Microbial transformation of albendazole

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Screening scale studies were performed to biotransform anthelmintic drug albendazole by using twelve bacterial strains representing six genera and five actinomycetes cultures. Among the cultures studied, Bacillus subtilis MTCC 619, Escherichia coli MTCC 118 and Klebsiella pneumoniae MTCC 109 could transform albendazole to one metabolite whereas, Enterobacter aerogenes NCIM 2695, Klebsiella aerogenes NCIM 2258, Pseudomonas aeruginosa NCIM 2074 and Streptomyces griseus NCIM 2622 could transform albendazole into two metabolites in significant quantities. The transformation of albendazole was identified by HPLC. Based on LC-MS-MS data, the two metabolites were predicted to be albendazole sulfoxide (M1) and albendazole sulfone (M2), the major mammalian metabolites reported previously. Since M1 is active metabolite, the results prove the versatility of microorganisms to perform industrially attractive chemical reactions.

Keywords: Albendazole, Albendazole sulfone, Albendazole sulfoxide, Biotransformation

Parasitic diseases account for millions of deaths each year and impose a significant burden on third world countries by reducing the productive potential of its people and animals1, 2. Half of humanity is exposed to the risk of parasitic infections, with the most significant being ascaris, malaria, schistosoma, filaria, hookworm, tricharis, amoeba, trypanosoma, leishmania, and strongyloides1, 2. The three groups of helminthes that infectious parasitise are nematodes (roundworms), trematodes (flukes) and cestodes (tapeworms). Most common are ascaris, tricharis, hookworm, filariosis, onchocerca and schistosoma. However, the control of parasitic diseases is still a matter of concern, largely due to the increased number of individuals who are immunocompromised1.

Albendazole (ABZ) is a benzimidazole carbamate with a broad anti-parasitic spectrum3. In general, most ascariasis, trichuriasis, enterobiasis and hookworm infections can be successfully treated with a single dose of ABZ, and strongyloidiasis with multiple doses. Absorption of ABZ from the gastrointestinal tract is poor and hence orally administered ABZ is of most value against intestinal nematodes. Once absorbed, the drug is rapidly metabolized in the liver before reaching the systemic circulation. The primary metabolite, albendazole sulfoxide has anthelmintic activity, and ABZ’s efficacy is attributed to this metabolite4-6. Poor and erotic absorption of albendazole from the gastrointestinal tract results in reduced systemic availability and efficacy. In an attempt to over come this problem, an injectable formulation of albendazole sulfoxide has been developed, exploiting its slightly greater solubility in water than that of other equally potent benzimidazole methyl carbamates. After subcutaneous injection, albendazole sulfoxide is widely distributed from blood to other tissues, especially the gastrointestinal tract7, so that plasma concentrations closely reflect those in fluids and tissues where the target parasite may be located8.

Biotransformation is the process by which an organism or its isolated enzyme brings out chemical change on molecules that are not part of their metabolism. Biotransformation may often result in the formation of novel or useful products that are difficult or impossible to obtain by conventional chemical methods. This process offers the advantages of high selectivity and specificity, mild operating conditions and also minimizes the problems of isomerization, racemization, epimerization and rearrangement that are common in chemical processes. Microbial transformation is one of the most attractive approaches for introducing functional groups into various positions of organic compounds and to generate structural diversity in a chemical library9,10.
Microbial transformation is one of the simplest and most direct methods for the preparation of a range of optically active sulfoxides of moderate to high enantiomeric purity. The present paper deals with the microbial transformation of albendazole with an aim to produce albendazole sulfoxide which possess increased solubility and efficacy than that of the parent compound albendazole.

Materials and Methods

Chemicals—Albendazole was gifted by Glaxo SmithKline, Mumbai, India. Methanol and acetonitrile were of HPLC grade obtained from Ranbaxy, New Delhi, India. Peptone, Beef extract, NaCl and all other chemicals of highest available purity were obtained from Himedia, Mumbai, India.

Microorganisms—The bacterial cultures were procured from National Collection of Industrial Microorganisms (NCIM), Pune, India and Microbial Type Culture Collection (MTCC) & Gene Bank, Chandigarh, India. The procured cultures include Bacillus cereus NCIM 2155, Bacillus subtilis MTCC 619, Bacillus subtilis MTCC 441, Bacillus subtilis NCIM 2162, Enterobacter aerogenes NCIM 2695, Escherichia coli MTCC 118, Klebsiella aerogenes NCIM 2258, K. pneumoniae MTCC 109, Proteus mirabilis NCIM 2241, P. vulgaris NCIM 2813, Pseudomonas aeruginosa NCIM 2074 and P. putida NCIM 2782, Streptomyces griseus NCIM 2622, S. griseus NCIM 2623, S. lavendulace NCIM 2827, S. rimosus NCIM 2213 and Streptomyces sps. NCIM 2214. Bacterial cultures were maintained on nutrient agar slants and actinomycetes were maintained on potato dextrose agar slants at 4°C and sub cultured for every 3 months.

Biotransformation—Biotransformation was performed using a two-stage fermentation protocol. In the first stage, fermentation was initiated by inoculating a 250 ml culture flask consisting of 50 ml of liquid broth. The liquid broth used for bacteria contains (per litre) peptone (5.0 g), beef extract (3.0 g) and sodium chloride (5.0 g) and for actinomycetes (per litre) glucose (20 g), peptone (5 g), yeast extract (5 g), K2HPO4 (5 g) and sodium chloride (5 g). The pH of both the media was adjusted to 6.0 with 0.1 M HCl. The prepared media was autoclaved and cooled to room temperature. The media was inoculated with a loopful of culture obtained from freshly grown agar slants. The flasks were incubated at 120 rev/min and 37°C for 24 h for bacteria and at120 rev/min and 27°C for 72 h for actinomycetes. Second stage cultures were grown in the same media using an inoculum of 1 ml of first stage culture per 20 ml of medium in a 100 ml culture flask. The second stage cultures were incubated for 24 h and the substrate albendazole in dimethyl formamide was added to give a final concentration of 20 mg/l. The flasks were incubated under similar conditions for 2 days in case of bacteria and for 7 days in case of actinomycetes. Culture controls consisted of a fermentation blank in which the microorganism was grown under identical conditions and no substrate was added. Substrate controls comprised of albendazole added to the sterile medium were incubated under similar conditions. Each culture was studied in triplicate.

Metabolites extraction and analysis—At the end of incubation period the culture medium and mycelium were separated by filtration. The separated broth was extracted with three volumes of ethyl acetate, the combined organic extracts were evaporated using a rotary vacuum evaporator and dried over a bed of sodium sulfate. The resultant residues were analyzed by HPLC and LC-MS-MS for identification of metabolites.

Analysis—HPLC analysis was performed according to the method described by Garcia et al., with a slight modification. The samples were analysed using an LC-10AT system (Shimadzu, Japan) by injecting 20 µl of sample into the syringe-loading sample injector (Model 7725i, Rheodyne, USA). The column used was Wakoil II, C18, 250 X 4.6 mm and 5 µm (SGE, Australia). The mobile phase consisted of a mixture of acetonitrile-water (pH adjusted to 3.0 with orthophosphoric acid) in the ratio of 15:85. The analysis was performed isocratically at a flow rate of 1 ml/min and the analytes were detected at 290 nm using a photodiode array detector (Model SPD M10Avp, Shimadzu, Japan). The metabolite peaks were identified based on the similarity in the UV spectra.

LC-MS-MS analysis was carried out using a Waters system, column XTerra C18, 25 X 0.46 cm, 5 µm and a mobile phase consisting of acetonitrile and water (pH adjusted to 3.0 with formic acid) in 15:85 ratio. The ESI detection was set to positive mode. A temperature of 300°C and a scan range of 50-500 were set for the analysis. The transformed compounds were identified from the masses of the fragmentation products obtained.
Results and Discussion

To investigate the course of biotransformation of albendazole, twelve bacterial strains representing six genera and five strains of Streptomyces were screened. Among the three strains of Bacillus subtilis studied, only one strain i.e., B. subtilis MTCC 619 could transform albendazole to albendazole sulfoxide (M1), the active metabolite of albendazole. Whereas Bacillus cereus NCIM 2155 failed to transform albendazole. Albendazole sulfoxide (M1) was also detected in culture broth of Escherichia coli MTCC 118 and Klebsiella pneumoniae MTCC 109 (Table 1). Enterobacter aerogenes NCIM 2695, Klebsiella aerogenes NCIM 2258, Pseudomonas aeruginosa NCIM 2074 and Streptomyces griseus NCIM 2622 could transform albendazole to albendazole sulfoxide (M1) and albendazole sulfone (M2). Proteus mirabilis NCIM 2241 failed to transform albendazole. Interestingly, Pseudomonas putida NCIM 2782 could produce albendazole sulfone (M2) only.

Present studies on the transformation of albendazole revealed that Bacillus subtilis MTCC 619, Escherichia coli MTCC 118 and Klebsiella pneumoniae MTCC 109 could transform albendazole to only one metabolite, albendazole sulfoxide (M1). Albendazole sulfone (M2) formation was not observed in these cultures. This may be due to insufficient quantity of albendazole sulfoxide (M1) produced to induce the enzyme(s) responsible for production of albendazole sulfone (M2). Similar type of sulfoxidation reactions were reported by Holland et al., Adam et al., Gonzalo et al., and Feingersch et al.. Whereas, Enterobacter aerogenes NCIM 2695, Klebsiella aerogenes NCIM 2258 and Pseudomonas aeruginosa NCIM 2074 could transform albendazole to two metabolites albendazole sulfoxide (M1) and albendazole sulfone (M2). CYP450 and the flavin monooxygenase system have been suggested to be responsible for the sequential sulfoxidation of ABZ in mammals. Pseudomonas putida NCIM 2782 could transform albendazole to albendazole sulfone, the inactive metabolite of albendazole. This may be either due to the involvement of CYP450, the main determinant of sulfonation reaction or due to the existence of dioxygenase enzyme in this bacterium. White et al., reported that cytochrome p-450 monoxygenase from Pseudomonas putida selectively oxidized adamantane to 1-adamantanol. Proteus vulgaris NCIM 2813 and Proteus mirabilis NCIM 2241 in the present study could tolerate albendazole without performing any transformation.

Two metabolites albendazole sulfoxide (M1) and albendazole sulfone (M2) were also detected in culture broth of Streptomyces griseus NCIM 2622 (Table 2). These metabolites could not be detected with S. griseus NCIM 2623, S. lavendulae NCIM 2827, S. rimosus NCIM 2213 and Streptomyces spp NCIM 2214. These metabolites could not be detected in culture control and substrate control. From the above result, it was demonstrated that S. griseus could transform albendazole to albendazole sulfoxide (M1) and albendazole sulfone (M2), while rest of the actinomycetes cultures could not transform albendazole.

In the present investigation, S. griseus could transform 28% of albendazole to albendazole sulfoxide (M1) and albendazole sulfone (M2) which was much higher transformation as compared to all other cultures studied. S. griseus has been reported to catalyze a wide array of oxidative biotransformation reactions with simple aromatic compounds, alkaloids and terpene substrates.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Metabolites formed (%)</th>
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<tbody>
<tr>
<td></td>
<td>Albendazole sulfone (M2)</td>
</tr>
<tr>
<td>Bacillus cereus NCIM 2155</td>
<td>0.00</td>
</tr>
<tr>
<td>Bacillus subtilis MTCC 619</td>
<td>0.00</td>
</tr>
<tr>
<td>Bacillus subtilis MTCC 441</td>
<td>0.00</td>
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<tr>
<td>Bacillus subtilis NCIM 2162</td>
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<tr>
<td>Enterobacter aerogenes NCIM 2695</td>
<td>2.40</td>
</tr>
<tr>
<td>Escherichia coli MTCC 118</td>
<td>0.00</td>
</tr>
<tr>
<td>Klebsiella aerogenes NCIM 2258</td>
<td>2.37</td>
</tr>
<tr>
<td>Klebsiella pneumoniae MTCC 109</td>
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</tr>
<tr>
<td>Proteus mirabilis NCIM 2241</td>
<td>0.00</td>
</tr>
<tr>
<td>Proteus vulgaris NCIM 2813</td>
<td>0.00</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa NCIM 2074</td>
<td>8.81</td>
</tr>
<tr>
<td>Pseudomonas putida NCIM 2782</td>
<td>7.10</td>
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The metabolites formed were identified basing on observation of new peaks in HPLC (Fig. 1) and characterized with the help of the mass values of fragmentation ions obtained in LC-MS-MS analysis. Mass spectrometric analysis of the metabolite M1 showed a molecular ion at m/z 281 (an increase of 16 units) indicating addition of single oxygen atom to albendazole which results in formation of albendazole sulfoxide (M1). Another molecular ion was found at m/z 298 (an increase of 32 units) indicating addition of two oxygen atoms which results in the formation of albendazole sulfone (M2; Fig. 2). Data obtained by MS-MS analysis of M1 showed fragment ions at m/z 240 (loss of 42 units i.e. propyle side chain attached to thiol group of M1) and 208 (further loss of 32 units i.e carbon and oxygen in carbamate side chain of M1). Another fragment was obtained at m/z 222 (loss of 59 units from M1).

HPLC and LCMS/MS analyses indicated that the metabolites were albendazole sulfoxide (M1) and albendazole sulfone (M2). In HPLC analysis, the metabolite M1 was eluted at 20.78 min and metabolite M2 at 11.56 min. whereas, the substrate albendazole was eluted at 27.81 min. The metabolites were quantified based on the peak areas obtained in HPLC analysis taking the drug and metabolites peak areas together as 100%. The pathway of the metabolite formation has been shown in Fig. 3.

It is interesting to note that the transformation was oxidative in nature. The metabolic studies of albendazole have been shown to follow similar pathways in various mammals. These metabolic conversions included oxidation at sulfur alkyl and aromatic hydroxylation, methylation at both nitrogen and sulfur and carbamate hydrolysis.[23] Albendazole sulfoxide and albendazole sulfone have been identified in plasma after oral administration in several species viz., rat, human, porcine, ovine, bovine, caprine and chicken[24-29].

In our search to find and develop an efficient microbial conversion of albendazole in an effort to obtain novel anthelmintic derivatives with increasing activity or differing pharmacological properties, Bacillus subtilis MTCC 619, Escherichia coli MTCC 118 and Klebsiella pneumoniae MTCC 109 could transform albendazole to only one metabolite albendazole sulfoxide (M1) whereas Enterobacter aerogenes NCIM 2695, Klebsiella aerogenes NCIM 2258 Pseudomonas aeruginosa NCIM 2074 and Streptomyces griseus NCIM 2622 could transform

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Table 2—Biotransformation of albendazole by actinomycetes

<table>
<thead>
<tr>
<th>Culture</th>
<th>Metabolites formed (%)</th>
<th>Albindazole sulfoxide (M1)</th>
<th>Albindazole sulfone (M2)</th>
<th>Albindazole remaining</th>
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<tr>
<td>Streptomyces griseus NCIM 2622</td>
<td>1.54</td>
<td>28.02</td>
<td>70.42</td>
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<tr>
<td>S. griseus NCIM 2623</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>S. lavendulae NCIM 2827</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>S. rimosus NCIM 2213</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>Streptomyces sps. NCIM 2214</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

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Fig.1—HPLC chromatograms showing (a) culture control and (b) transformed compounds obtained in Streptomyces griseus NCIM 262.
albendazole to albendazole sulfoxide (M1) and albendazole sulfone (M2). *P. putida* NCIM 2782 could transform albendazole to albendazole sulfone (M2), the known mammalian metabolite.

From the present study it is concluded that albendazole could be transformed to albendazole sulfoxide and albendazole sulfone in an ecofriendly way. However a detailed parametric study is needed to produce polar metabolite albendazole sulfoxide from albendazole in large quantities by inhibiting the enzymes responsible for production of albendazole sulfone. Some of cultures in the present study were found to be promising for producing albendazole sulfoxide, the active metabolite of albendazole.

**Acknowledgement**

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**References**


