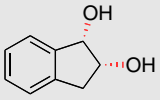
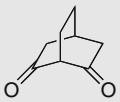
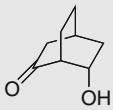
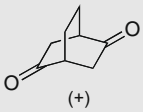
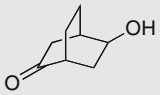
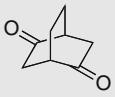
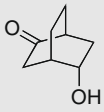
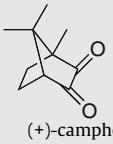
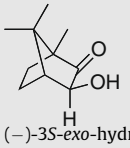


Figure 54. Enantioselective reduction of diketone to diol by *Acinetobacter* sp. SC13874.

Table 5
Reduction of diketones to hydroxyl ketone and diol

Substrate	Product	Biocatalyst	Ref.
	 *syn*-(3*R*,5*S*), >99% ee	*Acinetobacter* sp. SC13874	96
	 (2*R*,3*S*), 90% de, >99% ee, 85% yield	Baker's yeast, H_2O–DMSO	93
	 (1*S*,2*R*), 75% yield, >99% ee (from diketone), 90% yield, >99% ee (from recemic monoalcohol)	*Trichosporon cutaneum*	97,98
 BCO2,6D	 (1*R*,4*S*,6*S*)-6-hydroxybicyclo[2,2,2]octane-2-one	Recombinant yeast, YMR226C glucose	28
 (+)	 (+)-*endo*, >99% ee	*S. cerevisiae* overexpressed YMR226c	94
 (-)	 (−)-*exo*, 84% ee	*S. cerevisiae* overexpressed YMR226c	94
 (+)-camphorquinone	 (−)-3*S*-*exo*-hydroxycamphor	Carrot	37

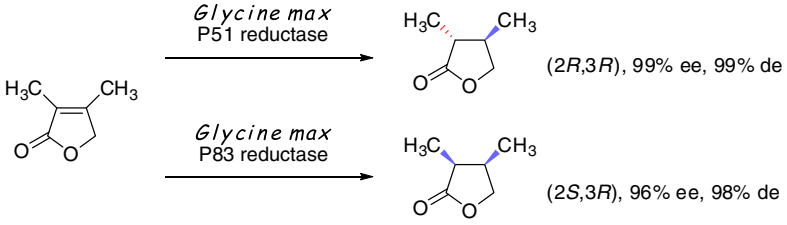
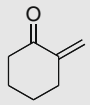
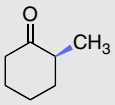
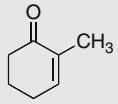
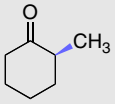
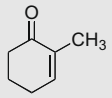
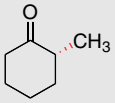
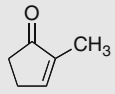
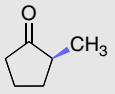
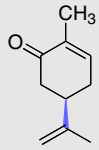
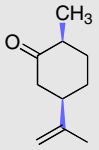
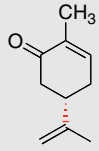
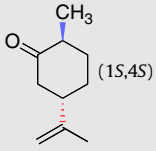
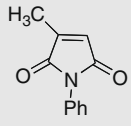
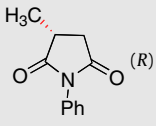


Figure 55. Reduction of C=C bond by enzymes from *Glycine max*.

completion in approximately half the time required for the engineered baker's yeast strain. Surprisingly, 4,4-disubstituted cyclohexanones were also accepted by the enzyme, and the enantioselectivities of these oxidations could be rationalized by considering the conformational energies of bound substrates along with the enzyme's intrinsic enantioselectivity. The enzyme ex-

Table 6
Reduction of C=C bonds by plant and microalgae

Substrate	Product		Biocatalyst	Ref.
	 (*S*)	97% ee	Tobacco p74 reductase	100,105
	 (*S*)	71% ee 99% ee	*S. elongatus* PCC 7942 Tobacco p90 reductase	102,103 100
	 (*R*)	85% ee >99% ee	*S. elongatus* PCC 7942 Tobacco p44 reductase	103 100
	 (*S*)	98% ee	*S. elongatus* PCC 7942	102,103
 (*R*)-carvone	 (1*S*,4*R*)	80% ee	*S. elongatus* PCC 7942	102,103
 (*S*)-carvone	 (1*S*,4*S*)	81% ee	*S. elongatus* PCC 7942	102,103
	 (*R*)	100% ee, 89% ee	Tobacco p44 reductase, Tobacco p90 reductase	101

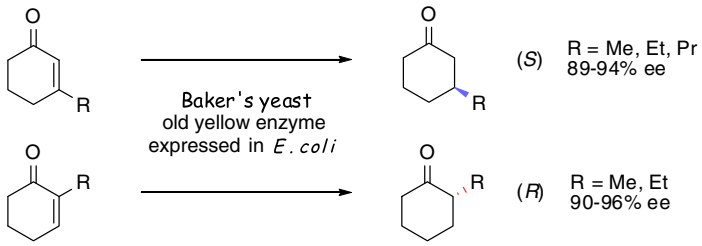


Figure 56. Reduction of C=C bond by enzymes from baker's yeast.

pressed in *E. coli* cells also oxidized several 4-substituted cyclohexanones bearing polar substituents, often with high enantioselectivities. In the case of 4-methylcyclohexanone, the lactone was obtained in >98% ee.

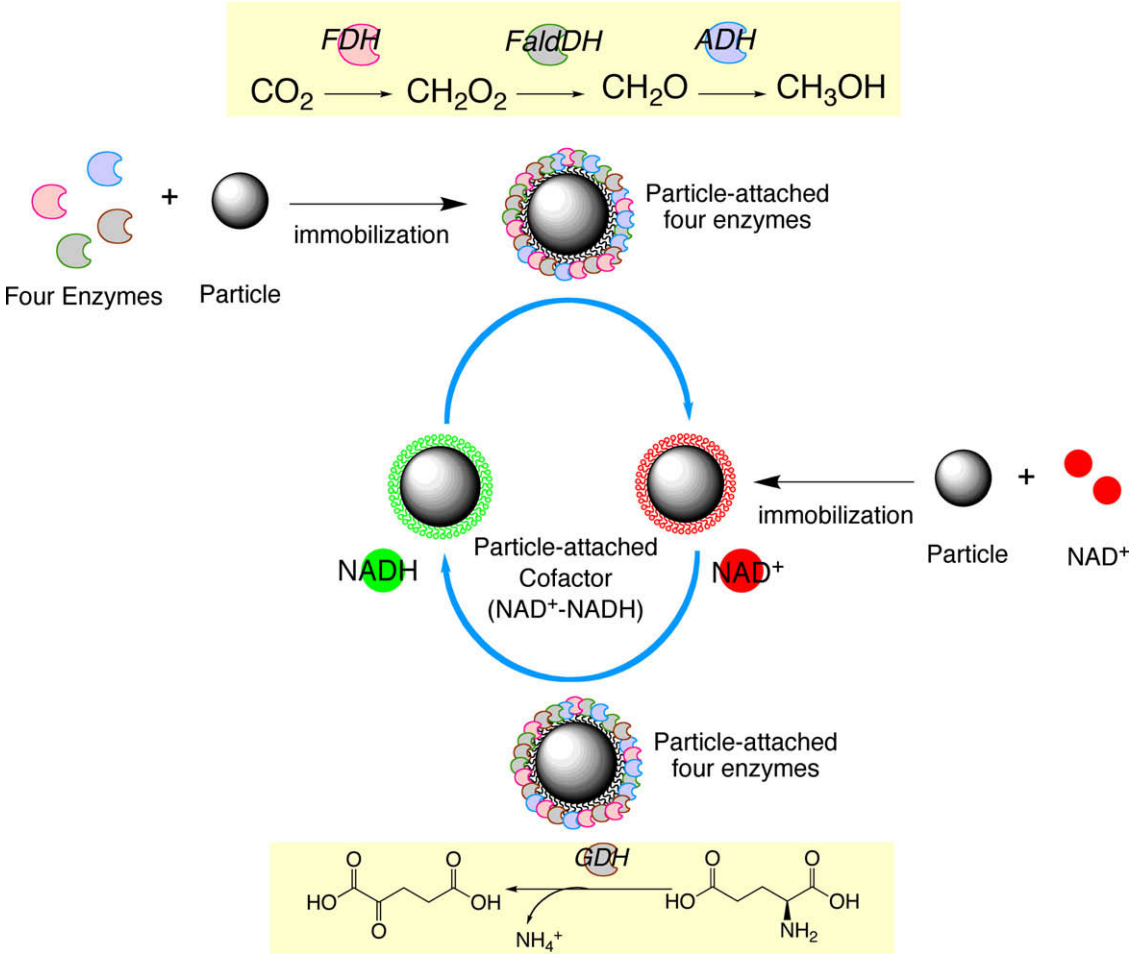


Figure 57. Reduction of CO_2 to methanol by co-immobilized enzymes and immobilized NADH.

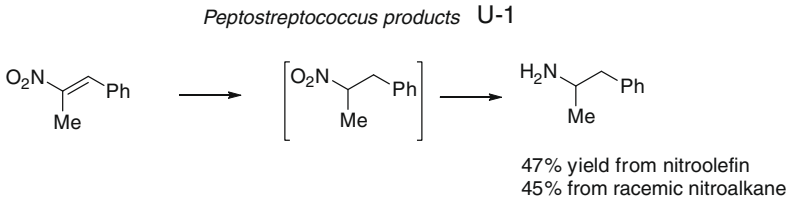


Figure 58. Reduction of nitro group by *Peptostreptococcus productus* U-1.

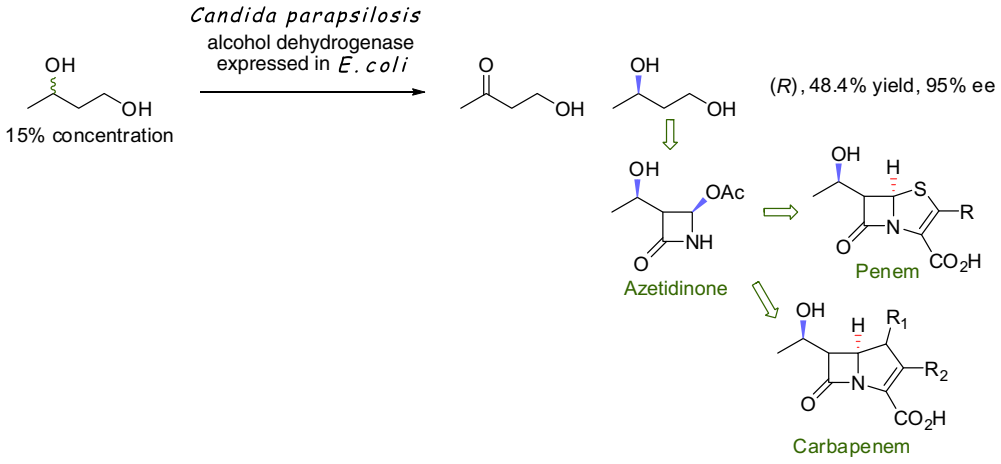


Figure 59. Oxidation of 1,3-butandiol by *Candida parapsilosis*.

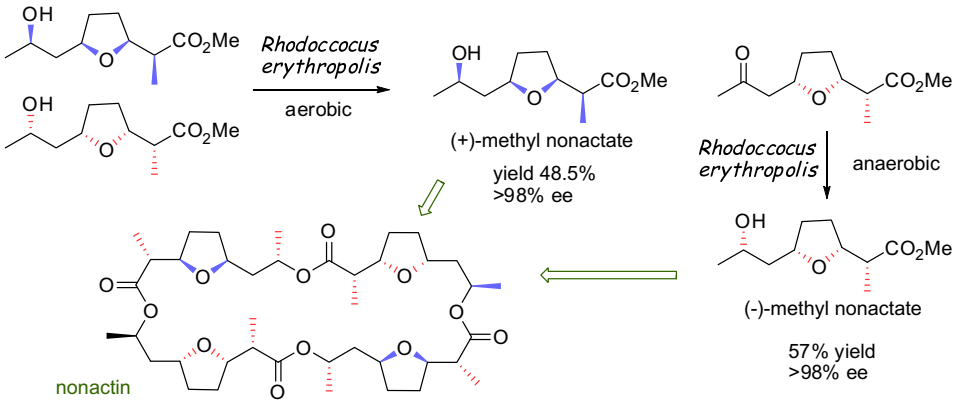


Figure 60. Oxidation and reduction for preparation of both enantiomers of nonactate by *Rhodoccocus erythropolis*.

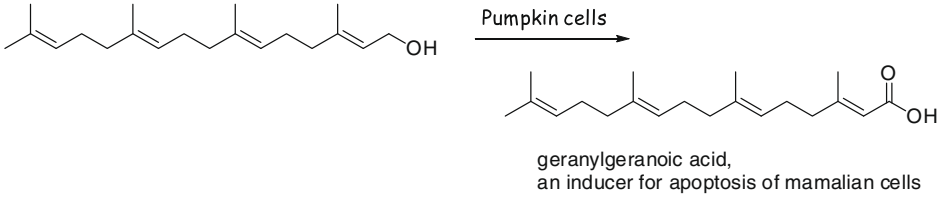


Figure 61. Oxidation of prenyl alcohol by *Cucurbita maxia* (pumpkin).[110]

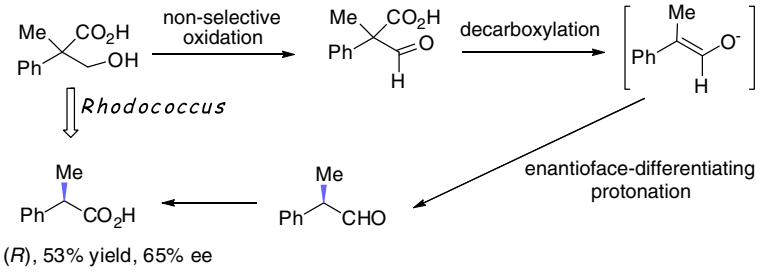


Figure 62. Oxidative decarboxylation of α-methyltropate by *Rhodococcus* sp. KU1314.[112]

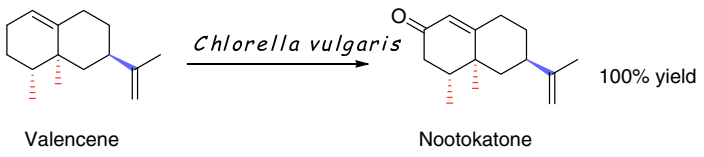


Figure 63. Oxidation of CH bonds by *Chlorella*.[114]

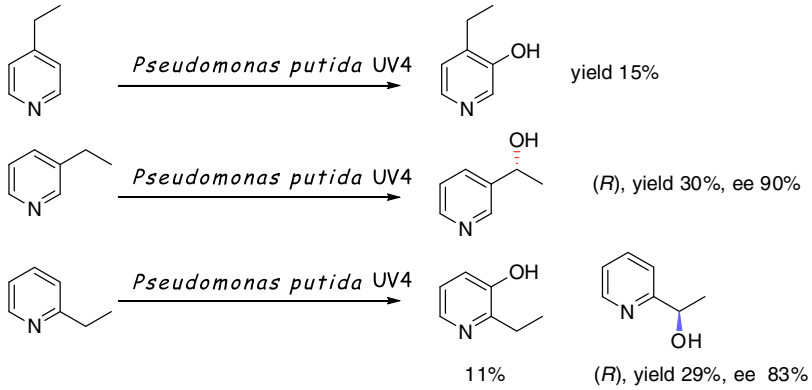


Figure 64. Oxidation of alkylpyridines by *Pseudomonas putida* UV4 toluene dioxygenase.[115]

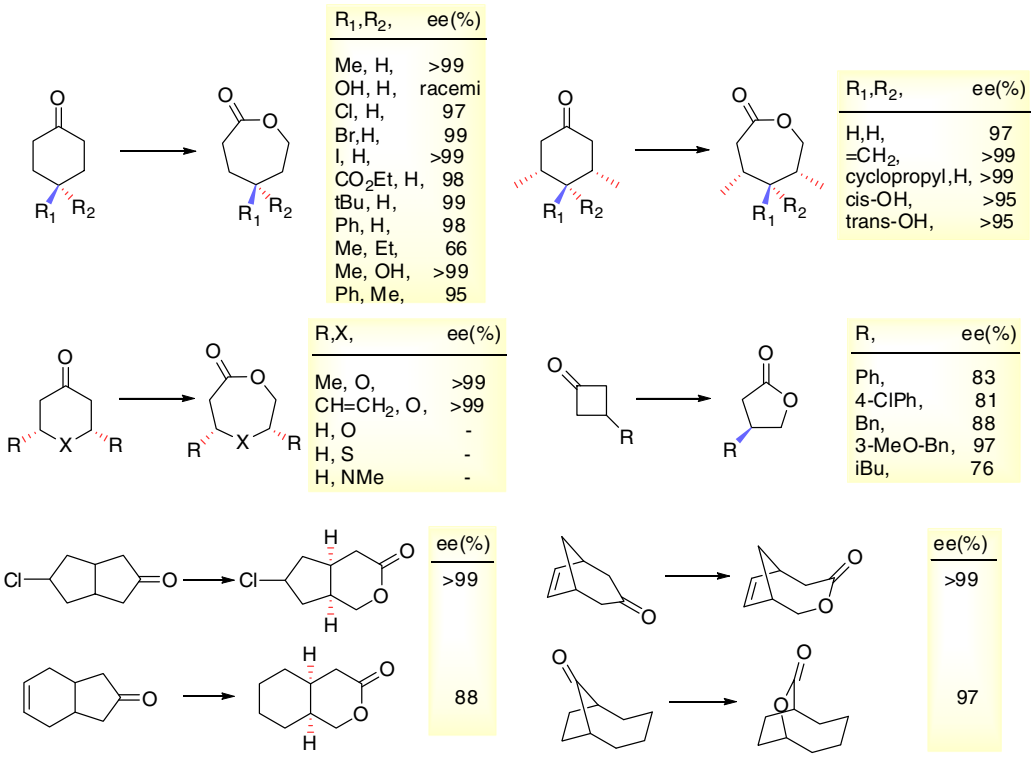
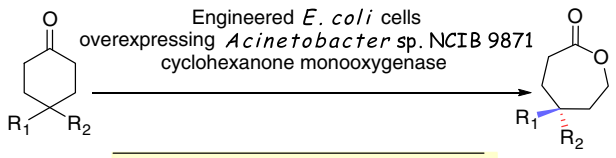


Figure 65. Baeyer–Villiger oxidations by *Xanthobacter* sp. ZL5 monooxygenase.[119]



R_1	R_2	Yield (%)	Ee (%)
Me	H	61	>98
Me	Me	61	
Et	H	91	97
Et	Me	91	75
Et	Et	60	
cyclopropyl		74	
OH	H	61	9.1
Et	OH	54	94
OMe	H	84	78
Br	H	63	97
I	H	60	97

Figure 66. Baeyer–Villiger oxidations by whole cell of *E. coli* overexpressing *Acinetobacter* sp. NCIB 9871 cyclohexanone monooxygenase.[120]

The enantioselective kinetic resolution of a set of racemic-substituted 3-phenylbutan-2-ones employing phenylacetone monooxygenase (PAMO) in non-conventional media was performed. The (*S*)-ketones were oxidized, but the (*R*)-isomers remained unreacted. The selectivity factor, *E* value increased by the use of miscible organic solvents such as methanol. For oxidation of 3-(4-methoxyphenyl)-butan-2-one, *E* values were 32 and 49 in aqueous system and 30% methanol system, respectively, as shown in Figure 5b.[11]

Effect of substituent length of 2-cyanoalkylcyclohexanone on the selectivity of Baeyer–Villiger oxidation by cyclohexanone monooxygenase was examined as shown in Figure 67.[121] In the reaction of the cyanoethyl derivative, only the (*R*)-substrate was oxidized to the proximal (*R*)-lactone (50% yield with 97% ee) and the (*S*)-isomer could not be oxidized (recovered: 46% yield with 95% ee). In the reaction of the cyanomethyl derivative, both enantiomers of the substrate were oxidized and the (*R*)-substrate was oxidized to the proximal lactone in >99% ee and the (*S*)-substrate was converted to the distal lactone in >99% ee. The 2-cyano substrate was inert to the oxidation.

Other examples of Baeyer–Villiger oxidations are summarized in Figure 68.[122–125]

5.4. Oxidative polymerization

Oxidative polymerization was catalyzed by peroxidases and laccases. For example, (+)-catechin was oxidatively polymerized by *Myceliophthora laccase* in a mixture of a polar organic solvent and buffer to give a new class of flavonoid polymers.[126] The polymers showed greatly amplified superoxide scavenging activity and xanthine oxidase inhibitory activity compared with monomeric catechin.

In another example shown in Figure 69, nanoscale surface patterning and polymerization of caffeic acid on 4-aminothiophenol-functionalized gold surfaces have been demonstrated with dip pen nanolithography (DPN).[127] The diphenolic moiety of caffeic acid can be polymerized by laccase or horseradish peroxidase.

6. Dynamic kinetic resolution

Dynamic kinetic resolution of racemic substrate proceeds through asymmetric reduction or oxidation when the substrate does racemize and the product does not under the applied experimental conditions. Recent development in dynamic kinetic resolution has been reviewed.[128]

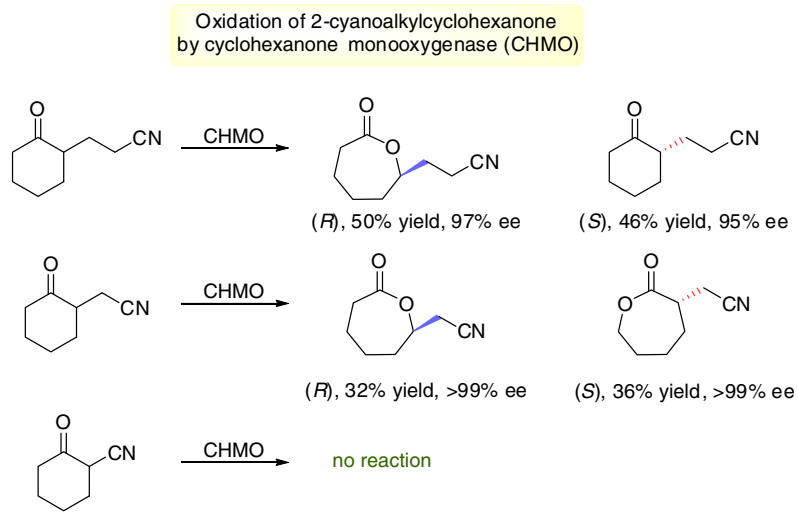


Figure 67. Effect of substituent length of 2-cyanoalkylcyclohexanone on selectivity of Baeyer–Villiger oxidation by cyclohexanone monooxygenase.[121]

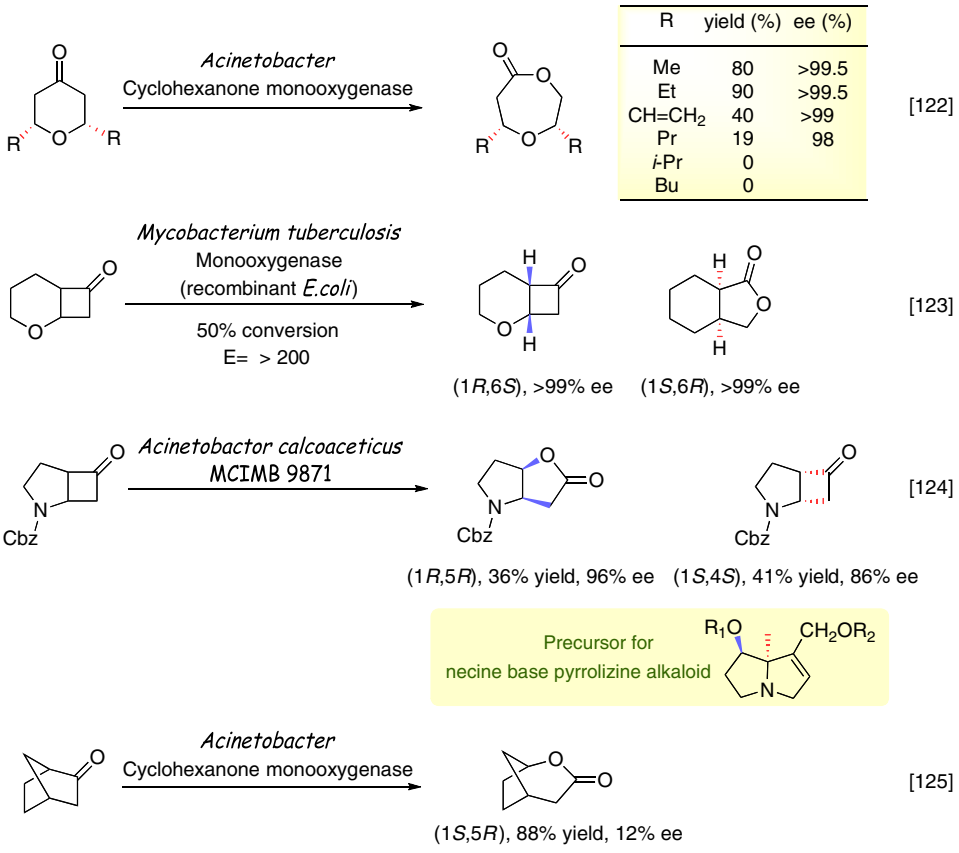


Figure 68. Examples for Baeyer–Villiger oxidations.[122–125]

6.1. Dynamic kinetic resolution through reduction

Dynamic kinetic resolution of α-alkyl β-keto ester has been performed through enzymatic reduction. One isomer, out of the four possible products for the unselective reduction (Fig. 70), can be selectively synthesized using biocatalyst.

Figure 71 shows dynamic kinetic resolution of cyclic β-keto esters by enzymes from baker's yeast.[129] Twenty purified dehydrogenases from baker's yeast expressed as fusion proteins with glutathione *S*-transferase were tested for their ability to reduce three homologous cyclic β-keto esters. The majority of dehydrogenases reduced ethyl 2-oxo-cyclopentanecarboxylate, yielding a

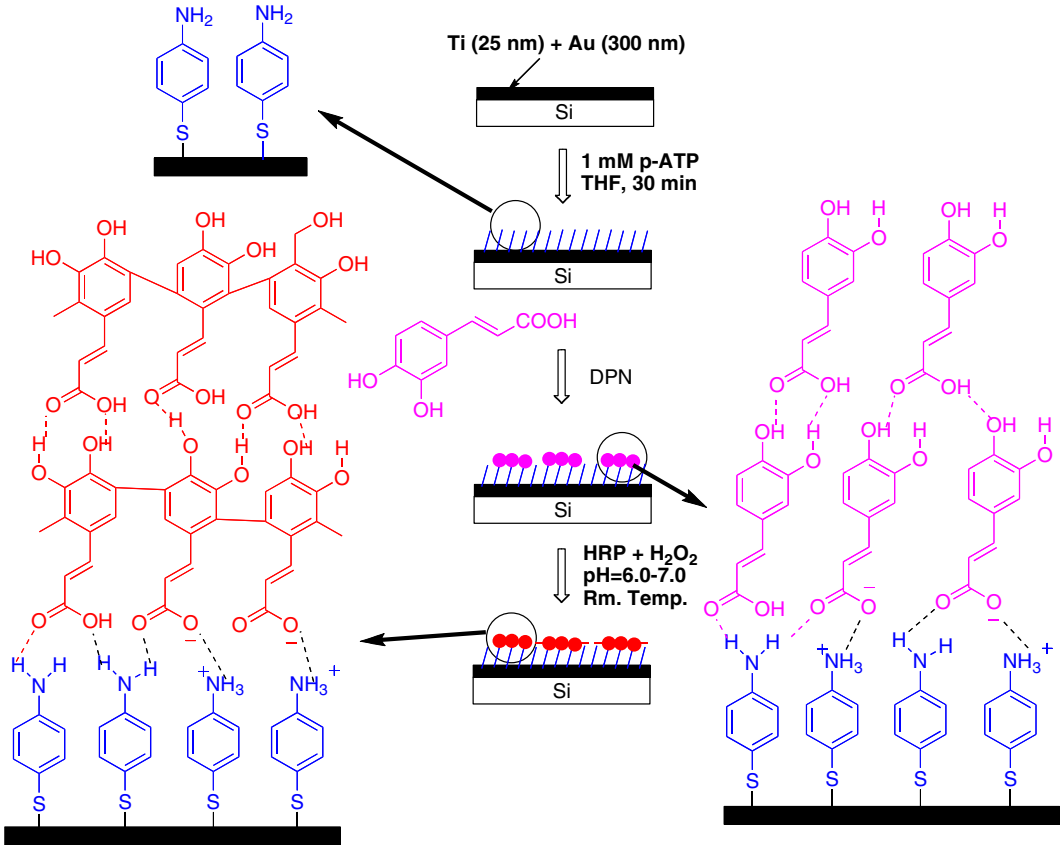


Figure 69. Oxidative polymerization of caffeic acid on 4-aminothiophenol-functionalized gold surfaces by horseradish peroxidase (HRP).[127]

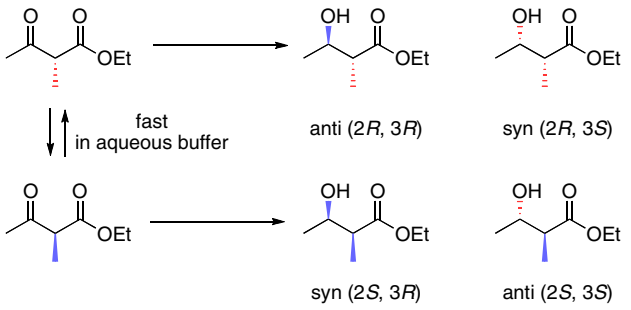


Figure 70. Possible products of dynamic kinetic resolution of α-methyl β-keto ester through reduction.

pair of diastereomeric alcohols with consistent (1*R*)-stereochemistry. Ethyl 2-oxo-cyclohexanecarboxylate reductions afforded only *cis*-alcohol enantiomers. Ethyl 2-oxo-cycloheptanecarboxylate was accepted by two enzymes in the collection, and both yielded mainly the *cis*-(1*R*,2*S*)-alcohol. *E. coli* cells overexpressing the YDL124w gene were used in a dynamic kinetic resolution of ethyl 2-oxo-cyclohexanecarboxylate to produce the key intermediate in a chemo-enzymatic synthesis of (1*R*,2*S*)-2-methyl-1-cyclohexanol, an important chiral building block.

Figure 72 shows the dynamic kinetic resolution of α-acetyl-γ-butyrolactone. The reaction with *Kluyvermyces marxianus* gave (+)-(3*R*,1′*S*)-α-1′-hydroxyethyl-γ-butyrolactone in 100% ee and 100% de. The corresponding (−)-(3*S*,1′*R*)-enantiomer was produced with *Hansenula* sp. in 100% ee and 90% de. The products are as potential CNS (central nervous system) ligands or as attractive intermediates to new bioactive compounds.[130]

Commercially available ketoreductases (KRED-101-120, Codexis, Inc. Pasadena, CA, USA) were used for dynamic kinetic resolution of diketones and keto esters.[4b,131] 3-Alkyl-2,4-pentandione was reduced to the corresponding (*syn*-3*R*,4*S*)-monoketone and (*anti*-3*S*,4*S*)-isomer by KRED 102 and KRED 118, respectively. KERD A1B reduced 3-allyl substrate to the (*syn*-3*S*,4*R*)-product (Fig. 73).

Dynamic kinetic resolution of 3-hydroxy-4-chromanone proceeded with *T. cutaneum* CCT 1903 through oxidation of (*S*)-hydroxychromanone and reduction of (*R*)-hydroxychromanone (Fig. 74). (3*R*,4*S*)-3,4-chromanediol was obtained in 40–58% yield with up to 99% ee.[132] The product is the precursor for HIV protease inhibitors.

Dynamic kinetic resolution of substituted α,β-unsaturated ketone was catalyzed by commercially available KRED 108 and NADPH with a coenzyme recycling system by 2-propanol and KRED 104 (Fig. 75). The corresponding *cis*-(*S*)-alcohol was obtained in 89% isolated yield with 99% de and 95% ee. The chemical methods were also attempted, and several catalysts could reduce the substrate with high ee. However none showed high diastereoselectivity.[133]

Figure 76 shows the dynamic kinetic resolution of 3-oxo-4-phenyl-β-lactam by recombinant *E. coli* overexpressing yeast reductase, Ara1p. As a result, *cis*-(3*S*,4*R*)-3-hydroxy-4-phenyl-β-lactam was obtained as a single enantiopure product.[134]

Figure 77 shows dynamic kinetic resolution of 2-substituted esters having an aldehyde group at 3 position to optically active 3-hydroxyesters.[6a,135–138] The enantioselectivity can be controlled by changing the ester group for the baker's yeast reduction.

Other examples of biocatalytic kinetic resolution through reduction are summarized in Table 7.

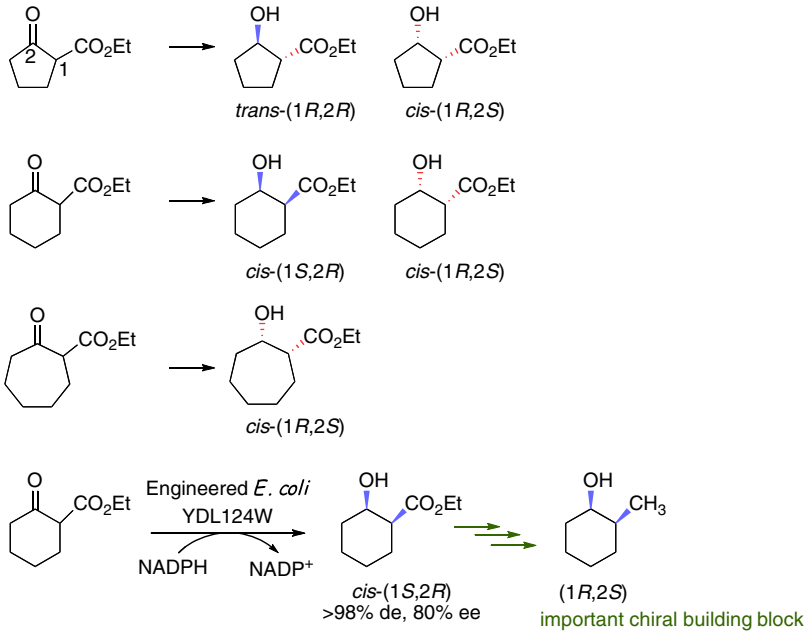


Figure 71. Dynamic kinetic resolution of cyclic β-keto esters by baker's yeast enzymes.[129]

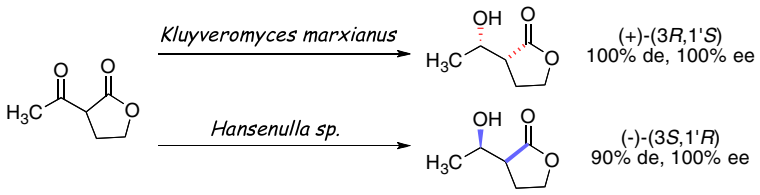


Figure 72. Dynamic kinetic resolution of α-acetyl-γ-butyrolactone with *Kluyvermyces marxianus* or *Hansenula* sp.[130]

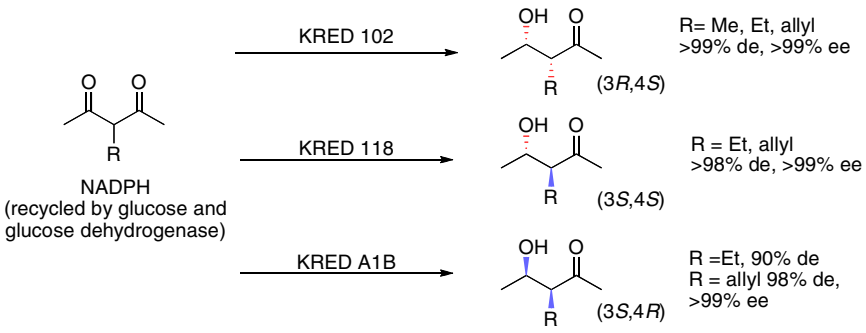


Figure 73. Dynamic kinetic resolution of diketones by commercially available ketoreductases, KRED-101-120.[4b,131]

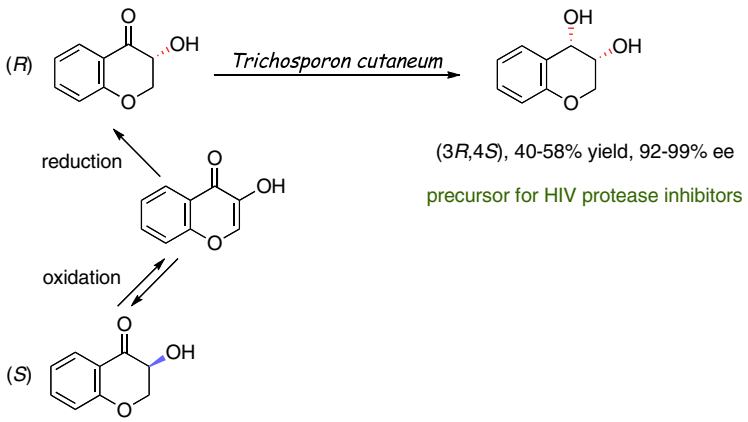


Figure 74. Dynamic kinetic resolution of 3-hydroxy-4-chromanone by *Trichosporon cutaneum* CCT 1903.[132]

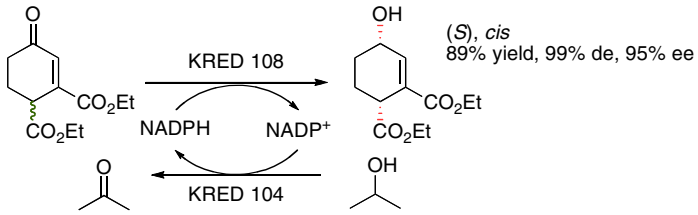


Figure 75. Dynamic kinetic resolution of α,β-unsaturated ketone by commercially available reductase, KRED 108.[133]

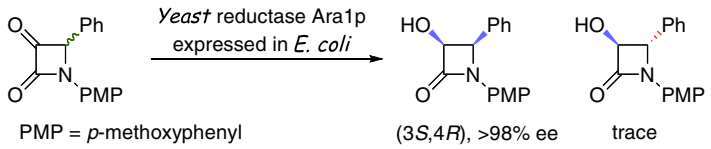


Figure 76. Dynamic kinetic resolution of 3-oxo-4-phenyl-β-lactam by yeast reductase, Ara1p.[134]

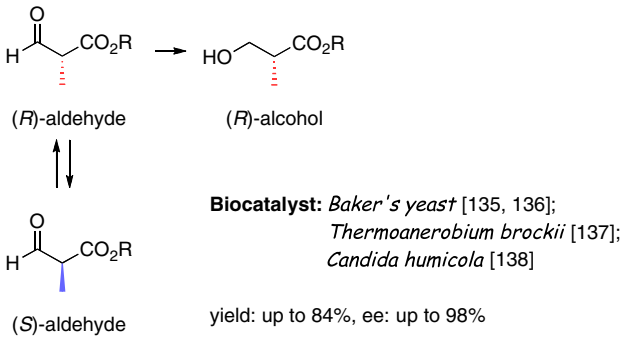


Figure 77. Dynamic kinetic resolution of aldehydes.[6a,135–138]

6.2. Dynamic kinetic resolution through Baeyer–Villiger oxidation

Dynamic kinetic resolution via Baeyer–Villiger oxidation has been reported.[11,151] Racemization of a ketone having an α-alkyl substituent is possible if the reaction was conducted at basic pH or an anion exchange resin was added. Thus, oxidation of 2-benzyloxymethylcyclopentanone by recombinant cyclohexanone monooxygenase in *E. coli* in the presence of Lewatit MP62 (tertiary amine type anion exchange resin) gave the (*R*)-lactone in 84% yield with 97% ee (Fig. 78a).[151]

Racemization of β-keto nitrile through Baeyer–Villiger oxidation is also possible since α-hydrogen is very acidic. For the reaction, phenylacetone monooxygenase was used as shown in Figure 78b.[11] The use of ethyl acetate (10%) as a co-solvent increases the yield of oxidation product, and enantiopure (*R*)-2-acetoxyphenylacetonitrile was obtained with 58% yield in >98% ee.

7. Deracemization

Deracemization reaction, transformation of racemic compounds into chiral forms in one pot without changing their chemical structures, has been performed using microorganism containing several enzymes with different stereochemistries. Instead, use of two isolated enzymes or use of one enzyme for enantioselective conversion and a chemical reagent is also possible to perform deracemization. This section shows some examples for deracemization.

7.1. Deracemization of alcohols

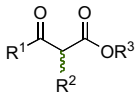
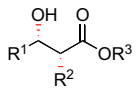
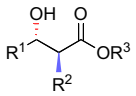
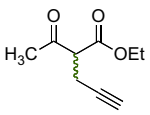
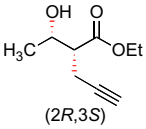
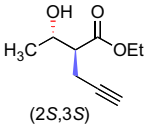
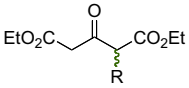
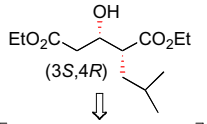
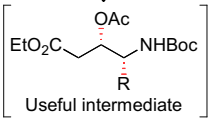
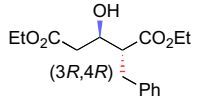
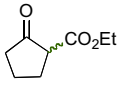
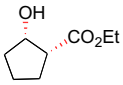
Deracemization of alcohols has been widely investigated since it is common that single kind of microorganism expresses several alcohol dehydrogenases with different enantioselectivities for a given substrate. One of the examples is deracemization of phenylethanol derivatives using *G. candidum* under aerobic conditions, in which the (*R*)-alcohols were obtained from the racemates.[152,153] The mechanism of deracemization is explained with the idea of oxidation–reduction process with two enzymes having different stereospecificities. In this case, the (*S*)-specific enzyme catalyzed reversible transformation of alcohol to ketones and ketones to alcohol, and the reaction catalyzed by (*R*)-enzyme was irreversible, so (*R*)-alcohol accumulated when the cell and racemic alcohols were mixed as shown in Figure 79a. The experiment using a deuterium labeled alcohol, racemic 1-*d*-phenylethanol, in microbial deracemization showed that the deuterium on the chiral center from the reacting enantiomer being inverted was exchanged by hydrogen from 'outside', while the deuterium in the mirror-image remained unaffected.

Figure 79b shows deracemization of 1,2-hexandiol by *C. parapsilosis* NBRC 0708. In this reaction, the (*R*)-isomer was oxidized to the hydroxyketone by NADH-dependent dehydrogenase and the hydroxyketone was reduced to the (*S*)-diol by NADPH-dependent enzyme. The (*S*)-isomer could not be oxidized.[154]

Figure 80 shows deracemization of various alcohols with multiple enzymes system. (*S*)-enantiomers were obtained by the (*R*)-selective oxidation with *Alcaligenes faecalis* and (*S*)-selective reduction using *Rhodococcus rubber* or *Rhodococcus erythropolis*. (*R*)-enantiomers were also accessible with a similar system using *R. erythropolis* DSM 43066 as the (*S*)-oxidation enzyme and *L. kefir* alcohol dehydrogenase as the (*R*)-reduction enzyme.[155]

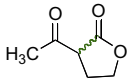
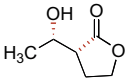
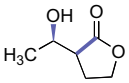
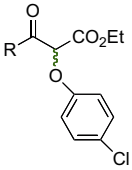
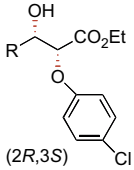
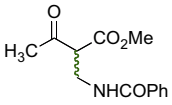
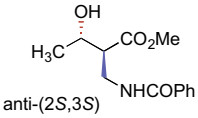
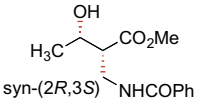
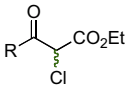
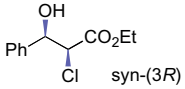
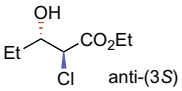
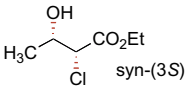
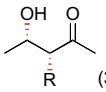
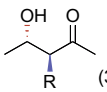
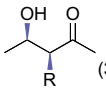
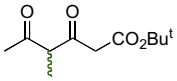
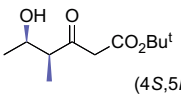
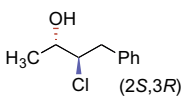
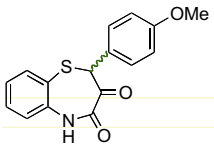
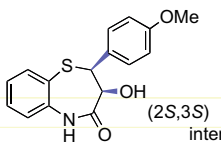
Figure 81 shows reduction of 1,2-indandione accompanying deracemization of alcohol group with the yeast *T. cutaneum*. By the reduction, (1*S*,2*R*)-1,2-indandiol was obtained in 75% yield with >99% ee. The precise research on reaction mechanism revealed that the reduction of diketone afforded racemic 2-hydroxyketone, and the deracemization of the monoalcohol followed by the enantioselective reduction gave (1*S*,2*R*)-1,2-indandiol. In fact, the reaction of the same microbe with the racemic 2-hydroxyindan-1-one afforded (1*S*,2*R*)-diol in 90% yield with >99% ee.[97,98]

Table 7
Dynamic kinetic resolution. The below references are cited in [139–150,4b,38,51,130,40,26,131,30]

Substrate	Product		Biocatalyst	Ref.
	 (syn-2*R*,3*S*)	92% de >99% ee	Baker's yeast Methyl vinyl ketone was used as an additive	[139]
		>99% de	Yeast ADH	[140]
	R^1; R^2; R^3 = CH_3; CH_3; C_2H_5	84% de >95% ee	*Glycine max*	[141]
		>99% de >99% ee	Yeast (YOR120w) ADH	[142]
		99% de >99% ee	*Klebsiella pneumoniae* 2 Kg scale	[143]
		100% yield >99% de, >99% ee	KRED 102	[4b]
	R^1; R^2; R^3 = CH_3, CH_3, neopentyl	92% de >99% ee	Baker's yeast	[144]
		>99% de	Yeast ADH	[140]
	R^1; R^2; R^3 = CH_3; allyl; C_2H_5	80% de >99%ee	*Mucor Javanicus*	[145]
		>99% de >99% ee	Yeast (YOR120w) ADH	[142]
	R^1; R^2; R^3 = CH_3; CH_3; C_2H_5	92% de 99% ee	*Marchantia polymorpha*	[141]
		98% de 86% ee	*Riccia fluitans*	[146]
	 anti-(2*S*,3*S*)	84% de >99% ee	*Candida tropicalis*	[145]
	R^1; R^2; R^3 = CH_3; allyl; t-Bu	88% de	Baker's yeast	[144]
	R^1; R^2; R^3 = CH_3; allyl; C_2H_5	94% de >99% ee	Yeast (YDR541c) ADH	[142]
	 (2*R*,3*S*)  (2*S*,3*S*)			[38]
		85% conv. 4% conv.	25.8 : 74.5 40 : 60	*D. carota* *D. carota* + microbial inhibitor (chloramphenicol + cyclohexylimide)
	 (3*S*,4*R*)  Useful intermediate	90% de, 100% ee	KRED 101	[147]
	 (3*R*,4*R*)	99% de, 100% ee	KRED 108	
	 (1*S*,2*R*)	100% conv. 98% de, 99% ee	Baker's yeast (Immobilized withalginate) glucose	[51]
			perfluorooctane	
		75% yield 84% ee	Baker's yeast (Immobilized with alginate) [bmim]PF_6: H_2O: MeOH = 100:10:2	

(*continued on next page*)

Table 7 (*continued*)

Substrate	Product		Biocatalyst	Ref.
	 (+)-(3*R*,1′*S*)	100% yield 100% de	*Kluyveromyces marxianus*	[130]
	 (-)-(3*S*,1′*R*)	90% de 100% ee	*Hansenulla* sp.	
	 (2*R*,3*S*)	R = Me 33% yield 92% de, >99% ee	Baker's yeast	[148]
		R = Ph 73% yield >99% de, 97% ee	*Kluyvromyces marxianus*	
	 anti-(2*S*,3*S*)	100% de, >99% ee	*Gossypium hirsultum*	[40]
	 syn-(2*R*,3*S*)	100% de, >99% ee	*Parthenocissus tricitspidata*	
	 syn-(3*R*)		*S. cerevisiae* YJR096w expressed in *E. coli*	[26]
	 anti-(3*S*)		*S. cerevisiae* YGL157w expressed in *E. coli*	
	 syn-(3*S*)	89% isolated yield 98% ee	*S. cerevisiae* YOR120w expressed in E. coli	
	 (3*R*,4*S*)	100% yield >99% de	KRED102	[131]
R = Me, Et, -CH_2CHMe_2, allyl, -$(CH_2)_2CH{=}CH_2$, -$CH_2CH{=}CMe_2$				
	 (3*S*,4*S*)	100% yield R=Et, >98% de, R=Pr, 90% de R=allyl, 98% de	KRED118	
	 (3*S*,4*R*)	100% yield R=Et, 90% de R=allyl, 98% de	KRED A1B	
	 (4*S*,5*R*)	66% yield 94% de, 99.2% ee	recombinant *Lactobacillus brevis* ADH	[149]
	 (2*S*,3*R*)	83%conv. 84% de, >99% ee	W110A TeSADH	[30]
	 (2*S*,3*S*)	80% yield >99% ee	Baker's yeast	[150]
		intermediate for Diltiazem		

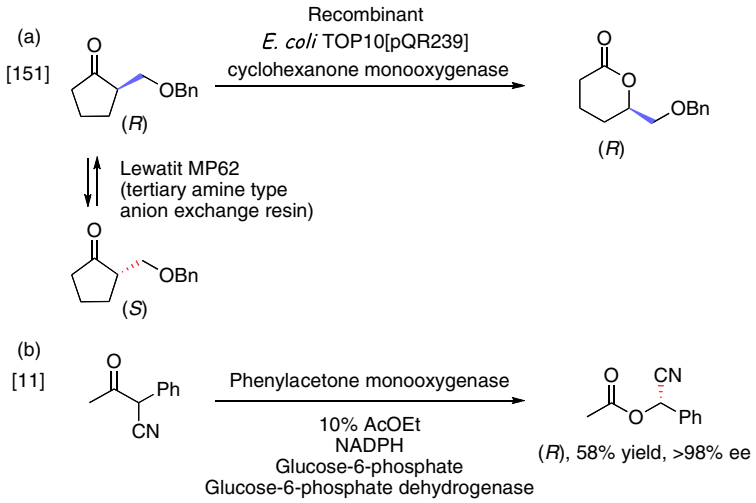


Figure 78. Dynamic kinetic resolution of (a) α-benzyloxymethyl ketone[151] and (b) β-keto nitrile[11] via Baeyer–Villiger oxidation.

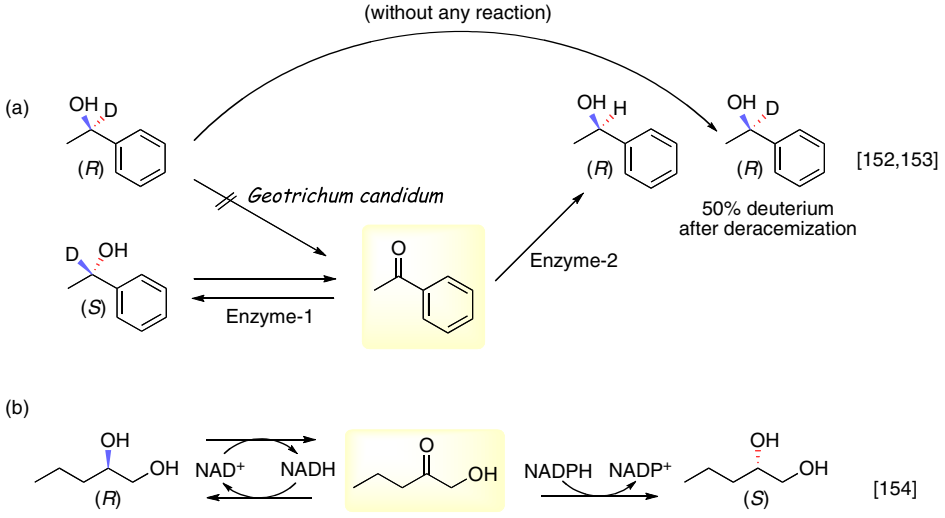
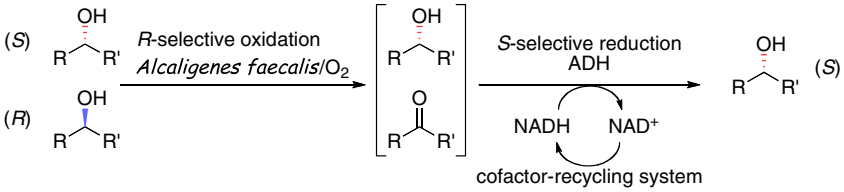
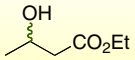


Figure 79. Deracemization of alcohol by (a) *Geotrichum candidum*[152,153] and (b) *Candida parapsilosis*.[154]



Substrate	ADH	Cofactor-recycling system	Yield(%)	ee(%)
2-octanol	*Rhodococcus ruber*	GDH-glucose	99	>99
2-octanol	*Rhodococcus ruber*	2-propanol	96	93
	*Rhodococcus erythropolis*	GDH-glucose	99	96

ADH: alcohol dehydrogenase, GDH: glucose dehydrogenase

Figure 80. Deracemization of alcohol by multiple enzymes system.[155]

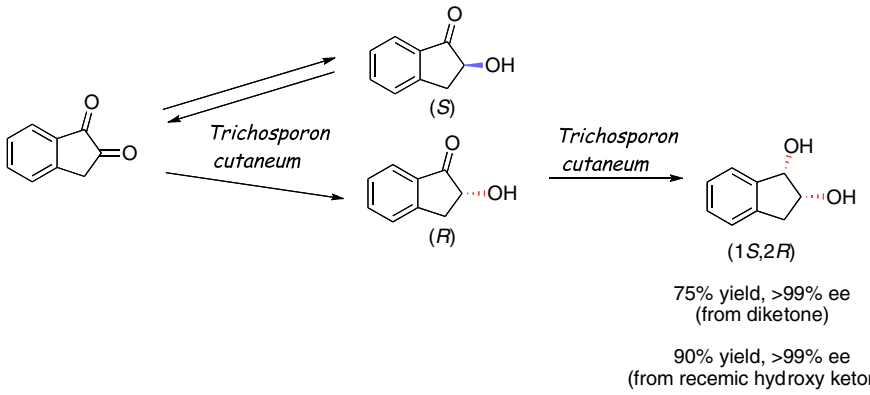
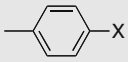
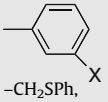
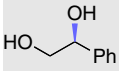
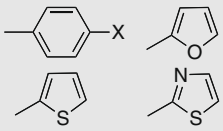
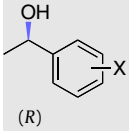
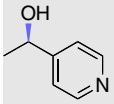


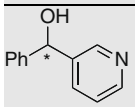
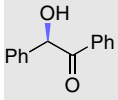
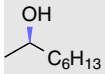
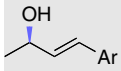
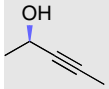
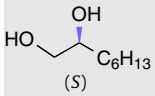
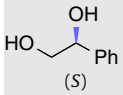
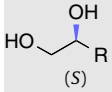
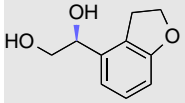
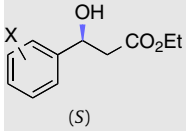
Figure 81. Reduction of 1,2-indandione by *Trichosporon cutaneum* accompanying deracemization of alcohol group.[97,98]

Table 8
Deracemization of alcohols to (*R*)-alcohols

Product			Biocatalyst	Ref.
 (*R*)	R =  –CH_2Ph, –$(CH_2)_2$Ph, –CH_2OPh, –CH_2CO_2Me	 –CH_2SPh, 2-pyridyl, 3-pyridyl, 4-pyridyl	>95% yield, 95–100% ee	*Geotrichum candidum*	153
 (*R*)	R = Ph, CH_2CO_2Me				
 (*S*)					
 (*R*)	R = 		79–100% yield, >89% ee	*Sphingomonas paucimobilis*	156
 (*R*)	R = Me, Et, CH_2CH_2Cl				
 (*R*)	X = *m*-F, *p*-Br, *p*-NO_2, *p*-OMe		88–100% yield, 99% ee	*Aspergillus terreus* CCT3320, CCT4083	84,157,158
 (*R*)			100% yield, 87% ee	*Catharanthus roseus*	159,160

(*continued on next page*)

Table 8 (*continued*)

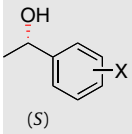
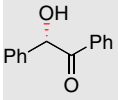
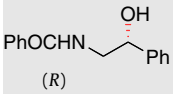
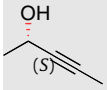
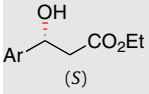
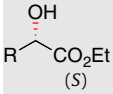
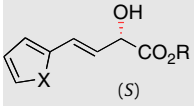
Product			Biocatalyst	Ref.
		(−) 93% yield, 100% ee	*Catharanthus roseus*	159,160
 (*R*)		73–76% yield, 95–97%, ee pH 7.5–8	*Rhizopus oryzae* ATCC 9363	161
 (*R*)		92% yield, 97% ee	*Candida rugosa*	162
 (*R*)		68–79% yield, 76–99% ee	*Candida parappsilosis* ATCC7330	163
 (*R*)		83% yield, 100% ee 70% yield, 98.7% ee*	*Nocardia fusca* AKU2131 *at 2%(v/v) substate concentrations NADPH, glucose, and GDH	164,165
 (*S*)		65.5% yield, >99% ee	*Candida albicans* CCT 0776	166
 (*S*)		92% yield, 98% ee	*Candida parapsilosis* CCTCC M203011	167–169
 (*S*)	R = alkyl, Ph, $Ph(CH_2)_2$–	93–100% yield, 97–100% ee	*Candida parapsilosis* NBRC 0708	154
 (*S*)		67% yield, 100% ee	*Pichia methanolica*	170
 (*S*)		X = *p*-NO_2, 68% yield, 99% ee, X = *p*-Me, 75% yield, 99% ee	*Candida parappsilosis* ATCC7330	171

Other examples for deracemization of alcohols to (*R*)-alcohols and (*S*)-alcohols are shown in Tables 8 and 9, respectively.

7.2. Chemoenzymatic deracemization of alcohols

Chemoenzymatic method for deracemization consists of two reactions, (1) enzymatic oxidation of one enantiomer and (2)

Table 9
Deracemization of alcohols to (*S*)-alcohols

Product			Biocatalyst	Ref.
 (*S*)	X = *m*-NO_2	96% yield, >98% ee	*Aspergillus terreus* URM 4602	84
	X = *p*-Cl	95% yield, 91% ee	*Cyanidioschyzon*	172
	X = *m*-F, *p*-OMe	84–99% yield, 97–>99% ee	*Aspergillus terreus* CCT 4083,	157,158
	X = H	100% yield, 98% ee	*Aspergillus orizae* CCT 4964 Ginger root	42
 (*S*)		85% yield, 71%, ee pH 4–5	*Rhizopus oryzae* ATCC 9363	161
 (*R*)		92% yield, 98% ee	*Cunninghamella echinulata*	173
 (*S*)		70% yield, 98% ee	*Nocardia psudosporangifera* AKU NOC 060	164
 (*S*)		−57% yield, −>99% ee	*Candida parapsilosis* ATCC 7330	174
 (*S*)	R = Ph	95% yield, 99% ee	*Candida parapsilosis* ATCC 7330	175
	R = CH_2CH_2Ar	66–70% yield, 91–95% ee		
	R = CH=CHAr	55–85% yield, 50–99% ee		
 (*S*)	X = S, O, NH	52–79% yield, 90–>99% ee	*Candida parapsilosis* ATCC 7330	176

chemical reduction to produce racemic alcohols, was developed. For example, the reaction of racemic lactate with L-lactate oxidase from *Aerococcus viridans* in the presence of sodium borohydride in a phosphate buffer gave D-lactate in 99% yield with >99% ee[177] as shown in Figure 82a. The similar reaction in an ionic liquid was reported for deracemization of aryl ethanol derivatives. Using *Geotricum candidum* as the biocatalyst and sodium borohydride as the chemical reducing agent in an ionic liquid, deracemization of racemic 1-(2-thienyl)ethanol gave the corresponding (*R*)-alcohol in 72% yield with >99% ee (Fig. 82b).[178]

7.3. Deracemization of amines

Amines can be deracemized by aminotransferase and dehydrogenase. As shown in Figure 83, *E. coli* cells expressing branched-chain amino acid aminotransferase utilizing the gene from *Sinorhizobium meliloti* ATCC 51124 were used for deracemization of 4-chlorophenylalanine. The D(*R*)-substrate was firstly oxidized to the corresponding keto acid by D-amino acid dehydrogenase (DadA) in *E. coli*, and the product was transformed to L(*S*)-amino acid catalyzed by amino acid aminotransferase. L(*S*)-substrate remained in the system without any reaction. Thus, L(*S*)-4-chlorophenylalanine was obtained in 94% ee.[179]

A combination of a selective enzymatic oxidation with an unselective electrochemical reduction was used for deracemization of racemic leucine to L-leucine. D-Amino acid oxidase from *Trigonopsis variabilis* was used as enzyme, and the reaction was conducted at −1.5 V versus Ag/AgCl in $(NH_4)_2SO_4/NH_3$ pH10 buffer. As a result, L-leucine was obtained in 80% yield with 91% ee (Fig. 84).[180]

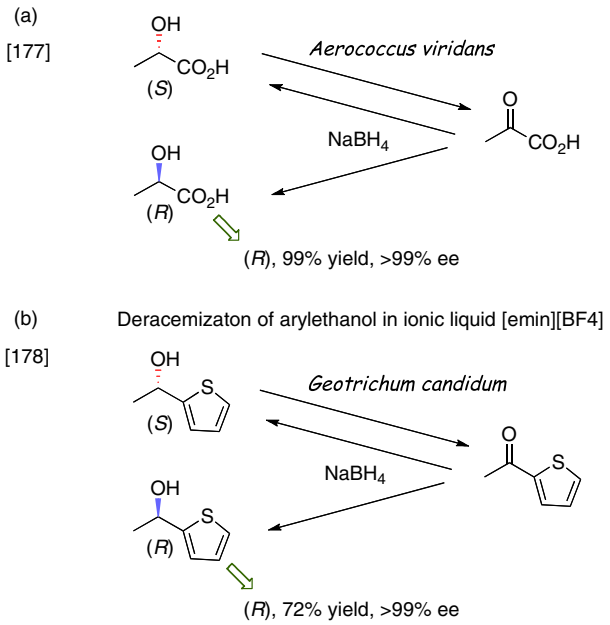


Figure 82. Chemoenzymatic deracemization of alcohols by (a) *Aerococcus viridans*[177] and (b) *Geotrichum candidum*.[178]

8. Conclusions

Asymmetric reduction and oxidations are covered in this review. Asymmetric reductions have been reported continuously for decades, and an increased number of reports about oxidation have been published recently. Now, enzyme source becomes diverse; enzymes expressed and unexpressed in natural environment can be used as long as the gene for the enzymes can be obtained and the enzymes can be expressed in laboratory. An increase in available gene in data bank has also promoted the development of biocatalysis. Collaboration between organic chemists and molecular biologist enabled the use of enzyme overexpressed in easy-to-use microorganisms such as *E. coli* or baker's yeast. An increase in the number of commercially available oxidoreductase also promoted the use of enzymes in organic synthesis. Furthermore, to improve the catalysts performance, various reaction conditions were applied to reactions including the use of innovative media such as supercritical CO_2 and ionic liquids. With these progresses in biocatalysis, industrial applications using oxidoreductase will increase in the future.

References

1. Carey, J. S.; Laffan, D.; Thomsonc, C.; Williamsd, M. T. *Org. Biomol. Chem.* **2006**, *4*, 2337–2347.
2. Patel, R. N. *Coord. Chem. Rev.* **2008**, *252*, 659–701.
3. http://www.daicelchiral.com/en/contents/chiralscreen/oh/index.html.
4. (a) http://www.codexis.com/pdf/Product_List.pdf; (b) Kalaitzakis, D.; Rozzell, J. D.; Kambourakis, S.; Smonou, I. *Org. Lett.* **2005**, *7*, 4799–4801.
5. (a) Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003**, *14*, 2659–2681; (b) Nakamura, K.; Matsuda, T. *Curr. Org. Chem.* **2006**, *10*, 1217–1246.
6. (a) Nakamura, K.; Matsuda, T. In *Asymmetric Organic Synthesis with Enzymes*; Gotor, V., Alfonsa, I., Garcia-Uradiales, E., Eds.; Wiley-VCH Verlag GmbH, 2008; pp 193–228; (b) Nakamura, K.; Matsuda, T. In *Organic Reactions in Water—Principles, Strategies and Methods*; Ulf, L., Ed.; Blackwell Publishing, 2007; pp 301–349.
7. Martins, B. M.; Macedo-Ribeiro, S.; Bresser, J.; Bucke, W.; Messerschm, A. *FEBS J.* **2005**, *272*, 269–281.
8. Malito, E.; Alfieri, A.; Fraaije, M. W.; Mattevi, A. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 13157–13162.
9. Matsuda, T.; Yamagishi, Y.; Koguchi, S.; Iwai, N.; Kitazume, T. *Tetrahedron Lett.* **2006**, *47*, 4619–4622.

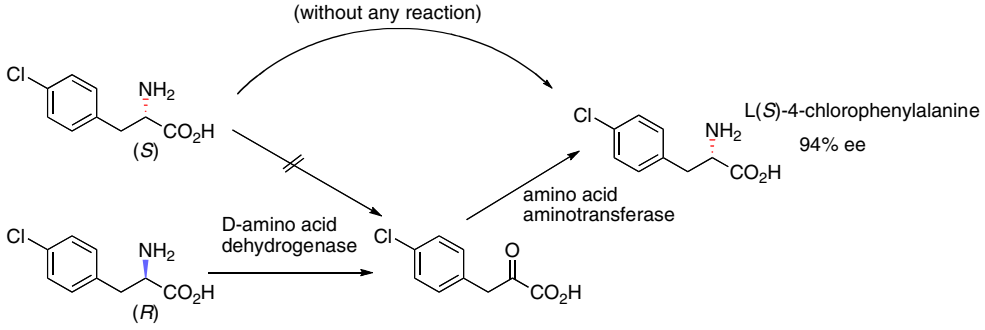


Figure 83. Deracemization of amines.[179]

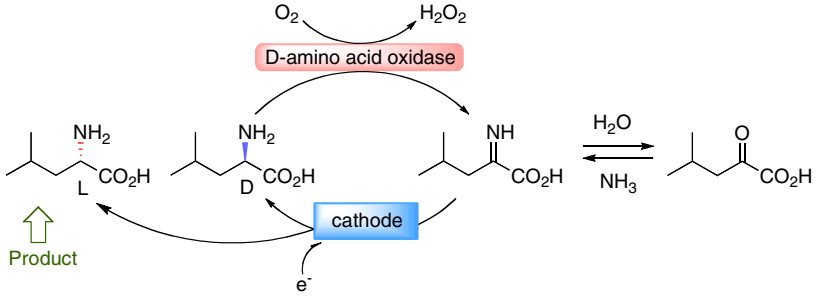


Figure 84. Electro-enzymatic deracemization of amines.[180]

10. Zhu, D.; Yang, Y.; Hua, L. *J. Org. Chem.* **2006**, *71*, 4202–4205.
11. Rodriguez, C.; de Gonzalo, G.; Pazmino, D. E. T.; Fraaije, M. W.; Gotor, V. *Tetrahedron: Asymmetry* **2008**, *19*, 197–203.
12. Ei-Zahab, B.; Donnelly, D.; Wang, P. *Biotechnol. Bioeng.* **2008**, *99*, 508–514.
13. Mertens, R.; Greiner, L.; van den Ban, E. C. D.; Haaker, H. B. C. M.; Liese, A. *J. Mol. Catal. B: Enzym.* **2003**, *24–25*, 39–52.
14. Höllrigl, V.; Otto, K.; Schmid, A. *Adv. Synth. Catal.* **2007**, *349*, 1337–1340.
15. Nakamura, K.; Yamanaka, R. *Chem. Commun.* **2002**, 1782–1783.
16. Nakamura, K.; Yamanaka, R. *Tetrahedron: Asymmetry* **2002**, *13*, 2529–2533.
17. Taglieber, A.; Schulz, F.; Hollmann, F.; Rusek, M.; Reetz, M. T. *ChemBioChem* **2008**, *9*, 565–572.
18. Fitzpatrick, T. B.; Amrhein, N.; Macheroux, P. *J. Biol. Chem.* **2003**, *278*, 19891–19897.
19. Hirano, J.; Miyamoto, K.; Ohta, H. *Tetrahedron Lett.* **2008**, *49*, 1217–1219.
20. Dhariwal, A.; Mavrov, V.; Schroeder, I. *J. Mol. Catal. B: Enzym.* **2006**, *42*, 64–69.
21. Homann, M. J.; Vail, R. B.; Previte, E.; Tamarez, M.; Morgan, B.; Dodds, D. R.; Zaks, A. *Tetrahedron* **2004**, *60*, 789–797.
22. Inoue, K.; Makino, Y.; Itoh, N. *Appl. Environ. Microbiol.* **2005**, *71*, 3633–3641.
23. Botes, A. L.; Harvig, D.; van Dyk, M. S.; Sarvary, I.; Frejd, T.; Katz, M.; Hahn-Hagerdal, B.; Gorwa-Grauslund, M. F. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1111–1114.
24. Katz, M.; Sarvary, I.; Frejd, T.; Hahn-Hagerdal, B.; Gorwa-Grauslund, M. F. *Appl. Microbiol. Biotechnol.* **2002**, *59*, 641–648.
25. Knietsch, A.; Waschkowitz, T.; Bowien, S.; Henne, A.; Daniel, R. *J. Mol. Microbiol. Biotechnol.* **2003**, *5*, 46–56.
26. Kaluzna, I. A.; Feske, B. D.; Wittayanan, W.; Ghiviriga, I.; Stewart, J. D. *J. Org. Chem.* **2005**, *70*, 342–345.
27. Hammond, R. J.; Poston, B. W.; Ghiviriga, I.; Feske, B. D. *Tetrahedron Lett.* **2007**, *48*, 1217–1219.
28. Katz, M.; Frejd, T.; Hahn-Hägerdal, B.; Gorwa-Grauslund, M. F. *Biotechnol. Bioeng.* **2003**, *84*, 573–582.
29. Ziegelmann-Fjeld, K. I.; Musa, M. M.; Phillips, R. S.; Zeikus, J. G.; Vieille, C. *Protein Eng. Des. Sel.* **2007**, *20*, 47–55.
30. Musa, M. M.; Ziegelmann-Fjeld, K. I.; Vieille, C.; Zeikus, J. G.; Phillips, R. S. *J. Org. Chem.* **2007**, *72*, 30–34.
31. Sun, L.; Bulter, T.; Alcalde, M.; Petrounia, I. P.; Arnold, F. H. *ChemBioChem* **2002**, *3*, 781–783.
32. Escalettes, F.; Turner, N. J. *ChemBioChem* **2008**, *9*, 857–860.
33. Morikawa, S.; Nakai, T.; Yasohara, Y.; Nnba, H.; Kizaki, T.; Hasegawa, J. *Biosci. Biotechnol. Biochem.* **2005**, *69*, 544–552.
34. Makino, Y.; Inoue, K.; Dairi, T.; Itoh, N. *Appl. Environ. Microbiol.* **2005**, *71*, 4713–4720.
35. Zhu, D.; Malik, H. T.; Hua, L. *Tetrahedron: Asymmetry* **2006**, 3010–3014.
36. Blanchard, N.; van de Weghe, P. *Org. Biomol. Chem.* **2006**, *4*, 2348–2353.
37. Utsukihara, T.; Watanabe, S.; Tomiyama, A.; Chai, W.; Horiuchi, C. A. *J. Mol. Catal. B: Enzym.* **2006**, *41*, 103–109.
38. Rodríguez, P.; Barton, M.; Aldabalde, V.; Onetto, S.; Panizza, P.; Menéndez, P.; Gonzalez, D.; Rodrígueza, S. *J. Mol. Catal. B: Enzym.* **2007**, *49*, 8–11.
39. Surette, M. A.; Sturz, A. V.; Lada, R. R.; Nowak, J. *Plant Soil* **2003**, *253*, 381–390.
40. Shimoda, K.; Kubota, N.; Hamada, H.; Hamada, H. *Tetrahedron Lett.* **2006**, *47*, 1541–1544.
41. Orden, A. A.; Bisogno, F. R.; Cifuente, D. A.; Giordano, O. S.; Sanz, M. K. *J. Mol. Catal. B: Enzym.* **2006**, *42*, 71–77.
42. Andrade, L. H.; Utsunomiya, R. S.; Omori, A. T.; Porto, A. L. M.; Comasseto, J. V. *J. Mol. Catal. B: Enzym.* **2006**, *38*, 84–90.
43. Machado, L. L.; Souza, J. S. N.; de Mattos, M. C.; Sakata, S. K.; Cordell, G. A.; Lemos, T. L. G. *Phytochemistry* **2006**, *67*, 1637–1643.
44. Erdélyia, B.; Szabóa, A.; Seresb, G.; Birincsika, L.; Ivanicsc, J.; Szatzkerd, G.; Popped, L. *Tetrahedron: Asymmetry* **2006**, *17*, 268–274.
45. Katz, M.; Johanson, T.; Gorwa-Grauslund, M. F. *Yeast* **2004**, *21*, 1253–1267.
46. Buque, E. M.; Chin-Joe, I.; Straathof, A. J. J.; Jongejan, J. A.; Heijnen, J. *J. Enzyme Microb. Technol.* **2002**, *31*, 656–664.
47. Milagrea, H. M. S.; Milagrea, C. D. F.; Morana, P. J. S.; Santanab, M. H. A.; Rodriguesa, J. A. R. *Enzyme Microb. Technol.* **2005**, *37*, 121–125.
48. Milagr, H. M. S.; Milagre, C. D. F.; Moran, P. J. S.; Santana, M. H. A.; Rodrigues, J. A. R. *Org. Process Res. Dev.* **2006**, *10*, 611–617.
49. Musa, M. M.; Ziegelmann-Fjeld, K. I.; Vieille, C.; Zeikus, J. G.; Phillips, R. S. *Angew. Chem., Int. Ed.* **2007**, *46*, 3091–3094.
50. Wolfson, A.; Dlugya, C.; Tavora, D.; Blumenfelda, J.; Shotlanda, Y. *Tetrahedron: Asymmetry* **2006**, *17*, 2043–2045.
51. Yajima, A.; Naka, K.; Yabuta, G. *Tetrahedron Lett.* **2004**, *45*, 4577–4579.
52. Canadell, J. G.; Le Quere, C.; Raupach, M. R.; Field, C. B.; Buitenhuis, E. T.; Ciais, P.; Conway, T. J.; Gillett, N. P.; Houghton, R. A.; Marland, G. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 18866–18870.
53. Matsuda, T.; Harada, T.; Nakamura, K. *J. Chem. Soc., Chem. Commun.* **2000**, 1367–1368.
54. Matsuda, T.; Watanabe, K.; Kamitanaka, T.; Harada, T.; Nakamura, K. *J. Chem. Soc., Chem. Commun.* **2003**, *10*, 1198–1199.
55. Matsuda, T.; Marukado, R.; Mukouyama, M.; Harada, T.; Nakamura, K. *Tetrahedron: Asymmetry* **2008**, *19*, 2272–2275.
56. Panza, J. L.; Russell, A. J.; Beckman, E. J. *Tetrahedron* **2002**, *58*, 4091–4104.
57. Pfruender, H.; Amidjojo, M.; Kragl, U.; Weuster-Botz, D. *Angew. Chem., Int. Ed.* **2004**, *43*, 4529–4531.
58. Musa, M. M.; Ziegelmann-Fjeld, K. I.; Vieille, C.; Phillips, R. S. *Org. Biomol. Chem.* **2008**, *6*, 887–892.
59. Kaluzna, I.; Andrew, A. A.; Bonilla, M.; Martzen, M. R.; Stewart, J. D. *J. Mol. Catal. B: Enzym.* **2002**, *17*, 101–105.
60. Ema, T.; Yagasaki, H.; Okita, N.; Takeda, M.; Sakai, T. *Tetrahedron* **2006**, *62*, 6143–6149.
61. Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Wada, M.; Kataoka, M.; Shimizu, S. *Tetrahedron: Asymmetry* **2001**, *12*, 1713–1718.
62. Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Wada, M.; Kataoka, M.; Shimizu, S. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 1430–1436.
63. Zhu, D.; Ankati, H.; Mukherjee, C.; Yang, Y.; Biehl, E. R.; Hua, L. *Org. Lett.* **2007**, *9*, 2561–2563.
64. Kita, K.; Fukura, T.; Nakase, K. I.; Okamoto, K.; Yanase, H.; Kataoka, M.; Shimizu, S. *Appl. Environ. Microbiol.* **1999**, *65*, 5207–5211.
65. Zhu, D.; Yang, Y.; Buynak, J. D.; Hua, L. *Org. Biomol. Chem.* **2006**, *4*, 2690–2695.
66. Gröger, H.; Chamouleau, F.; Orologas, N.; Rollmann, C.; Drauz, K.; Hummel, W.; Weckbecker, A.; May, O. *Angew. Chem., Int. Ed.* **2006**, *45*, 5677–5681.
67. Groger, H.; Rollmann, C.; Chamouleau, F.; Sebastien, I.; May, O.; Wienand, W.; Drauz, K. *Adv. Synth. Catal.* **2007**, *349*, 709–712.
68. Krausser, M.; Hummel, W.; Groger, H. *Eur. J. Org. Chem.* **2007**, 5175–5179.
69. Fischer, T.; Pietruszka, J. *Adv. Synth. Catal.* **2007**, *349*, 1533–1536.
70. Gelo-Pujic, M.; Le Guyader, F.; Schlama, T. *Tetrahedron: Asymmetry* **2006**, *17*, 2000–2005.
71. Pollard, D.; Truppo, M.; Pollard, J.; Chen, C.; Moore, J. *Tetrahedron: Asymmetry* **2006**, *17*, 554–559.
72. Miyadera, A.; Satoh, K.; Imura, A. *Chem. Pharm. Bull.* **2000**, *48*, 563–565.
73. Satoh, K.; Imura, A.; Miyadera, A.; Kanai, K.; Yukimoto, Y. *Chem. Pharm. Bull.* **1998**, *46*, 587–590.
74. Matsuda, M.; Yamazaki, T.; Fuhshuku, K.; Sugai, T. *Tetrahedron* **2007**, *63*, 8752–8760.
75. Hiraoka, C.; Matsuda, M.; Suzuki, Y.; Fujieda, S.; Tomita, M.; Fuhshuku, K.; Obata, R.; Nishiyama, S.; Sugai, T. *Tetrahedron: Asymmetry* **2006**, *17*, 3358–3367.
76. Uwai, K.; Konn, N.; Kitamura, S.; Ohta, S.; Takeshita, M. *Chirality* **2005**, *17*, 494–500.
77. Pawlowski, J. E.; Huizinga, M.; Penning, T. M. *J. Biol. Chem.* **1991**, *266*, 8820–8825.
78. Yamano, S.; Nakamoto, N.; Toki, S. *Xenobiotica* **1999**, *29*, 917–930.
79. Uwai, K.; Konno, N.; Yasuta, Y.; Takeshita, M. *Bioorg. Med. Chem.* **2008**, *16*, 1084–1089.
80. Fujisawa, H.; Nagata, S.; Misono, H. *Biochem. Biophys. Acta* **2003**, *1645*, 89–94.
81. Nakamura, K.; Kondo, S.; Kawai, Y.; Nakajima, N.; Ohno, A. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 2236–2240.
82. Murata, K.; Fukuda, Y.; Simosaka, M.; Watanabe, K.; Saikusa, T.; Kimura, A. *Eur. J. Biochem.* **1985**, *151*, 631–636.
83. Bisel, P.; Walter, L.; Nieger, M.; Hummel, W.; Müller, M. *Tetrahedron: Asymmetry* **2007**, *18*, 1142–1144.
84. Comasseto, J. V.; Assis, L. F.; Andrade, L. H.; Schoenlein-Crusius, I. H.; Porto, A. L. M. *J. Mol. Catal. B: Enzym.* **2006**, *39*, 24–30.
85. Yang, Y.; Zhu, D.; Piegat, T. J.; Hua, L. *Tetrahedron: Asymmetry* **2007**, *18*, 1799–1803.
86. Kurbanoglu, E. B.; Zilbeyaz, K.; Kurbanoglu, N. I.; Kilic, H. *Tetrahedron: Asymmetry* **2007**, *18*, 1159–1162.
87. Kurbanoglu, E. B.; Zilbeyaz, K.; Kurbanoglu, N. I.; Kilic, H. *Tetrahedron: Asymmetry* **2007**, *18*, 2332–2335.
88. Soni, P.; Banerjee, U. C. *Biotechnol. J.* **2006**, *1*, 80–85.
89. Kamble, A. L.; Soni, P.; Banerjee, U. C. *J. Mol. Catal. B: Enzym.* **2005**, *35*, 1–6.
90. Utsukihara, T.; Misumi, O.; Kato, N.; Kuroiwa, T.; Horiuchi, C. A. *Tetrahedron: Asymmetry* **2006**, *17*, 1179–1185.
91. Yang, W.; Xu, J.-H.; Xie, Y.; Xu, Y.; Zhao, G.; Lin, G.-Q. *Tetrahedron: Asymmetry* **2006**, *17*, 1769–1774.
92. Kizaki, N.; Yasohara, Y.; Hasegawa, J.; Wada, M.; Kataoka, M.; Shimizu, S. *Appl. Microbiol. Biotechnol.* **2001**, *55*, 590–595.
93. Katoh, T.; Mizumoto, S.; Fudesaka, M.; Takeo, M.; Kajimoto, T.; Node, M. *Tetrahedron: Asymmetry* **2006**, *17*, 1655–1662.
94. Friberg, A.; Johanson, T.; Franzén, J.; Gorwa-Grauslund, M. F.; Frejd, T. *Org. Biomol. Chem.* **2006**, *4*, 2304–2312.
95. Wolberg, M.; Hummel, W.; Wandrey, C.; Mulller, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 4306–4308.
96. Guo, Z.; Chen, Y.; Goswami, A.; Hanson, R. L.; Patel, R. N. *Tetrahedron: Asymmetry* **2006**, *17*, 1589–1602.
97. Conceicao, G. J. A.; Moran, P. J. S.; Rodrigues, J. A. A. *Tetrahedron: Asymmetry* **2003**, *14*, 2327–2330.
98. Cazetta, T.; Lunardi, I.; Conceicao, G. J. A.; Morana, P. J. S.; Rodrigues, J. A. R. *Tetrahedron: Asymmetry* **2007**, *18*, 2030–2036.
99. Shimoda, K.; Kubota, N.; Hirata, T.; Kondo, Y.; Hamada, H. *Tetrahedron Lett.* **2007**, *48*, 1345–1347.
100. Hirata, T.; Shimoda, K.; Gondai, T. *Chem. Lett.* **2000**, 850–851.
101. Shimoda, K.; Kubota, N.; Hamada, H. *Tetrahedron: Asymmetry* **2004**, *15*, 2443–2446.
102. Shimoda, K.; Kubota, N.; Hamada, H.; Kaji, M.; Hirata, T. *Tetrahedron: Asymmetry* **2004**, *15*, 1677–1679.
103. Shimoda, K.; Kubota, N.; Hamada, H.; Yamane, S. Y.; Hirata, T. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 2269–2272.
104. Nakamura, K.; Yamanaka, R.; Tohi, K.; Hamada, H. *Tetrahedron Lett.* **2000**, *41*, 6799–6802.
105. Shimoda, K.; Izumi, S.; Hirata, T. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 813–816.

106. Swiderska, M. A.; Stewart, J. D. *J. Mol. Catal. B: Enzym.* **2006**, *42*, 52–54.
107. Korbekandi, H.; Mather, P.; Gardiner, J.; Stephens, G. *Enzyme Microb. Technol.* **2008**, *42*, 308–314.
108. Matsuyama, A.; Yamamoto, H.; Kobayashi, Y. *Org. Process Res. Dev*. **2002**, *6*, 558–561.
109. Nikodinovic, J.; Dinges, J. M.; Bergmeier, S. C.; McMills, M. C.; Wright, D. L.; Priestley, N. D. *Org. Lett.* **2006**, *8*, 443–445.
110. Nagaki, M.; Imaruoka, H.; Kawakami, J.; Saga, K.; Kitahara, H.; Sagami, H.; Oba, R.; Ohya, N.; Koyama, T. *J. Mol. Catal. B: Enzym.* **2007**, *47*, 33–36.
111. Shidoji, Y.; Okamoto, K.; Muto, Y.; Komura, S.; Ohishi, N.; Yagi, K. *J. Cell. Biochem.* **2006**, *97*, 178–187.
112. Miyamoto, K.; Hirokawa, S.; Ohta, H. *J. Mol. Catal. B: Enzym.* **2007**, *46*, 14–19.
113. Furusawa, M.; Hashimoto, T.; Noma, Y.; Asakawa, Y. *Chem. Pharm. Bull.* **2005**, *53*, 1423–1429.
114. Furusawa, M.; Hashimoto, T.; Noma, Y.; Asakawa, Y. *Chem. Pharm. Bull.* **2005**, *53*, 1513–1514.
115. Garrett, M. D.; Scott, R.; Sheldrake, G. N.; Dalton, H.; Goode, P. *Org. Biomol. Chem.* **2006**, *4*, 2710–2715.
116. Flitsh, S.; Grogan, G. In *Enzyme Catalysis in Organic Synthesis A comprehensive Handbook*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH Verlag GmbH: Weinheim, 2002; pp 1202–1245.
117. Alphand, V.; Furstoss, R. *J. Org. Chem.* **1992**, *57*, 1306–1309.
118. Petit, F.; Furstoss, R. *Tetrahedron: Asymmetry* **1993**, *4*, 1341–1352.
119. Rial, D. V.; Bianchi, D. A.; Kapitanova, P.; Lengar, A.; van Beilen, J. B.; Mihovilovic, M. D. *Eur. J. Org. Chem.* **2008**, 1203–1213.
120. Mihovilovic, M. D.; Chen, G.; Wang, S. Z.; Kyte, B.; Rochon, F.; Kayser, M. M.; Stewart, J. D. *J. Org. Chem.* **2001**, *66*, 733–738.
121. Berezina, N.; Kozma, E.; Furstoss, R.; Alphand, V. *Adv. Synth. Catal.* **2007**, *349*, 2049.
122. Mihovilovic, M. D.; Grotzl, B.; Kandioller, W.; Muskotal, A.; Snajdrova, R.; Rudroff, F.; Spreitzer, H. *Chem. Biodiv.* **2008**, *5*, 490–498.
123. Snajdrova, R.; Grogan, G.; Mihovilovic, M. D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4813–4817.
124. Luna, A.; Gutierrez, M. C.; Furstoss, R.; Alphand, V. *Tetrahedron: Asymmetry* **2005**, *16*, 2521–2524.
125. Mihovilovic, M. D.; Kapitan, P.; Kapitanova, P. *CHEMSUSCHEM* **2008**, *1*, 143–148.
126. Kurisawa, M.; Chung, J. E.; Uyama, H.; Kobayashi, S. *Macromol. Biosci.* **2003**, *3*, 758–764.
127. Xu, P.; Uyama, H.; Whitten, J. E.; Kobayashi, S.; Kaplan, D. L. *J. Am. Chem. Soc.* **2005**, *127*, 11745–11753.
128. Pellissier, H. *Tetrahedron* **2008**, *64*, 1563–1601.
129. Padhi, S. K.; Kaluzna, I. A.; Buisson, D.; Azerad, R.; Stewart, J. D. *Tetrahedron: Asymmetry* **2007**, *18*, 2133–2138.
130. Ribeiro, J. B.; Andrade De Sousa, L. M.; da Volta Soares, M.; Ramos, M. D. C.; Radler de Aquino Neto, F.; Fraga, C. A. M.; Leite, S. G. F.; Cordeiro, Y.; Antunes, O. A. C. *Tetrahedron: Asymmetry* **2006**, *17*, 984–988.
131. Kalaitzakis, D.; Rozzell, J. D.; Smonou, I.; Kambourakis, S. *Adv. Synth.* **2006**, *348*, 1958–1969.
132. Lunardi, I.; Conceicao, G. J. A.; Moran, P. J. S.; Rodrigues, J. A. R. *Tetrahedron: Asymmetry* **2005**, *16*, 2515–2519.
133. Kosjek, B.; Tellers, D. M.; Biba, M.; Farr, R.; Moore, J. C. *Tetrahedron: Asymmetry* **2006**, *17*, 2798–2803.
134. Yang, Y.; Drolet, M.; Kayser, M. M. *Tetrahedron: Asymmetry* **2005**, *16*, 2748–2753.
135. Zuger, M. F.; Giovannini, F.; Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 1012.
136. Nakamura, K.; Miyai, T.; Ushio, K.; Oka, S.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2089–2093.
137. Seebach, D.; Zuger, M. F.; Giovannini, F.; Sonnleitner, B.; Fiechter, A. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 151.
138. Matzinger, P. K.; Leuenberger, H. G. W. *Appl. Microbiol. Biotechnol.* **1985**, *22*, 208–210.
139. Nakamura, K.; Kawai, Y.; Miyai, T.; Ohno, A. *Tetrahedron Lett.* **1990**, *31*, 3631–3632.
140. Nakamura, K.; Kawai, Y.; Miyai, T.; Honda, S.; Nakajima, N.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 1467–1470.
141. Nakamura, K.; Miyoshi, H.; Sugiyama, T.; Hamada, H. *Phytochemistry* **1995**, *40*, 1419–1420.
142. Kaluzna, I. A.; Matsuda, T.; Sewell, A. K.; Stewart, J. D. *J. Am. Chem. Soc.* **2004**, *126*, 12827–12832.
143. Miya, H.; Kawada, M.; Sugiyama, Y. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 95–98.
144. Nakamura, K.; Miyai, T.; Nagar, A.; Oka, S.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 1179–1187.
145. Nakamura, K.; Miyai, T.; Fukushima, K.; Kawai, Y.; Babu, B. R.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 1713–1715.
146. Speicher, A.; Roeser, H.; Heisel, R. *J. Mol. Catal. B: Enzym.* **2003**, *22*, 71–77.
147. Kambourakis, S.; Rozzell, J. D. *Tetrahedron* **2004**, *60*, 663–669.
148. Perrone, M. G.; Santandrea, E.; Scilimati, V.; Tortorella, V.; Capitelli, F.; Bertolasi, V. *Tetrahedron: Asymmetry* **2004**, *15*, 3501–3510.
149. Ji, A.; Wolberg, M.; Hummel, W.; Wandrey, C.; Müller, M. *Chem. Commun.* **2001**, 57–58.
150. Kometani, T.; Sakai, Y.; Matsumae, H.; Shibatani, T.; Matsuno, R. *J. Ferment. Bioeng.* **1997**, *84*, 195–199.
151. Gutierrez, M. C.; Furstoss, R.; Alphand, V. *Adv. Synth. Catal.* **2005**, *347*, 1051–1059.
152. Nakamura, K.; Inoue, Y.; Matsuda, T.; Ohno, A. *Tetrahedron Lett.* **1995**, *36*, 6263–6266.
153. Nakamura, K.; Fujii, M.; Ida, Y. *Tetrahedron: Asymmetry* **2001**, *12*, 3147–3153.
154. Hasegawa, J.; Ogura, M.; Tsuda, S.; Maemoto, S.; Kutsuki, H.; Ohashi, T. *Agric. Biol. Chem.* **1990**, *54*, 1819–1827.
155. Voss, C. V.; Gruber, C. C.; Kroutil, W. *Angew. Chem., Int. Ed.* **2008**, *47*, 741–745.
156. Allan, G. R.; Carnell, A. J. *J. Org. Chem.* **2001**, *66*, 6495–6497.
157. Comasseto, J. V.; Omori, A. T.; Andrade, L. H.; Porto, A. L. M. *Tetrahedron: Asymmetry* **2003**, *14*, 711–715.
158. Comasseto, J. V.; Andrade, L. H.; Omori, A. T.; Assis, L. F.; Porto, A. L. M. *J. Mol. Cat. B: Enzym.* **2004**, *29*, 55–61.
159. Takemoto, M.; Achiwa, K. *Tetrahedron: Asymmetry* **1995**, *6*, 2925–2928.
160. Takemoto, M.; Achiwa, K. *Chem. Pharm. Bull.* **1998**, *46*, 577–580.
161. Demir, A. S.; Hamamci, H.; Sesenoglu, O.; Neslihanoglu, R.; Asikoglu, B.; Capanoglu, D. *Tetrahedron Lett.* **2002**, *43*, 6447–6449.
162. Nie, Y.; Xu, Y.; Mu, X. Q.; Tang, Y.; Jiang, J.; Sun, Z. H. *Biotechnol. Lett.* **2005**, *27*, 23–26.
163. Titu, D.; Chadha, A. *Tetrahedron: Asymmetry* **2008**, *19*, 1698–1701.
164. Ogawa, J.; Xie, S. X.; Shimizu, S. *Biotechnol. Lett.* **1999**, *21*, 331–335.
165. Xie, S.-X.; Ogawa, J.; Shimizu, S. *Appl. Microbiol. Biotechnol.* **1999**, *52*, 327–331.
166. Chen, L. S.; Mantovani, S. M.; de Oliveira, L. G.; Duarte, M. C. T.; Marsaioli, A. J. *J. Mol. Catal. B: Enzym.* **2008**, *54*, 50–54.
167. Nie, Y.; Xu, Y.; Mu, X. Q. *Org. Process Res. Dev*. **2004**, *8*, 246–251.
168. Mu, X. Q.; Xu, Y.; Nie, Y.; Ouyan, J.; Sun, Z. H. *Process Biochem.* **2005**, *40*, 2345–2350.
169. Mu, X. Q.; Xu, Y.; Yang, M.; Sun, Z. H. *J. Mol. Catal. B: Enzym.* **2006**, *43*, 23–28.
170. Goswami, A.; Mirfakhrae, K. D.; Patel, R. N. *Tetrahedron: Asymmetry* **1999**, *10*, 4239–4244.
171. Padhi, S. K.; Pandian, N. G.; Chadha, A. *J. Mol. Catal. B: Enzym.* **2004**, *29*, 25–29.
172. Utsukihara, T.; Misumi, O.; Nakajima, K.; Koshimura, M.; Kuniyoshi, M.; Kuroiwa, T.; Horiuchi, C. A. *J. Mol. Catal. B: Enzym.* **2008**, *51*, 19–23.
173. Cardusa, G. J.; Carnell, A. J.; Trauthwein, H.; Riermeir, T. *Tetrahedron: Asymmetry* **2004**, *15*, 239–243.
174. Padhi, S. K.; Chadha, A. *Tetrahedron: Asymmetry* **2005**, *16*, 2790–2798.
175. Baskar, B.; Pandian, N. G.; Priya, K.; Chadha, A. *Tetrahedron* **2005**, *61*, 12296–12306.
176. Vaijayanthi, T.; Chadha, A. *Tetrahedron: Asymmetry* **2007**, *18*, 1077–1084.
177. Oikawa, T.; Mukoyama, S.; Soda, K. *Biotechnol. Bioeng.* **2001**, *73*, 80–82.
178. Tanaka, T.; Iwai, N.; Matsuda, T.; Kitazume, T. *J. Mol. Catal. B: Enzym.* **2009**, *57*, 317–320.
179. Kato, D.; Miyamoto, K.; Ohta, H. *Biocatal. Biotransform.* **2005**, *23*, 375–379.
180. Markle, W.; Lutz, S. *Electrochem. Acta* **2008**, *53*, 3175–3180.