



Fermentation Technology: Use of Various Cultures for Deracemisation of Secondary Alcohols

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Abstract: Enantioselective synthesis of secondary alcohols plays an important role in pharmaceuticals, pheromones, flavors and fragrances, etc. Biocatalysts has unique characteristics when compared with chemical (homogeneous and heterogeneous) catalysts. The present work provides a list of various cultures used for deracemisation/stereoselective synthesis of various natural and synthetic secondary alcohols.

Keywords: Biocatalyst, Enantioselective synthesis, Stereoselective synthesis, Deracemisation, Fermentation technology.

1. Introduction

Development of efficient methods for the synthesis of enantiomerically pure chiral compounds is of tremendous importance. It has been reported that in the year 2004, nine out of ten top drugs contained enantiomerically pure compounds [1]. The requirement of one of the stereospecific products is because it interacts with biological molecules that are highly specific in action. The requirement of enantiomerically pure chiral compounds is not only in the field of pharmaceuticals, but also is of great importance in the field of flavor and aroma chemicals, agriculture and specialty materials [2].

Over the past decades, biocatalysis have been recognized as a highly valuable tool for organic chemists and microbiologists to prepare enantiomerically pure molecules. Among the various strategies for the synthesis of chiral molecules, use of chiral catalysts is an important and efficient technique and in enzyme-catalyzed enantioselective synthesis, these biological catalysts are the recently used ones. In addition, biocatalysis is relatively a green technology, as enzyme-catalyzed reactions can be carried out in aqueous medium at ambient temperature and neutral pH under atmospheric pressure and extreme conditions are

also not required thus energy is saved. Moreover, the by-products are also not formed and the reaction is proceeding at an extremely high rate, maybe up to the order of 10^{20} [3]. It has proven to be a useful technology for the chemical industry for the reactions not easily feasible in organic chemistry. These days, highly stereo-, regio- and chemoselective biotransformations result in economically attractive, environmentally acceptable and simplified manufacturing processes. Enzymes have been efficiently used for resolution of racemic mixtures, although might not have competed well with chemical methods in synthesis. Phenylethylamine and ibuprofen are commonly cited compounds prepared using enzyme-based chiral processes. The use of pure enzymes requires additional steps that may further complicate the situation, therefore; there is an emerging trend for the use of microbes as a whole for the synthesis of enantiomerically pure compounds. Though, the story of microbial biotransformation has been associated with mankind since long and dates back to around 2000 years BC for the production of vinegar. It is the oldest, best-known examples of oxidations carried out by microbes. Pasteur in 1958 was the first to demonstrate the microbial resolution of tartaric acid. Ammonium salt of tartaric acid was resolved with the

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help of mold *Penicillium glaucum*. Pasteur's investigation for the conversion of alcohol into vinegar, also confirmed by Brown was also reported back in the nineteenth century, where the causative organism was named *Bacterium xylinum*.

2. Biocatalyst

Biocatalysts differ from chemical catalysts in many positive ways, viz. selectivity (strict recognition of the substrate with very high enantio-, regio- and chemoselectivities); safety (since reaction conditions are mild, the solvent is water, while ethanol and glucose, etc. are used as hydrogen sources instead of explosive hydrogen gas); natural and environmentally usable (biocatalysts include: microorganisms, plants, animals or their isolated enzymes so can be easily decomposed in the environment after use). Commercially available biocatalysts are easily available or can be cultured easily, including baker's yeast and the alcohol dehydrogenases from baker's yeast, *Thermoanaerobium brockii* (TBADH), horse liver and the hydroxysteroid dehydrogenase from *Pseudomonas testosterone* and *Bacillus sphaericus*.

Biocatalysts can perform a reduction in non-aqueous solvents like ionic liquids, organic solvents

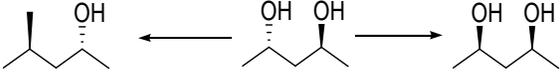
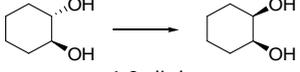
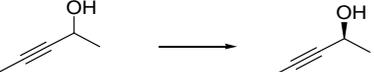
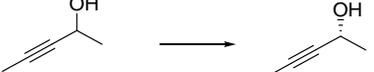
like benzene, cyclodextrin and hydrophobic polymers as well. It can be used to control the enantioselectivities as well as to improve the system's environmental friendliness. For example, the alcohol dehydrogenase from *G. candidum* was used in supercritical CO₂ around 100 atm and 35°C and found to catalyze the reduction of fluoroacetophenones. In some cases, there could be competing enzymes with different enantioselectivities, in such condition, an inhibitor of the unnecessary enzyme(s) like allyl bromide, ethyl chloroacetate, allyl alcohol, methyl vinyl ketone, sulfur compounds, Mg²⁺ and Ca²⁺ etc. could be used.

3. Enantioselective Reductions

Enantiomerically pure secondary alcohols are important intermediates for the synthesis of pharmaceuticals, agrochemicals, pheromones, flavors and fragrances, liquid crystals and chiral auxiliaries in asymmetric synthesis [4]. Enzymatic reductions also go via prologs rule [5], but there are incidences where anti pre logs product is formed [6]. The present study is a compendium of work carried out by combining sequential oxidation reductions by the use of microbes to access a range of secondary alcohols from racemates [Table 1].

Table 1.

Microorganism / Cultures	Reactions catalysed	References
<i>Bacillus stearothermophilus</i> , <i>Yarrowia lipolytica</i>		[7] & [8]
<i>Alcaligenes bronchisepticus</i> , <i>Streptococcus faecalis</i>		[9]
<i>Pseudomonas polycolor</i> , <i>Micrococcus freudenreichii</i>		[10]
<i>Byssochlamys fulva</i>		[11]
<i>Rhodococcus erythropolis</i>		[12]
<i>N. asteroides</i> , <i>Candida parapsilosis</i>		[13]
<i>Catharanthus roseus</i>		[14]
<i>Candida parapsilosis</i>		[15]

<i>Candida boidinii</i>		[16]
<i>Corynespora cassiicola</i>		[17] & [18]
<i>Candida and Pichia</i> spps.	1,2-diols	
<i>Corynespora cassiicola</i>		[13]
<i>Geotrichum candidum</i>		[19]
	R=Aryl gp	
Various bacteria, fungi, and mammalian cultures	Diterpenoids, triterpenoids, steroids and synthetic substrates	[20]
<i>Nicotiana tabacum</i> and <i>Catharanthus roseus</i>	Camphorquinones → α -ketoalcohols	[21]
<i>Nocardia pseudosporangifera</i>		[22]
<i>Nocardia fusca</i>		
<i>Rhizopus arrhizus</i>	Diarylketone → Diarylmethanol	[23]

4. Conclusion

The production of single enantiomer of various intermediates is increasingly important in the pharmaceutical and other industries. Organic synthesis is one approach for the synthesis of single enantiomer, but the process may be more complicated. Use of biocatalysts provides another dimension for the synthesis of chiral compounds. The resulting advantages of two catalysts over chemical catalysis are stereo- and regioselectivity. Moreover, it is also an aspect of green chemistry, another emerging trend in synthetic chemistry to achieve environmentally friendly processes. Different classes of enzymes provide stereospecificity to different types of reactions, may it be the resolution of racemic compounds or in the asymmetric synthesis of enantiomerically rich chiral compounds. Oxidoreductases have been used in the synthesis of chiral alcohols.

Recently, biological catalysts in the form of whole cells have also been shown to be extremely stereoselective in the synthesis of secondary alcohols. The reduction yield high regio- and stereoselectivity that is difficult to be achieved with chemical catalyst. The need is to identify the particular cultures that will be reaction specific.

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