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Baker's yeast mediated synthesis of bioactive chiral hydroxyamides

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ABSTRACT

The growing awareness of the importance of chirality in conjunction with biological activity has led to an increasing demand for efficient methods for the industrial synthesis of enantiomerically pure compounds. Hydroxyamides represent one such class which serves as useful chiral synthetic building blocks for other fine chemicals and pharmaceuticals and finds application in the production of antibiotics(1). Present work describes development of an economically viable method for the production of 3-hydroxy butanamide and 3-hydroxy N-phenyl butanamide by biocatalytic reduction of acetoacetamide and acetoacetanilide using microbial catalyst [Baker's Yeast (Saccharomyces cerevisiae)] in its free (FBY) as well as in immobilized (ImBY) form. Optically active products thus obtained were isolated, purified and characterized by combined application of chromatographic and spectroscopic techniques. The products thus obtained also exhibits significant antibacterial activity against four strains of bacteria i.e. Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa

Keywords: Baker's Yeast (Saccharomyces cerevisiae), hydroxyamides, microbial catalyst, chirality, spectroscopic techniques & antibacterial activity.

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INTRODUCTION

Enantioselective reduction of ketones to optically active secondary alcohols is one of the most interesting areas of research. Since the separation of alcohol racemate is not easy, catalytic enantioselective hydrogenation of the corresponding prochiral ketone is an attractive route for synthesis of pure alcohol enantiomers. Different catalytic systems have been reported for this purpose employing both chemo- and bio-catalysts. Noyori's chiral Ru-BINAP complex [2-4] and supported nickel catalyst modified with tartaric acid and sodium bromide [5-6] are some of the successful systems known. General drawbacks of these reactions are the requirement of often expensive chiral metal-complex catalysts; contamination of end product with catalysts, vigorous reaction conditions such as high pressure, flammable reaction media, or cryogenic conditions [7-8] and the range of possible products is limited. The necessity to synthesize precursor molecules may further complicate the synthetic procedure and may reduce the product yield [9-10]. Another major disadvantage could be lower enantiomeric excesses (ee) compared to biochemical processes [11]. Employing bio-catalysts for the asymmetric reduction is advantageous since reactions proceed at low temperature and in the absence of high hydrogen pressure [12]. Use of the whole cell such as baker's yeast (Saccharomyces Cerevisiae) for chiral reduction is more attractive from economical, environmental and handling points of view [13]. Thus biotransformations are eco- friendly, regio- and stereo selective processes.

Chiral hydroxyamides are useful intermediates and auxiliaries in the production of various fine chemicals [14]. Because of the fact that they contain a chiral center and two easily modifiable functional groups (–OH and $–CONH_2$), they are valuable synthons, i.e., they can serve as starting materials for the synthesis of certain drugs which are used in treatment of autoimmune disorders, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, cancer, respiratory diseases and fibrosis, including multiple sclerosis, arthritis, emphysema, chronic obstructive pulmonary disease, liver and pulmonary fibrosis.

Present study describes biocatalytic reduction of acetoacetamide and acetoacetanilide using free as well as immobilized whole cell biocatalyst, Baker’s Yeast and inhibitory effect of the products (3-hydroxy butanamide and 3-hydroxy N-phenyl butanamide) on the biological activities of some bacteria, since they are found to possess antibacterial activity.

MATERIALS AND METHODS

All the chemicals used were of AR (Analytical Reagent) grade and triply distilled water was used for the making of solution and Baker’s Yeast purchased was of food grade.

Experimental

1. **Reduction using Free Baker’s Yeast**

Biotransformation of acetoacetamide(A) and acetoacetanilide(B) was carried out as follows:

In a one liter round bottom flask, equipped with a magnetic stirrer (Remi-2MLH make) water (200 ml), fresh BY (10 g) and isopropanol (25ml) were placed and corresponding suspension was stirred for 30 minutes. The alcoholic solution of compounds (A/B) (2mM) was poured gradually into BY suspension. The resulting solution was magnetically stirred for suitable period (Table 1). The suspension changed its colour during the course of reaction. After completion of the reaction, the product was filtered using celite (HIMEDIA grade), the filtrate was saturated with sodium chloride and extracted with diethyl ether, and ether extracts were combined and dried over sodium sulphate. After evaporation, the product was isolated, purified and characterized by combined application of chromatographic techniques and spectroscopy.

2. Reduction using Immobilized Baker's Yeast

The experiment was performed under similar conditions with Immobilized Baker'sYeast, obtained insitu immobilization of Baker's Yeast (2g) in polyacrylamide gel. The details of immobilization are given below

Immobilization of BY in polyacrylamide gel

The gel was prepared using the following solutions.

Solution A: - Acrylamide (10 g) and N, N'-methylene bisacrylamide (2.5 g) in DDW (100 ml),
Solution B: - Tris (5.98 g), TEMED (0.46 ml) and 1N HCl (48 ml) solution to 100ml,
Solution C: -APS (560 mg) in DDW (100 ml), **Solution D**: - Isopropanol (25 ml)

Where- TRIS= Trihydrixy Methyl Amino Methane, TEMED= N, N, N', N"-tetramethyl Ethylenediamine, APS= Ammonium Persulphate, DDW= doubly distilled water.

Then Solutions were mixed in following way-
Sol. A (10 ml) + sol.B (5 ml) + BY (2g) + sol.C (5 ml)
And then solution D was added and then deaerated for 30min

3. Characterization of the Product

The purity of products was checked by single spot obtained by thin layer chromatography (TCL) and then characterization of products was done on the basis of IR, NMR& Mass spectral analysis. NMR spectra were recorded in $CDCl_3$ solution on Joel (Japan) 300 MHz spectrophotometer and IR spectra were recorded by using Nicolet (USA) FTIR Spectrophotometer. Samples were sent to CDRI for mass spectral analysis. Optical activity of products was measured by using a polarimeter and enantiomeric excess (ee) was calculated. These results are shown in Table-1

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4. **Antibacterial activity**

The synthesized compounds, 3-hydroxybutanamide and 3-hydroxy N-phenyl butanamide were then screened for their antibacterial constituents against four strains of bacteria i.e. Staphylococcus aureus (ATCC 29213), Bacillus cereus (ATCC 6633), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853) by Well diffusion method. Nutrient agar was used as culture medium. All compounds were dissolved in Dimethyl sulfoxide (DMSO). Ciprofloxacin (5mg) was used as reference antibiotic and Dimethyl formamide (DMF) as control. The zones of inhibition formed were measured in mm. Results are shown in Table-2.

RESULTS AND DISCUSSION

1. Reduction using Baker's Yeast in free and in immobilized form

Baker's Yeast (BY) is a common microorganism can be easily used in free as well as in immobilized form (ImBY) for synthesis of optically pure alcohols. When whole microbial cells, such as baker s yeast, are used as the catalyst for the asymmetric reduction of carbonyl compounds, two enzyme systems are mainly involved in the production reaction. One is the enzyme catalyzing the asymmetric reduction of prochiral carbonyl compounds to chiral alcohols, i.e. carbonyl reductases. The other is a cofactor regeneration system, which supplies NADH or NADPH through the oxidation of the energy source, such as carbohydrates and alcohols [22].

Mechanism of Reduction

The actual reducing agent in this system is NADH (Nicotinamide Adenine Dinucleotide Hydride) which donates hydride (H^-) to aldehydes and ketones and thereby reduces them. The electron lone pair on nitrogen atom of NADH pushes out the hydride ion, which is subsequently added to a carbonyl group of another molecule to cause its reduction. The process in completed by addition of proton to the carbonyl oxygen. The scheme of the reduction is depicted in the Fig. 1.

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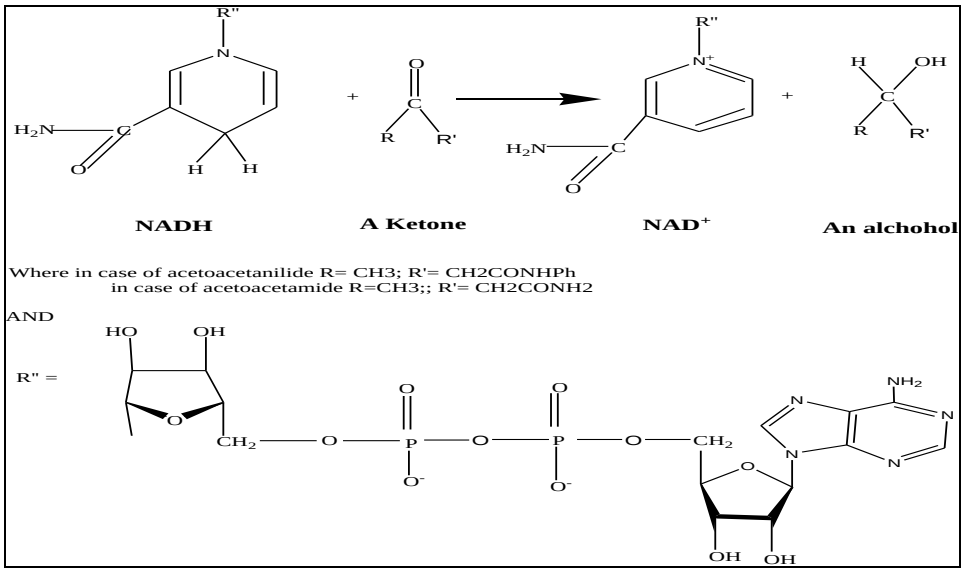

Figure 1: Depicting biological pathway for reduction of carbonyl group by NADH

Role of Isopropanol

The reducing agent, NADH (Nicotinamide Adenine Dinucleotide phosphate hydride) is present in limited amount. After reducing the substrate it is itself oxidised to NAD^+. Therefore, to continue reduction process it is necessary to reduce NAD^+ (Nicotinamide Adenine Dinucleotide Phosphate ion) into NADH. Yeast contains some saccharides in the cell, which reduce NAD^+ to NADH via pentose- phosphate pathway. To activate this pathway isopropanol is added to the reaction mixture, which is oxidized to acetone and regenerates NADH from NAD^+. Thus it results in an increased concentration of NADH which ultimately ensures an increase in the enantiomeric excess of the product.

Immobilization

It enhances the operational stability of FBY and isolation of the products becomes easier. In addition, reuse of the catalyst is often possible under these conditions and the product formation rates are usually high, not only because of the inhibitory influences but also high cell population. It is also permits easy and continuous operation since immobilized cells can be easily removed from the reaction medium and can be repeatedly reused.

The classical methods generally involve use of either corrosive reagent or yield product which is burden to the ecosystem. The use of baker's yeast however offers an alternative to carry out reduction at room temperature using simple installation with an easy work-up. The process is essentially green and the yields are very good.

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2. Antibacterial Activity

3-hydroxybutanamide and 3-hydroxy N-phenyl butanamide synthesized via baker yeast mediated reduction were examined against four bacterial species i.e. Staphylococcus aureus (ATCC 29213), Bacillus cereus (ATCC 6633), Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853). The results so obtained (as given in Table-2) suggest that 3-hydroxybutanamide is highly active against Staphylococcus aureus , Bacillus cereus and Escherichia coli with inhibition zones as 49 mm, 45 mm and 42 mm respectively and moderately active against Pseudomonas aeruginosa with inhibition zone as 30 mm. While Ethyl 4-hydroxyvalerate showed high activity against all bacterial species under test conditions with inhibition zones as 52mm, 48mm, 44mm and 40 mm. Thus the results clearly demonstrate that both compounds have good bactericidal activity.

Table-1 Spectroscopic data for microbial reduction of compounds

S.No.	Substrate Name	Reaction Medium	Reaction Time (In Hours)	IR Data (cm^{-1})	NMR Data (δ- Value)	ee (%) Free BY	ee (%) IMBY	Compound Confirmed
1	Aceto-acetamide	Glycerol:water	72 hrs	3300-3450 (O-H N-H str) 2980 (C-H str) 1670 (C=O str) 1470 (C-H def)	6.0(NH2) 3.62(CH) 2.33(CH2) 2.0(OH) 1.21(CH3)	82%	89%	3-hydroxy butanamide
2	Aceto-actanilide	Alchohol:water	72 hrs	3300-3450 (O-H N-H str) 3060 (C-H str) 2980 (C-H str) 1670 (C=O str) 1450-1600 (C-H def)	8.0(NH) 7.64(CH) 7.24(CH) 7.0(CH) 3.62(CH) 2.38(CH2) 2.0(OH) 1.21(CH3)	76%	82%	3-hydroxy N-phenyl butanamide

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Table-2 Effect of 3-hydroxy butanamide and 3-hydroxy N-phenyl butanamide on the growth of tested Bacteria

Sample / Bacteria	Ciprofloxacin (Reference)	3-hydroxy butanamide	3-hydroxy N-phenyl butanamide
1. Staphylococcus aureus (Gram positive)	+ ++ (38 mm)	+ + + (49mm)	+ + + (52mm)
2. Bacillus cereus (Gram positive)	+ + + (33mm)	+ + + (45mm)	+ + + (48mm)
3. Pseudomonas aeruginosa (Gram negative)	+ + + (35mm)	+ + (30mm)	+ + + (40mm)
4. Escherichia coli (Gram negative)	+ + + (35mm)	+ ++ (42mm)	+ + + (44mm)

Moderately active = + + (inhibition zone< 30 mm)
Highly active = + + + (inhibition zone> 30mm)

CONCLUSION

The present study is a novel alternative synthetic route involving microbial assisted biotransformation of prochiral substrates into useful chiral products and has merits like specificity & cost effectiveness. It is expected to reduce the ever-increasing problem of pollution caused by hazardous, corrosive chemicals and harsh reaction conditions.

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