

## Sulphoxidation of a drug intermediate using microorganisms

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### ABSTRACT

The sulphoxide group is present in various pharmacologically active agents. Recently, there has been an increased interest in the development of methodologies for the preparation of chiral sulphoxide. One of the approaches for the preparation of chiral sulphoxide is to exploit the enzymatic capacity of the microbial cells in bringing about stereospecific sulphoxidation of the achiral sulphide. In the current work, an attempt has been made to screen certain selected microorganisms for sulphoxidation of omeprazole intermediate. Bio-oxidation was performed in phosphate buffer of pH 7.6 with the resting cells of various fungi. The sulphide intermediate (concentration 1g/L) was incubated with the resting cells for 48 h at 30 °C, 160 rpm in a rotary shaker. The work up consisted simply of filtration to remove the spent mycelium, extraction of the filtrate with alkaline dichloromethane and further concentration by evaporation to get the oxidized product. Ten different species of fungi were tested for sulphoxidation, of which only two species, *Aspergillus niger* and *Rhizopus stolonifer*, showed conversion. The product was identified by TLC and LCMS, quantification was done by HPLC.

**Key words:** Sulphoxidation, Omeprazole, *Aspergillus niger*, *Rhizopus stolonifer*.

### INTRODUCTION

A number of sulphur compounds are known in which the sulphur is bonded to three substituents and also retain a lone pair of electrons. These types of compounds can in suitable cases exhibit chirality. Sulphoxides also have a structure with three different substituents and a lone pair of electrons around a sulphur atom and so can exhibit chirality<sup>1</sup>.

Sulphoxides have been obtained in optically active forms by resolution of the racemates. However, optically active sulphoxide have also been prepared by asymmetric synthesis, which are carried out by oxidation of unsymmetrical substituted sulphide. Sulphoxidation in chiral environments is either limited to specific substituents

or gives only moderate to low enantiomeric excess of product and use of chiral oxidants can be similarly unrewarding<sup>2</sup>.

The asymmetric oxidation of a prochiral sulphide is undoubtedly a more direct and commercial method for the synthesis of sulphoxide than the kinetic resolution of a racemic sulphoxide. The transformations with metal catalyst or isolated enzymes are tedious and expensive. Asymmetric sulfoxidation by whole cells are much cheaper and more convenient, as it does not require expensive co-factors<sup>3</sup>.

One of the earliest methods of preparing chiral sulphoxides, microbial biotransformation, has not been extensively adopted for synthetic purposes. Despite the proven success of this

method in performing stereospecific asymmetric oxidation of certain prochiral sulphides and the associated developments in the application of isolated oxidase enzyme methodology, further work is still necessary to identify new substrates and specific microorganisms which will prove useful for the production of chiral sulphoxides.

The use of enzymes as reagents in organic synthesis provides exciting opportunities for the exercise of regio and stereoselectivity. In case of oxidative reactions catalyzed by oxygenase enzymes, whole microbial cells in an actively growing or resting stage are commonly employed<sup>4,5,6,7</sup>. In general, organic sulfides, along with other xenobiotic organic compounds are oxidized both *in vivo* and *in vitro* by either one or both of two types of mono oxygenases enzymes, those dependent upon cytochrome P-450 for activation and transfer and other using a flavin molecule for the purpose<sup>2</sup>. It has generally been assumed that the fungal enzymes responsible for oxidation of sulphide to sulphoxide are cytochrome P-450 containing monooxygenases.

some selected fungi were screened for their capacity to carry out sulphoxidation of 5-Methoxy-2-[[[4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]-sulphinyl]-1 H benzimidazole to omeprazole.

The sulphide selected for screening is an intermediate in the synthesis of omeprazole, a potent proton pump inhibitor. It acts as an inhibitor of gastric H<sup>+</sup>, K<sup>+</sup> ATPase, the gastric acid pump. This being sulphoxide, has an asymmetric center in the sulphur atom and exists in two optical isomers. The S isomer is the desirable sulphoxide for the observed clinical activity.

Fungi selected for screening are *Aspergillus niger* MTCC 961, *Pichia farinosa* MTCC 246, *Saccharomyces cerevisiae* MTCC 174, *Candida viswanathii* MTCC 1629, *Rhizopus stolonifer* MTCC 2198, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus oryzae*, *pencillium* species, (All isolated from soil) and bakers yeast.

## MATERIAL AND METHODS

### Microorganisms

#### *Saccharomyces cerevisiae*

MTCC 174 was obtained from MTCC, Chandigarh. The organism was maintained on YEPD media containing, yeast extract 3.0 g, peptone 10.0 g, dextrose 20.0 g, agar 20.0 g and distilled water 1000 ml.

#### *Pichia farinosa*

MTCC 246 was obtained from MTCC, Chandigarh. The organism was maintained on MYA media containing malt extract 3.0 g, yeast extract 3.0 g, peptone 5.0 g, dextrose 10.0 g, agar 20.0 g and distilled water 1000 ml.

#### *Aspergillus niger* (soil isolate) and *Aspergillus niger* MTCC 961

The organisms were maintained on MRBA media containing, dextrose 10.0 g, peptone 5.0 g, potassium dihydrogen phosphate 1.0 g, Magnesium sulphate 0.5 g, Rose Bengal 0.0035 g, Agar 20.0 g, distilled water 1000 ml and streptomycin 0.03 g.

#### *Candida viswanathii* MTCC 1629

The organism was obtained from MTCC, Chandigarh and maintained on YEPD medium.

#### *Pencillium species* (soil isolate)

The organism was maintained on MRBA medium.

#### *Rhizopus stolonifer* MTCC 2198

The organism was maintained on MRBA Medium

### Chemicals

All chemicals and solvents were from local suppliers and of analytical grade. Omeprazole standard was obtained from the industry.

#### Cultivation of *Saccharomyces cerevisiae*, *Pichia farinosa* and *Candida viswanathii*

The organisms from the slant culture was subcultured into 300ml YEPD medium containing Yeast extract 0.3 g, peptone 1.0 g, dextrose 2.0 g and distilled water 100 ml, pH was adjusted to 7.0 and was sterilized at 121°C for 15 min. The cultures

were grown at 30°C, 160 rpm for 24 h. 10% volume of the fermentation medium was used for inoculation of 2.5 L of YEPD medium. The inoculated medium was incubated at 30°C, 160 rpm for 48 h. After 48 h of growth, the cells were separated by filtration using buchner funnel and the biomass was washed with phosphate buffer twice.

#### Cultivation of *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium*

The spores from the maintenance culture were inoculated to, 2 L of potato dextrose medium containing potato 400.0 g, dextrose 20.0 g and distilled water 2000ml.

The pH of the medium was adjusted to 6.0. The medium was sterilized at 121 °C for 15 min. The inoculated medium was incubated at 25°C for 5 days to get sufficient biomass. The mycelial biomass was separated by filtration and washed with phosphate buffer twice.

#### Procedure for Bio-oxidation

10 g of the biomass was taken in 20 ml phosphate buffer of pH 7.6, 20 mg of omeprazole intermediate dissolved in 2 ml alcohol was added to the above suspension and incubated at 30°C, 160 rpm for 48 h.

The reaction mixture was filtered to remove the biomass. Filtrate was extracted with alkaline methylene dichloride (20 ml x 3), washed with 20 ml of brine twice and dried over anhydrous sodium sulphate. The organic extract was then concentrated by evaporation.

#### HPLC Analysis

The oxidized product of omeprazole was quantified by HPLC.

#### Chromatographic condition

Mobile phase : phosphate buffer (pH 7.6) : methanol ( 25:75)

Column: C18 phenomenex  
250 × 4.6 mm, .5 µm,  
Flow rate: 0.8 ml/min.  
Wave length: 280 nm  
Injection volume: 20 µl.

#### Standard Preparation

25 mg of the Standard was dissolved in 25 ml of methanol and diluted to get concentration of 2.5 to 10.0 µg.

#### Sample preparation

The concentrated sample was made up to 10 ml with methanol.

## RESULTS AND DISCUSSION

Totally ten organisms were selected for carrying out sulphoxidation of omeprazole intermediate (Table – 1), out of which one species of *Aspergillus niger* showed negligible conversion while *Rhizopus stolonifer* was found to be effective in bringing about sulphoxidation of the selected substrate. Even though *Aspergillus* species are known to have good sulphoxidation activity, the above selected species were inactive in general on the omeprazole intermediate.

The reaction was monitored by TLC (Benzene: ethyl acetate: methanol:: 5:3:1), and the product was confirmed by HPLC and LCMS. The standard for the analysis was obtained from industry.

Maintenance of alkalinity during reaction and extraction process is critical in sulphoxidation of omeprazole intermediate as omeprazole is unstable in acidic or neutral medium.

Table 1

Microorganisms	Yield in mcg
<i>Aspergillus niger</i> MTCC 961	00.00
<i>Aspergillus niger</i> (soil isolate)	13.06
<i>Aspergillus flavus</i>	00.00
<i>Aspergillus oryzae</i>	00.00
<i>penicillium</i> species	00.00
<i>Pichia farinosa</i> MTCC 246	00.00
<i>Candida viswanathii</i> MTCC 1629	00.00
<i>Saccharomyces cerevisiae</i> : MTCC 174	00.00
Bakers yeast	00.00
<i>Rhizopus stolonifer</i> MTCC 2198	138.02

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