

BIOTRANSFORMATIONS OF RIFAMYCINS: PROCESS POSSIBILITIES

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Abstract

Rifampicin, an important antibiotic, is manufactured by chemical conversion of rifamycin S which is obtained by the chemical modification of rifamycin B. Rifamycin B is a product of *Nocardia mediterranei* fermentations. The chemical conversion of rifamycin B to rifamycin S has many disadvantages: Strong acidic conditions are required, heavy foam formation accompanies transformation and the yields are low. This review highlights the developments in alternative, biochemical transformations using enzymes and cells; the main focus is on transformations carried out by rifamycin oxidase.

Key Words

Rifamycin antibiotics, biotransformation, rifamycin oxidase, *Monocillium* sp., *Humicola* sp., *Curvularia lunata*.

Introduction

Chemical or biochemical transformation of naturally produced antibiotics can potentially be used to alter some of the characteristics of the natural substances. Changes in antimicrobial activity, spectrum of action, oral absorption, toxicity and allergenic responses may be achieved. A commercially used example of such a transformation is the conversion of penicillins (predominantly benzyl penicillin) to

6-amino penicillanic acid, 6-APA, which is a precursor for other semi-synthetic penicillins. The enzyme penicillin acylase produced by *Escherichia coli*, other bacteria and fungi [1], is the basis of this conversion which involves selective hydrolysis of the amide bond in the penicillin side-chain.

Rifampicin, a powerful antibiotic against certain important diseases, is currently produced by a scheme utilising fermentation followed by chemical conversion: The relatively inactive precursor, rifamycin B, is produced by *Nocardia mediterranei* in submerged culture; it is converted to the active rifamycin S in a chemical process. The low pH requirements of the chemical conversion necessitate expensive acid-resistant equipment, the yields are low and excessive foam formation causes operational problems. As a result, rifampicin is one of the more expensive antibiotics. In view of the shortcomings of the chemical conversion step, alternative biotransformations of rifamycin B to S have been investigated [2, 3]. Here we review these biotransformation methods based in several forms of biocatalysts: immobilized enzymes, growing and resting whole cells, and pretreated cells.

Rifamycins

From the culture filtrate of the original strain of *N. mediterranei*, seven metabolites (rifamycins A-E, G and Y) were isolated [4, 5-7]. The structures of these natural rifamycins are shown in Figure 1 [8]. Of these, rifamycin B was chosen for development because of its stability, ease of purification and solubility at physiological pH's. Addition of barbiturates to the fermentation media was used to dramatically alter the product mix, favouring the formation of rifamycin B, and hence establishing a practical method for its production [9, 10]. Rifamycin B was demonstrated to be microbiologically inactive [11]; its apparent activity depended on its transformation into active products in test cultures or in body fluids. The transformation products (rifamycin O, SV and S) were isolated and found to be microbiologically very active. Due to its good *in-vivo* activity, tolerability and solubility properties, rifamycin SV was chosen for further studies. Rifamycin SV has been marketed in several countries for the treatment of infections from gram-

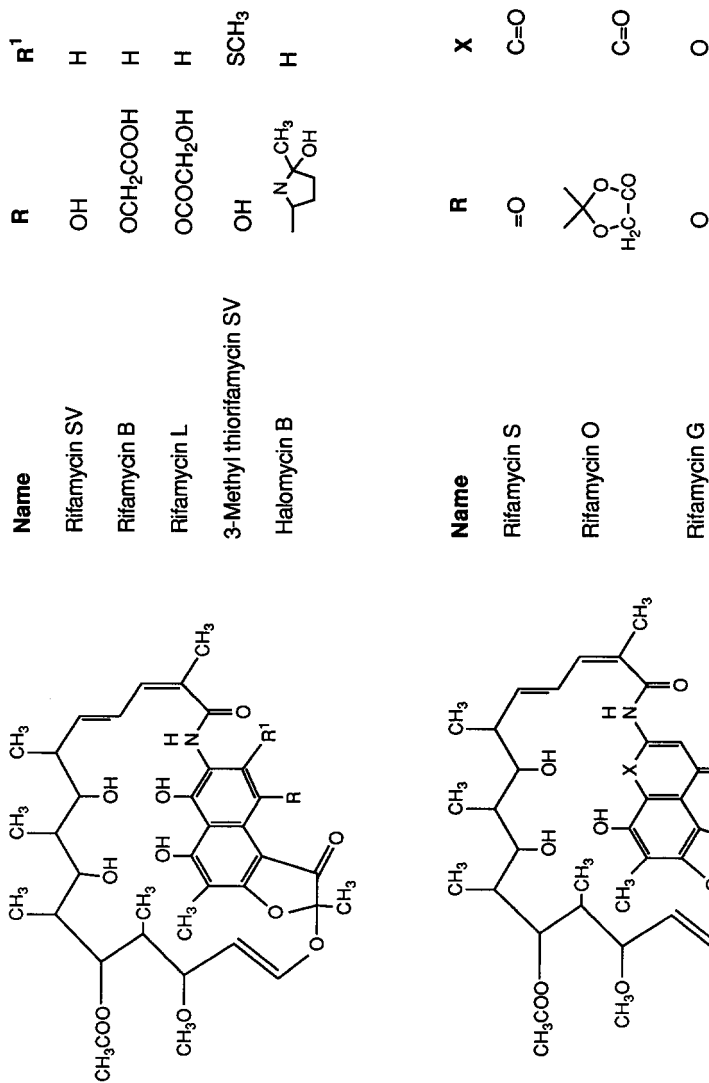


Figure 1. The structure of natural rifamycins.

positive bacteria, but it is poor in anti-tuberculous activity. Hundreds of compounds were synthesized from rifamycin B, O and S with the aim of obtaining a product superior to rifamycin SV in at least two respects: activity by oral administration and greater efficacy in curing tuberculosis. These goals were attained with rifampicin which is now widely used for the treatment of leprosy, tuberculosis and several other infectious diseases [12]. Rifampicin is at present also used in several other infections, notably in severe infections by *Staphylococci* resistant to β -lactam antibiotics and infections caused by intracellular bacteria.

Rifamycin SV, rifamide and rifampicin are the rifamycin group of antibiotics which are industrially produced and used as drugs. Their minimum inhibitory concentration (MIC) values against a range of organisms are given in Table 1 [11]. Rifampicin is also effective against trachoma and inhibits certain viruses and experimental tumours. Other semi-synthetic rifamycins are 27-demethyl-rifamycin SV and, 25-deacetyl and 27-demethyl rifamycin B. Rifamycin S is of considerable

Table 1. Minimum Inhibitory Concentrations of Rifamycin Group of Antibiotics

Infecting Microorganism	Minimum Inhibitory Concentration ($\mu\text{g/mL}$)		
	Rifamycin SV	Rifamide	Rifampicin
<i>Staphylococcus aureus</i> ATCC 6538	0.005	0.01	0.002
<i>Streptococcus faecalis</i> ATCC 10541	0.05	0.1	0.01
<i>Streptococcus pyogenes</i> C 203	0.002	0.01	0.02
<i>Diplococcus pneumoniae</i> XXLII	0.025	0.02	0.01
<i>Neisseria gonorrhoeae</i> ATCC 9826	-	0.005	0.02
<i>Haemophilus influenzae</i> ATCC 9334	-	-	0.02
<i>Escherichia coli</i> (McLeod)	50	10	1
<i>Klebsiella pneumoniae</i> ATCC 10031	25	20	5
<i>Proteus vulgaris</i> X 19	25	20	5
<i>Salmonella typhi</i>	100	20	5
<i>Pseudomonas aeruginosa</i>	50	50	10
<i>Mycobacterium tuberculosis</i> H ₃₇ Rv	0.05	0.2	0.5

Based on Sensi *et al.* [11].

economic importance because it is a precursor for the majority of semi-synthetic rifamycin antibiotics. Although, it is possible to produce the biologically active rifamycin S by fermentation from a blocked mutant, its yield is low compared to that of rifamycin B. Addition of sodium diethyl barbiturate to the fermentation medium results essentially in the formation of a single product, rifamycin B.

Biotransformation of Rifamycins

Biotransformation of rifamycin was first reported by Lancini *et al.* [13] who found that washed mycelia of *Nocardia mediterranei* could convert rifamycin B to rifamycin Y. It was further shown that rifamycin Y was not produced by "ex novo" synthesis, but originated from rifamycin B; this transformation did not require the addition of diethyl barbituric acid. This work provided strong evidence that rifamycin B was the natural precursor of rifamycin Y. In 1969 biotransformation of rifamycin S to rifamycin B and rifamycin L by washed cells of *N. mediterranei* was reported [14]. Acetylation (rifamycin S to rifamycin B) and esterification (rifamycin S to rifamycin L) reactions were involved. Based on these observations Lancini *et al.* [14] proposed that in *N. mediterranei* fermentations, rifamycin S or its reduced form (rifamycin SV) is the first microbiologically active product of the biosynthetic pathway, and is the precursor of rifamycins B and L. In the same year Lancini and Hengeller noted that *N. mediterranei* also deacetylates rifamycin B [15]. A little later, White *et al.* discovered that the washed mycelium from a rifamycin B producing strain of *N. mediterranei* transformed rifamycin W into rifamycin B [16]. This biotransformation proved that the mycelia of *N. mediterranei* are capable of carrying out the necessary modifications, *i.e.*, removal of methyl group and introduction of oxygen molecule, to transform the common progenitor into rifamycin S. Lancini *et al.* demonstrated that washed cells of *N. mediterranei* transformed the quinone ring of rifamycin S into pyrone ring of rifamycin G [7]. Later, Ghisalba *et al.* showed that a strain of *N. mediterranei* N813 (rifamycin B producer) partially transformed protorifamycin I to protorifamycin W [17]. The formation of a small amount of rifamycin B from protorifamycin I could also be detected [17].

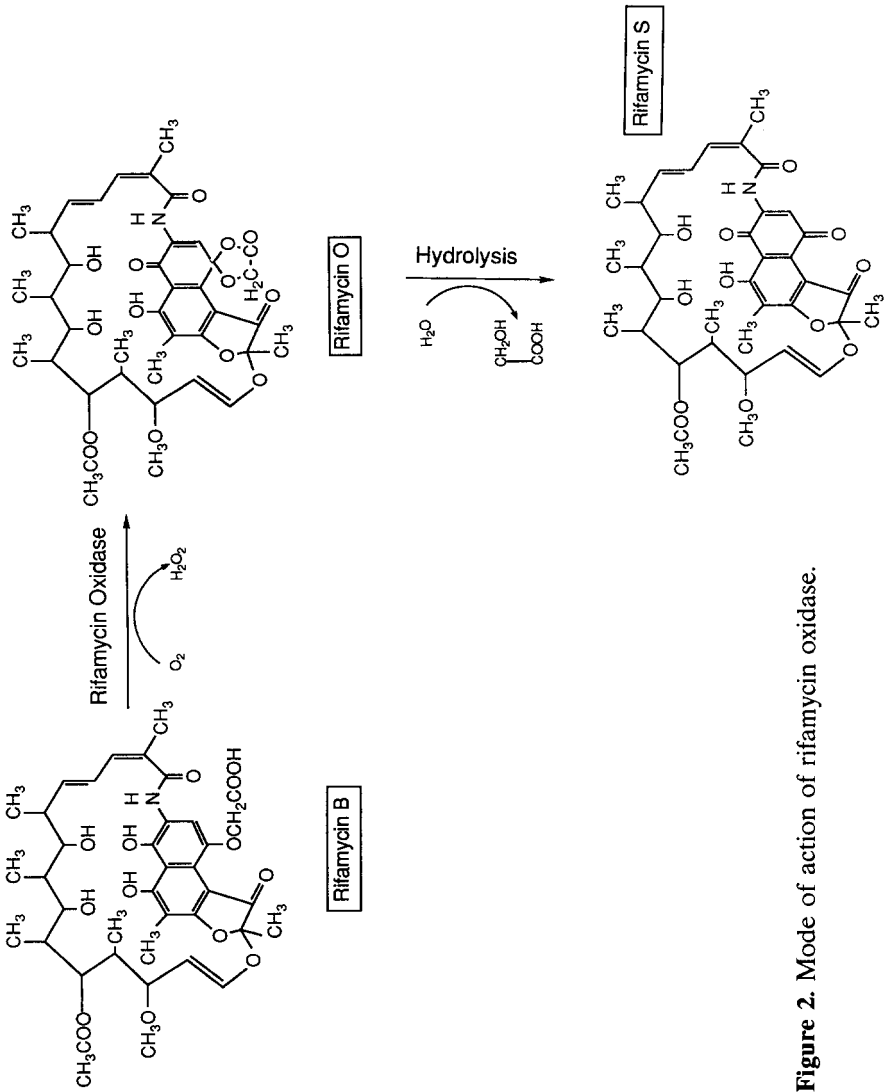


Figure 2. Mode of action of rifamycin oxidase.

More recently, Schupp *et al.* of Ciba-Geigy determined that the washed mycelia of a recombinant strain of *N. mediterranei* R-21 transformed 50% of the added rifamycin S to rifamycin B in 24 hours and 90% in 48 hours [18]. No other transformation of rifamycins was detectable under the conditions used. Ghislba *et al.* were able to coax permeabilized cells of *N. mediterranei* and *E. coli* into transforming rifamycin S to rifamycin SV [19]. This transformation was NADH dependent, but was not specific for the rifamycin biosynthetic pathway [19]. The reduction was probably due to the reducing conditions of the cells.

The above cited biotransformation studies were carried out to elucidate the biosynthetic pathways of rifamycins.

Bioprocesses for rifamycin S production

Possible advantages of biotransformation over the conventional chemical means prompted researchers to look for new microbial strains with rifamycin B transforming activity. In 1983 the first real biotransformation of inactive rifamycin B to the active S-form was reported by Han *et al.* [2]. They isolated two fungi imperfecti, *Humicola* sp. (ATCC 20620) and *Monocillium* sp. (ATCC 20621), which could catalyze the conversion of rifamycin B to rifamycin O and on to rifamycin S, which is the reverse reaction of rifamycin B biosynthesis. The enzyme from *Monocillium* sp. catalysed the oxidative reaction of rifamycin B to rifamycin O [2]. The identification of the reaction products suggested that the reaction proceeded by oxidative cyclization of rifamycin B to give rifamycin O, which on spontaneous hydrolysis, gave rifamycin S in neutral aqueous solution. The characterization of the enzyme was different as compared with that of other polyphenol oxidases such as laccase. They classified this enzyme into a sub group EC.1.10.3.6 with a trivial name rifamycin oxidase [2].

The mode of action of rifamycin oxidase, as presented in Figure 2, was suggested [2]. In the two-step transformation reaction, one mole of rifamycin B reacts with one mole of oxygen to produce one mole each of rifamycin O and hydrogen peroxide. Further hydrolysis of one mole of rifamycin O produces one mole each of rifamycin S and glycolic acid. From the stoichiometry it is also

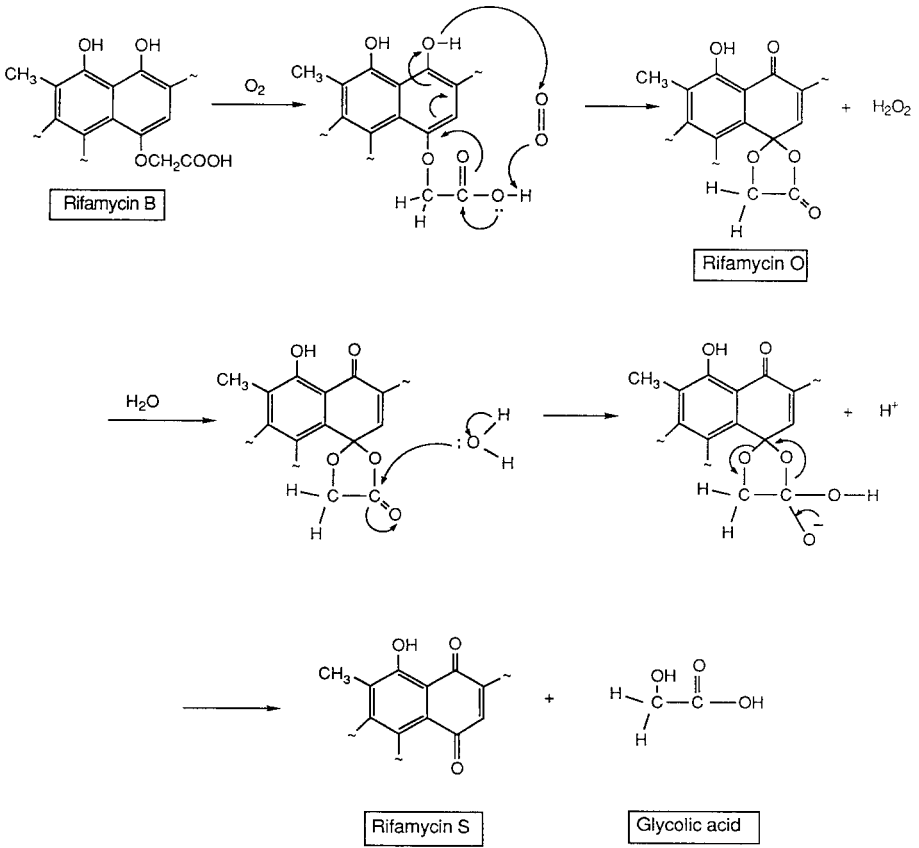


Figure 3. The reaction mechanism for transformation of rifamycin B to rifamycin S.

known that 100 g of rifamycin B yields 92 g of rifamycin S. From the action of rifamycin oxidase on rifamycin B, it seems that substituted hydroquinone moiety of rifamycin B is converted to quinone form of rifamycin B, *i.e.*, to rifamycin S. The proposed organic reaction mechanism of this transformation is shown in Figure 3.

The same group of workers also published the isolation procedure of *Monocillium* sp. and its morphological and culture characteristics [20]. They further demonstrated that rifamycin S can be prepared in high yield by microbial transformation of rifamycin B [20].

In 1989 Vohra *et al.* reported a highly active extracellular rifamycin oxidase from *Curvularia lunata* var *aeria* [3]. This enzyme could effectively transform rifamycin B to S. The time course of this conversion, monitored by HPLC [21], showed rapid consumption of rifamycin B, with 90% of it disappearing within the first twenty minutes of incubation. The production of rifamycin O was correspondingly fast and it underwent spontaneous hydrolysis to rifamycin S. Within an hour all the rifamycin B was converted to rifamycin O and most of the latter was hydrolysed to rifamycin S.

Production of rifamycin oxidase

In order to increase the intracellular rifamycin oxidase production by *Humicola* sp., biochemical engineering studies were conducted by Kim *et al.* [22]. The maximum specific enzyme activity in fermentations without pH control was reported to be 9.5 IU/g dry cell, and 15 IU/g dry cell for cells cultured at controlled pH of 8.0 [22]. Rifamycin oxidase production was accompanied by biomass growth [22]. Antifoam (Neorin 202) concentrations lower than 1% (v/v) did not have any significant effect on cell growth or on enzyme productivity [22]. pH control enhanced cell growth as well as enzyme production. The maximum specific cell growth rate was 0.24 h^{-1} at a constant pH 7.0, but it was only 0.19 h^{-1} in absence of pH control. Highest specific enzyme activity and productivity were observed at pH 8.0. No significant repression of enzyme production by high glucose concentrations was apparent up to 60 g/L glucose [22]. At this glucose

concentration, 15 IU of enzyme activity per gram dry cell weight were obtained (with pH control) and total enzyme production was 300 IU/L.

The effects of different carbon and nitrogen sources and of environmental factors (pH, temperature) on the production of rifamycin oxidase by *C. lunata* have been studied [23, 24]. Carboxymethylcellulose, yeast extract and peptone (1% w/v, each) and ammonium sulphate (0.04 % nitrogen content) were determined to be the best combination for maximum rifamycin oxidase production [24]. Other synthetic media were also tested, but no growth or enzyme production occurred in those media [23]. When carboxymethylcellulose was used as a carbon source, the organism (*C. lunata*) formed mycelial type of growth rather than pellet type [23]. With other substrates, pellet type of growth prevailed [23]. Mycelial growth always yielded higher enzyme activity [24]. Enzyme activity was reduced under conditions which led to intracellular black pigment production. *C. lunata* could produce high amounts of extracellular rifamycin oxidase within 40-50 hours of fermentation. The enzyme yield was found to be 3400 IU/g (dry weight) cell mass. The *C. lunata* rifamycin oxidase being an extracellular enzyme requires simpler downstream processing relative to the enzyme from *Humicola* sp. Filtered broths of *C. lunata* can be used for the transformation reaction. The intracellular enzyme from the *Humicola* sp. may require cell disruption for extraction of enzyme activity. Alternatively, delipidation of *Humicola* sp. may be used to reduce the diffusional resistances during the transformation reactions while fixing the enzyme within the cells. Compared to *Humicola* sp. enzyme, the enzyme from *C. lunata* has an important advantage for industrial applications. So far there is no literature on the production of rifamycin oxidase by *Monocillium* sp.

Characteristics of rifamycin oxidase

The characteristics of rifamycin oxidase from *Humicola* sp. are different from that of *Monocillium* sp. The enzymes differ noticeably in optimum pH, temperature, degree of inhibition by substrates and in activities toward *p*-hydroquinone. Further, the rifamycin oxidase from *Monocillium* sp. has quite different properties as compared with other similar enzymes such as catechol oxidase, laccase, ascorbate

oxidase, *o*-aminophenol oxidase and 3-hydroxyanthranilate oxidase, which utilize diphenols and related compounds as electron donors and oxygen as an electron acceptor [2]. The substrate specificity of rifamycin oxidase from *Monocillium* sp. was also different from that of the laccase from different sources. This enzyme oxidised hydroquinone and rifamycin B very rapidly, while laccase showed relatively low activity on those compounds [2]. Rifamycin oxidase from *Monocillium* sp. also oxidised rifamycin SV, 3-formyl rifamycin SV, pyrogallol and catechol. Resorcinol and the mannich derivative of rifamycin SV (3-diethylaminomethyl rifamycin SV) were not attacked at all. The enzyme activity was neither inhibited by Ag^+ or Hg^{+2} nor activated by Cu^{+2} . Other metal ions such as Ca^{+2} , Mg^{+2} , Fe^{+2} , Fe^{+3} , Co^{+2} , Mn^{+2} , Zn^{+2} and Mo^{+2} did not affect the enzyme activity [2]. EDTA caused no marked inhibition of the enzyme. The enzyme was neither a cupro-protein nor a flavo-protein; it did not contain any heme or non-heme ion nor any metals as co-factors [2]. Rifamycin oxidase from *Monocillium* sp. has pH and temperature optima of pH 7.8 and 40 °C, respectively.

The catalytic properties of partially purified rifamycin oxidase produced intracellularly by *Humicola* sp. were reported by Seong *et al.* [25]. The enzyme was most specific for rifamycin B among the various rifamycin derivatives tested [26]. The substrate specificity of the enzyme was investigated using various substrate analogues, including rifamycin B derivatives and other polyol compounds. *P*-hydroxyphenoxyacetic acid and *p*-hydroquinone which are corresponding structural analogues in the quinonoid moiety, caused a significant decrease in the enzyme activity compared to rifamycin B and rifamycin SV, respectively [25]. The enzyme showed highest catalytic activity on rifamycin B among the various substrates used. The K_m -value of this enzyme from *Humicola* sp. was found to be 0.05 mM [25]. Substrate inhibition was observed at substrate concentrations above 2 mM. A pH of 7.8 and a temperature of 45 °C were optimal for enzyme activity, catalytic activity was greatly reduced above this temperature. The rifamycin oxidase activity was strongly inhibited by Fe^{+2} and Hg^{+2} , but not by other metal

ions [25]. Presence of chelating agents such as EDTA did not affect the enzyme activity [25].

The optimum pH and temperature of rifamycin oxidase from *Curvularia lunata* are pH 6.5 and 50 °C, respectively [3]. The kinetic constants for the *C. lunata* enzyme have also been reported [26]. Under optimal conditions, Michaelis-Menten type kinetics were observed [26]; the K_m and V_{max} values, with rifamycin B as substrate, were found to be 0.67 mM and 11 IU/mL, respectively [26]. No inhibition of enzyme activity was noted up to 10 mM rifamycin B [26]. The activation and deactivation energies of the partially purified enzyme, calculated from Arrhenius plots, were 5.80 and 35.10 kcal/mole respectively [26]. The *C. lunata* rifamycin oxidase activity was inhibited by Fe^{+2} , Ag^+ and Hg^{+2} , but was not affected by the chelating agent, EDTA [26].

Biotransformation with soluble enzymes

Reports on biotransformation of rifamycin B to rifamycin S with soluble rifamycin oxidases are very limited. Intracellular rifamycin oxidases from *Monocillium* sp. and *Humicola* sp. were isolated from the cells and purified, but no systematic work was done to transform rifamycin B to S with the soluble enzyme. Rifamycin B was used as a substrate simply to ascertain enzyme activity; rifamycin S did form in the reaction mixture.

Extracellular, soluble rifamycin oxidase from *C. lunata* was used by Banerjee *et al.* for the transformation of rifamycin B to S [27]. The transformation conditions were optimized using partially purified rifamycin oxidase [27]. Transformation reactions were carried out at 28, 37 and 50 °C, with or without agitation. Incubation temperature, as well as the mixing rate, significantly affected the transformation rate [27]. Although at higher temperatures (37 and 50 °C), the transformation time (2 hours) was less than at 28 °C (3 hours), for practical purposes, the lower temperature is preferred: Rifamycin oxidase has better thermostability at 28 °C and oxygen (a required reactant) is more soluble in aqueous solutions at lower temperatures. The transformation time in the absence of mixing was almost 50 hours at 28 °C. Optimal agitation speed was found to be

200 rpm. Different percentages (2-8 % v/v) of enzyme solution were used, and 5% (v/v) enzyme concentration was found to be optimal. Suitable aeration rate for the transformation, optimized in a 0.5 L MBR bioreactor, was 1-1.25 vvm. Antifoam agent (polypropylene glycol) was added to suppress the foam. No adverse effect of the antifoam on enzyme activity was noted.

Biotransformation with immobilized enzymes

Rifamycin oxidase from *C. lunata* was immobilized on nylon fibres using glutaraldehyde as the crosslinking agent [28]. An activity of 18 IU/g fibres, with a binding efficiency of 37 %, was achieved. The immobilized enzyme showed an operational stability of 7-days and was protected against thermal inactivation. It exhibited a $K_{m(app)}$ of 2.0 mM. A polyacrylamide-based immobilization procedure has also been described for this enzyme [26]. Various percentages of acrylamide (6-10 % w/v) were used for the immobilization; 10 % (w/v) gel strength was found to be optimal. Compared to free enzyme, the optimum pH (6.0-6.5) for the immobilized preparation shifted slightly to the acidic region, but the temperature optima were unaffected at 50 °C. In biotransformation studies with the immobilized enzyme preparations, it took nearly 9 hours to complete the transformation whereas with soluble enzyme, with the same percentage of rifamycin B (1 % w/v), the reaction took 3 hours. The immobilized enzyme preparations were tested for reusability. Upon reuse, the beads acquired colour and after 3-4 uses, the transformation was additionally diffusionally limited. The time required for complete transformation increased with the number of use cycles. Transformation time for the fourth cycle was 10.5 hours, and in the sixth and seventh cycles the transformation time was 20 and 25 hours, respectively. Some adsorption of rifamycin on the beads was noted and it led to lower than expected recovery of rifamycin S after the transformation. So far, there are no reports of immobilization of soluble rifamycin oxidase from *Monocillium* or *Humicola* sp.

Biotransformation with whole cells in suspension

As an enzyme source for industrial applications, whole cell preparations of *Humicola* sp. were tested by Seong *et al.* [25]. A time lag in the conversion time profile was observed in the initial stages of reaction with whole cells, but no such trend was noticed with acetone dried (delipidated) cells. Diffusional resistances to the substrates and products through the cell membrane, may have been responsible for the initial lag in conversion. Acetone treatment removes the major portion of lipids of the cell membrane making the cell more porous and removing any diffusional limitations. Also, the lag could be reduced by increasing the temperature. Enhanced mobility of membrane of whole cells at higher temperatures was a possible explanation for the reduced lag.

Substrate inhibition was observed with whole cells as well as with acetone treated cells when substrate concentration in the transformation reaction increased, but up to 4 mM rifamycin B concentration there was no significant reduction in the initial rate of reaction [25]. Rifamycin O and rifamycin S are relatively hydrophobic and have an increased affinity to the cell membrane. This could cause their accumulation in the membrane resulting in further interference with the diffusion of substrate.

Han *et al.* clearly indicated the use of delipidated whole cells of *Humicola* sp. for attaining maximal conversion yield of rifamycin B to rifamycin S [29, 30]. Banerjee reported the transformation of rifamycin B to rifamycin S using growing and resting cells of *C. lunata* [26]. The organism could grow in the presence of rifamycin B and simultaneously convert rifamycin B to rifamycin S [26]. With growing cells, rifamycin B was added at the time of inoculation in the medium. With 1% (w/v) of rifamycin B in the medium, growth of the organism was reduced, but the transformation was completed within 20 hours of inoculation [26]. In other experiments, cells were grown up to the stationary phase and rifamycin B was added at different phases of growth. Twelve-hours grown cells in the culture broth took 16-hours to complete the transformation, whereas with 24-hours grown cells it took only 4-hours. It took nearly 6-hours for 48-hours grown cells to complete the transformation. Seventy-two hours grown cells of *C. lunata*

transformed only 10% of the added rifamycin B, and 96 and 120-hours grown cells did not transform any rifamycin B. Resting cells in both exponential and stationary phases could transform rifamycin B to rifamycin S and both could be reused for the transformation reaction. It took 3.6-hours to convert rifamycin B (1%, w/v) to rifamycin S by 24-hours grown, resting cells. The cells could be conveniently recycled up to ten-times. At the 10th cycle, 18-hours were taken to complete the transformation. Transformation of rifamycin B with growing and resting cells did not give stoichiometric yields; less than expected rifamycin S was obtained.

Biotransformation with immobilized whole cells

De-fatted whole cells of *Humicola* sp. with rifamycin oxidase activity have been immobilized by copolymerization with acrylamide for use in biotransformations of rifamycin [31]. The enzyme activity remaining after the immobilization step was ~50 % of the initial activity. The recovery of activity declined with increasing cell volume added into the original mixture [31]. This may have been due to increase in mass transfer resistances, or, to overpacking of the polyacrylamide gel matrix with cells. The optimal reaction pH for the immobilized, acetone de-fatted, cells was pH 7.8, while the optimal temperature for both free and immobilized de-fatted whole cells was found to be 50 °C. Experiments were conducted at a lower temperature of 40 °C to reduce enzyme inactivation. No appreciable activity loss occurred for the immobilized acetone de-fatted cells during one-month storage at 4 °C and pH 7.8 [30]. The half life of the treated cells at 40 °C and pH 8 was *ca.* 8-days.

The Michaelis-Menten constants, $K_{m(app)}$, and the substrate inhibition constant, $K_{in(app)}$, of the immobilized, acetone de-fatted, cells were found to be 0.6 mM and 19.6 mM, respectively [31]. The K_m -value of the acetone de-fatted cells

found to be pH 7.2 and 50-55 °C, respectively. Compared to the free enzyme, the immobilized preparations were less sensitive to temperature and pH changes. Twenty percent of the enzyme activity was recovered when the treated cells were immobilized on 3 mm beads [33]. Recovery of the immobilized enzyme activity increased as the bead size became smaller. Mass transfer limitations were concluded to be the main reason for lower activity of the immobilized enzyme [33]. The optimal loading of the acetone treated cell powder for cellulose acetate beads was one gram powder in 50 mL acetone/dimethylsulfoxide (3:2 v/v) mixture [33]. Higher enzyme loadings were not practical because of the precipitation of acetone treated cell powder during the immobilization process which caused problems with homogeneity of the catalyst. The physical strength of the cellulose acetate beads containing acetone de-fatted *Humicola* sp. cells was sufficient for use in packed bed reactors.

Besides the characterization of the enzyme rifamycin oxidase in free cells, whole cells and treated cells, Chung *et al.* did biotransformation studies in fluidized bed and rotating packed disc reactors [33]. A fluidized bed system with immobilized whole cells of *Humicola* sp. was used also by Lee *et al.* [34]. A linear relationship between the loading of the immobilized whole cells and conversion in both batch and continuous operations were found [34]; however, the conversion efficiency was higher in the batch mode for any given residence time [34]. Moreover, the aeration effect on the reaction rates in continuous operation was different from that in batch operation. Among the two reactor geometries used, the one which produced better mixing also gave 10 % better yield.

For the biotransformation with immobilized whole cells in a rotating packed disc reactor (RPDR), Chung *et al.* found that the initial reaction rate and the total productivity were dependent upon the degree of submergence of the discs [35]. The optimal submergence was 0.5; the rotational speed of the disc did not affect the conversion very much. Higher conversions were attained with longer residence times; however, the productivity declined with increasing residence times. Higher volumetric productivities may be achieved if more enzyme is loaded in the immobilized enzyme beads, or, more discs are installed in the RPDR. A RPDR

may be more amenable to plug flow operation than a fluidized bed reactor, which may increase the relative conversion efficiency in the RPDR. No reports of transformation of rifamycin B using immobilized whole cells or treated cells of *Monocillium* sp. or *C. lunata* have appeared so far.

Conclusions

The transformation of rifamycin B to rifamycin S can be successfully performed by rifamycin oxidase enzyme. Intracellular enzyme from *Humicola* sp. and *Monocillium* sp. may be used after extraction from the cells. Alternatively, delipidation of *Humicola* sp. may be employed to decrease the diffusional limitations in a process with the enzyme fixed within the cells. The transformation may also be achieved less expensively with the extracellular enzyme of *C. lunata*. The latter system has important processing advantages and either partially purified enzyme, or crude extracts of *C. lunata* can be used. Immobilized rifamycin oxidase preparations can also be employed with the advantages of greater stability, reusability and productivity. It is now clear that several biotransformation options may be used to prepare rifamycin S from rifamycin B. In view of the importance of rifamycin S as a key intermediate for the preparation of many semi-synthetic rifamycin derivatives of therapeutic value, further process improvements and scale-up considerations need to be investigated.

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