Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *85* (3) 279–290 (2020)
JSCS–5300
UDC 66.094.000.57+574.594:66.094.1.00.14:544.354.3
Original scientific paper

Application of the redox system of *Nocardia corallina* B-276 in the enantioselective biotransformation of ketones and alcohols

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(Received 6 August 2018, revised 15 July 2019, accepted 19 August 2019)

Abstract: The aim of this research was to evaluate the redox system of *Nocardia corallina* B-276 in the biotransformation of 1-phenyl-1-propanone (**1a**), 2-hydroxy-1-phenylethanone (**2a**) and methyl (2-chlorophenyl)(oxo)acetate (**3a**) into 1-phenylpropan-1-ol (**1b**), 1-phenyl-1,2-ethanediol (**2b**) and methyl (2-chlorophenyl)(hydroxy)acetate (**3b**). The biomass of *N. corallina* was obtained in a liquid medium with an initial pH of 8.50, but the pH changed during the 96 h of the culture media, the final pH was between 4.74 and 7.62. The *N. corallina* biomass biocatalyzed the enantioselective reduction of **1a**–**3a** to the corresponding alcohols. Whereas, during the process of oxidation of the *rac*-alcohols **1b**–**3b, 1b** was oxidized in enantioselective way, the oxidation of **2b** was not selective, but **3b** was biotransformed mainly to (*R*)-**3b**. These results are indicative that *N. corallina* produced reductases and oxidases, whereby the biocatalytic activity was influenced by the final pH of the culture media, the reaction time and structure of the substrate.

Keywords: actinomycetes; oxidation–reduction; enantioselectivity; pH influence.

INTRODUCTION

Chirality is a key factor in the effectiveness of biologically active compounds, because each enantiomer can perform a different biological function, and thus, the production of enantiomerically pure compounds would be relevant that avoid or diminish the probability of side effects, toxicity or high doses of chiral drugs.[1–3] A vast majority of the drugs developed nowadays contain chiral centers, and according to Global Industry Analysts nearly 95 % of drugs will be chiral by 2020, and hence, the FDA demanded manufacturers of chiral drugs to investigate the properties (physicochemical, pharmacokinetic, *etc*.) of all enantio-

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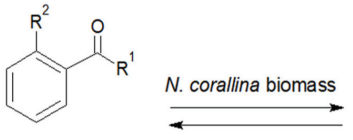
https://doi.org/10.2298/JSC180806089M

mers/diastereomers to determine their individual safety and efficacy. Due to this, the development of efficient chiral synthesis technologies is a challenge for many academic and industrial researchers.[4,5] The application of enzymes in the synthesis of compounds for the manufacture of chemicals, agrochemicals, pharmaceuticals, cosmetics, flavors and fragrances is an attractive alternative to conventional synthetic methods, because of their high levels of chemo-, regio- and stereo-selectivity, reduced formation of by-products and waste, lower energy consumption and decrease in the environmental impact.[1–3]

Enantiomerically pure alcohols are amongst the most important building blocks for the production of chiral pharmaceuticals, flavors, agrochemicals and functional materials. They can be obtained by either asymmetric reduction of the respective prochiral ketones or kinetic resolution of the racemic alcohols. The bioreduction of ketones is more valuable than chemical methods for the synthesis of chiral alcohols, due to its remarkable chemo-, regio- and stereo-selectivity together with mild reaction conditions; and environmental compatibility.[6,7]

Pure enantiomers of 1-phenylpropan-1-ol (**1b**, Fig. 1) have been used in the preparation of interleukin-2 inducible T cell kinase (ITK) inhibitors[8] and antiandrogen.[9] Enantiomerically pure 1,2-diols are important intermediates for various synthetic applications; for example, (*R*)-1-phenyl-1,2-ethanediol can be used as a building block for the preparation of (*R*)-norfluoxetine, (*R*)-fluoxetine and β-lactam antibiotics.[10] Methyl (*R*)-(2-chlorophenyl)(hydroxy)acetate is a key chiral intermediate for the synthesis of vicagrel and (*S*)-clopidogrel,[11] which are drugs with antiplatelet activity. Clopidogrel has been widely administered to atherosclerotic patients with the risk of a heart attack or stroke caused by blood clots and is the second-best-selling drug in the world, with global sales of $10 billion per year.[12]



	R^1	R^2		R^1	R^2
1a	CH_2CH_3	H	**1b**	CH_2CH_3	H
2a	CH_2OH	H	**2b**	CH_2OH	H
3a	$COOCH_3$	Cl	**3b**	$COOCH_3$	Cl

Fig. 1. Biotransformation of ketones **1a**–**3a** and alcohols **1b**–**3b** with *N. corallina*.

Nocardia corallina B-276 (ATCC 31338) is a versatile microorganism that catalyzes redox processes, such as the oxidation of aldehydes, allylic and benzylic alcohols to obtain carboxylic acids or ketones, and enantioselective reduction of ketones to secondary alcohols in an ecofriendly manner, with good yields.[13–17]

The aim of this research was to evaluate the redox system of *Nocardia corallina* B-276 in the biotransformation of 1-phenyl-1-propanone (**1a**), 2-hydroxy-1-phenylethanone (**2a**), methyl (2-chlorophenyl)(oxo)acetate (**3a**), 1-phen-

ylpropan-1-ol (**1b**), 1-phenyl-1,2-ethanediol (**2b**) and methyl (2-chlorophenyl)-(hydroxy)acetate (**3b**) (Fig. 1).

EXPERIMENTAL

Compounds **1**(**a**, **b**) and **2**(**a**, **b**), (*R*)-(2-chlorophenyl)(hydroxy)acetic acid and *rac*-(2--chlorophenyl)(hydroxy)acetic acid were purchased from Aldrich (St. Louis, Missouri, USA), **3a**, *rac*-**3b** and (*R*)-**3b** were prepared according to previously reported methods[18] and characterized by their infrared spectra (FT-IR) recorded on a Perkin–Elmer Paragon 1600 FT as liquid films. Hydrogen and carbon nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectra were recorded on an Agilent DD2 600 MHz instrument, in $CDCl_3$ using tetramethylsilane as the internal reference. Conversion was determined by GC analysis using a Hewlett––Packard HP 6890 gas chromatograph (Waldbronn, Germany) equipped with a flame ionization detector. The enantiomeric excess (ee) was determined by HPLC analysis, performed on an Agilent 1100 liquid chromatograph (Hannover, Germany) equipped with a diode array detector; and by TLC on silica gel 60 GF_{254} (Merck, Darmstadt, Germany).

Organism and growth

The cells of *Nocardia corallina* B-276 (ATCC 31338, were grown at 28–30 °C for three days on Petri dishes containing 15 g L^{-1} of agar, 3 g L^{-1} of beef extract and 5 g L^{-1} of peptone (Bioxon, Oaxaca, México). Then, the cells were inoculated in liquid media, which consisted on three solutions: solution A ($FeSO_4 \cdot 7H_2O$ 0.05 g L^{-1}, K_2HPO_4 1.74 g L^{-1}, $(NH_4)_2SO_4$ 2.00 g L^{-1}, yeast extract 1.00 g L^{-1}); solution B ($MgSO_4$ 1.50 g L^{-1}) and solution C (glucose 2.00 g L^{-1}, J. T. Baker, USA); the pH was adjusted to 8.50±0.5. The culture was incubated in an orbital shaker at 28–30 °C, 150 rpm for 96 h.[19] The cells were collected by centrifugation at 4500 rpm for 15 min, and the pH of the supernatant was measured. The cells were washed twice with potassium phosphate buffer (0.1 mol L^{-1}, pH 7.00) and centrifuged at 4500 rpm for 15 min.

*Biotransformation of 1-phenyl-1-propanone (**1a**) and 1-phenylpropan-1-ol (**1b**)*

The *N. corallina* cells were incubated in a 50 mL phosphate buffer (0.1 mol L^{-1}, pH 7.00) for 30 min at 28–30 °C in an orbital shaker (150 rpm), then **1a** or **1b** was added separately to the whole cells with a substrate:cells mass ratio of 1:500 using, 0.6 vol. % of *N*,*N*--dimethylformamide. The mixture was shaken under the same conditions, and experiments were performed in triplicate at different final pH of the culture media and times. The sample was centrifuged at 4500 rpm for 15 min and then extracted with ethyl acetate (3×15 mL), and the organic layer was concentrated to dryness.[15] The products **1a** or **1b** were identified by FT--IR and NMR and the spectra were in full accordance with literature.[20,21] The product was dissolved in 0.5 mL of HPLC 2-propanol and analyzed by GC using a Supelcowax™-10 column (30m×0.25mm, 0.25 μm) at 180 °C with N_2 as carrier gas at 0.8 mL min^{-1}, the retention times were t_R(**1a**) = 4.39 min and t_R(**1b**) = 5.63 min. It was then analyzed by HPLC equipped with two columns: Chiralcel OB-H (25.0 cm×0.46 cm, 0.5 μm) column; the mobile phase was hexane:2-propanol (90:10), with a flow rate of 0.5 mL min^{-1}, λ = 220 nm at 25 °C, the retention times were t_R(*R*)-(**1b**) = 9.6 min, t_R(*S*)-(**1b**) = 11.4 min, and t_R(**1a**) = 20.4 min: chiralcel OD (25.0 cm×0.46 cm, 10 μm) column; the mobile phase was hexane:2-propanol (97:3) at a flow rate of 1.0 mL min^{-1}, λ = 220 nm at 25 °C. The retention times were t_R(*R*)-(**1b**) = 12.7 min, t_R(*S*)-(**1b**) = 14.9 min, and t_R(**1a**) = 7.7 min. The absolute configuration of **1b** was assigned according to the literature.[22]

*Biotransformation of 2-hydroxy-1-phenylethanone (**2a**) and 1-phenyl-1,2-ethanediol (**2b**)*

The *N. corallina* cells were incubated in 50 mL phosphate buffer (0.1 mol L^{-1}, pH 7.00), 30 min, at 28–30 °C in an orbital shaker (150 rpm), and **2a** or **2b** was added to the whole cells at a substrate:cells mass ratio of 1:100 for **2a** and 1:400 for **2b**, using 0.6 vol. % of *N,N*--dimethylformamide and shaken under the same conditions. The experiments were performed in triplicate at different final pH values of the culture media and times. The sample was centrifuged at 4500 rpm for 15 min and then extracted with ethyl acetate (4×15 mL), and the organic layer was concentrated to dryness. The product was dissolved in 0.5 mL of HPLC grade 2-propanol. The GC analysis was performed with a HP-5 column (30 m×0.33 mm, 0.25 μm) (Hewlett–Packard, Germany) at 80–200 °C, with N_2 as the carrier gas at 1.0 mL min^{-1}. The oven temperature was ramped from 80–200 °C at 10 °C min^{-1}, held for 3 min, decreased to 80 °C at 25 °C min^{-1} and held for 2 min. The retention times were t_R(**2a**) = 4.90 min and t_R(**2b**) = = 5.79 min. Then, the analyse was realized by HPLC using a chiracel OB-H (25.0 cm×0.46 cm, 0.5 μm) column (Daicel Chemical Industries, Tokyo, Japan). The mobile phase was hexane:2-propanol (90:10), 0.5 mL min^{-1}, λ = 220 nm, 24 °C. For the reduction of **2a**, the retention times were t_R(*R*)-**2b** = 15.10 min, t_R(*S*)-**2b**= 18.90 min and t_R(**2a**) = 28.90 min. For the oxidation of **2b**, the mobile phase was hexane:2-propanol (90:10), 0.8 mL min^{-1}, λ = 260 nm, 24 °C. The retention times were t_R(*R*)-**2b** = 9.04 min, t_R(*S*)-**2b** = 11.38 min and t_R(**2a**) = = 17.12 min, The absolute configuration of **2b** was assigned according to the literature.[23]

*Biotransformation of methyl (2-chlorophenyl)(oxo)acetate (**3a**) and methyl (2-chlorophenyl)-(hydroxy)acetate (**3b**)*

The *N. corallina* cells were incubated in 50 mL phosphate buffer (0.1 mol L^{-1}, pH 7.00), 30 min at 28–30 °C in an orbital shaker (150 rpm), and then **3a** or **3b** was added to the whole cells, with a substrate:cells mass ratio of 1:500, using 0.6 vol. % of *N,N*-dimethylformamide. The mixture was shaken under the same conditions, and experiments were performed in triplicate at different final pH values of the culture media and times. The samples were centrifuged at 4500 rpm for 15 min and then extracted with ethyl acetate (3×15 mL), and the organic layer was concentrated to dryness. The product was dissolved in 0.5 mL of HPLC grade 2-propanol and analyzed by GC using a Supelcowax^{TM}-10 column (30 m×0.25 mm, 0.25 μm) at 230 °C, with N_2 as carrier gas at a flow rate of 1.0 mL min^{-1}. The retention times were t_R(**3a**) = 4.6 min and t_R(**3b**) = 6.1 min. Then the product was analyzed by HPLC using a chiralcel OD (25.0 cm×0.46 cm, 10 μm) column; the mobile phase was hexane:2-propanol (90:10) at a flow rate of 1.0 mL min^{-1}, λ = 235 nm at 25 °C. To assign the retention times of the enantiomers of *rac*-**3b**, methyl (*R*)-(2-chlorophenyl)(hydroxy)acetate was previously prepared in order to obtain the retention time of (*R*)-**3b** (10.2 min), then *rac*-**3b** was mixed with (*R*)-**3b** to enrich the enantiomer *R* and confirm the retention time of (*R*)-**3b** within the mixture. The retention times were t_R(*R*) **3b** = 10.2 min, t_R(*S*)-**3b**= 8.9 min, and t_R(**3a**)= 7.0 min.

RESULTS AND DISCUSSION

Nocardia corallina B-276 biomass

The *N. corallina* biomass was produced in a liquid medium with an initial pH of 8.50. After 96 h, it was observed that in several experiments the final pH of the culture media was between 4.74 and 7.62. Several authors have reported variations in the initial and final pH of the culture media during microorganism growth, and they found that the production of metabolites was dependent on

these pH variations.[24–26] For example, *Pediococcus damnosus* NCFB 1832 was grown at initial pH 6.7 uncontrolled and the highest production of pediocin PD-1 was allowed at a final pH 4.14.[24] The highest hydrogen production with *Clostridium beijerinckii* L9 and *Clostridium tyrobutyricum* FYa102 was achieved under uncontrolled pH conditions (initial pH of 6.4–6.6 and final pH of 4–4.2).[25] Glycerol fermentation for the production of 2,3-butanediol (2,3-BD) by *Klebsiella pneumoniae* was sensitive to pH, a decrease in the pH was the most important condition for high 2,3-BD productivity, while experiments with constant pH and avoiding pH fluctuations resulted in low amounts of 2,3-BD produced from glycerol.[26] It has likewise been observed that the production and catalytic activity of enzymes depends on the pH value, depending on the dissociation constant of the ionizing groups present at the active site of the enzyme, such as aspartic acid that ionizes at pH 3–6 or histidine with a ionization pH of 5–8.[27–29] The outcome of a given reaction with whole-cell not only depends on the strain being used and the substrate structure but also on the biological status of the cells, such as age and growth conditions. Therefore, the predictability of whole-cell alcohol oxidations and the potential production pattern with respect to regio-, chemo- and enantio-selectivity is generally low.[30]

Therefore, controlling pH of the medium may have a negative effect over the biocatalytic activity of the microorganism, because there are interactions between the constituent amino acids on the active site of the enzyme and the substrate, where most amino acids perform acid–base catalysis, and such catalysis can also affect the activity and selectivity of oxidation–reduction reactions.[24–26,31,32] Based on these results, in this work it was decided not to control the pH during the growth of *N. corallina* and to evaluate its redox biocatalytic behaviour at the final pH of the culture media.

To characterize the compounds **1**(**a**, **b**) and **2**(**a**, **b**), their spectra were compared with spectra data obtained for the compounds purchased from Aldrich. The IR and NMR spectra, as well as GC and HPLC chromatograms of 1-phenyl-1--propanone (**1a**), 2-hydroxy-1-phenylethanone (**2a**), methyl (2-chlorophenyl)-(oxo)acetate (**3a**) into 1-phenylpropan-1-ol (**1b**), 1-phenyl-1,2-ethanediol (**2b**) and methyl (2-chlorophenyl)(hydroxy)acetate (**3b**), as well as the biotransformation experiments are presented in the Supplementary material to this paper.

*Reduction of ketones **1a**, **2a** and **3a** with* N. corallina *biomass*

The reduction of the ketones **1a**, **2a** and **3a** to the corresponding alcohols **1b**, **2b** and **3b** was performed using as a source of biocatalyst the *N. corallina* cells obtained with uncontrolled pH of the culture medium. The biotransformations were performed at different times and final pH values, and the results are presented in Fig. 2 and in the Supplementary material, Figs. S-1–S-3.

The aromatic ketone **1a** without substituent was reduced in an enantioselective way (Fig. S-1), the specificity of the *N. corallina* redox system was depen-

dent on the final pH of the culture media. At final pH of the culture medium of 5.36 and 5.67, the reduction of **1a** to the corresponding alcohol **1b** was 98 and 80 % respectively, however a decrease of ee was observed from 80 to 10 %. However, at a final pH of 7.62, total conversion and enantioselectivity was observed regardless of the reaction time. In every experiment, only the (*R*)-**1b** enantiomer was obtained. Then, it was decided to study the biotransformation of **1a** with *N. corallina* biomass obtained at final pH of 5.36 because in Fig. S-1 an influence of the reaction time was noted. In the first 24 h, it could be observed that **1a** was completely transformed to (*R*)-**1b** (Fig. 2); but after 48 h, a slight decrease in the quantity of (*R*)-**1b** from 90 to 75 % and an increase in the amount of (*S*)-**1b** from 10 to 25 % were observed, probably due to a competition between the oxidation and reduction reactions. Between 72 and 96 h, oxidation of the alcohol **1b** to the ketone **1a** was observed, but this reaction was not enantioselective because the proportion of (*R*)-**1b**/(*S*)-**1b** was constant (72/28, Fig. 2).

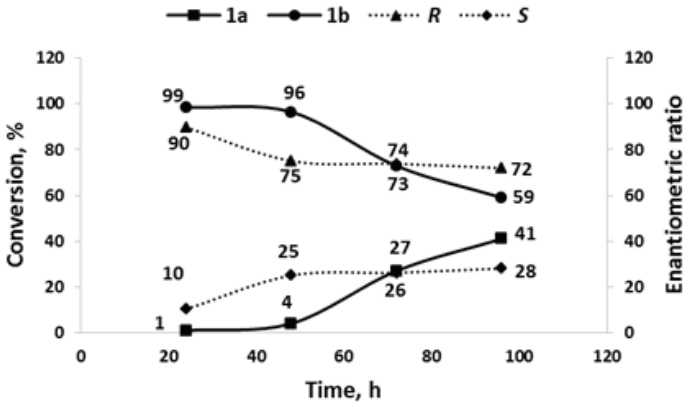


Fig. 2. Biotransformation of **1a** with *N. corallina* biomass, obtained with uncontrolled pH of the culture medium (pH 5.36).

In the Fig. S-2, it could be observed that the conversion, as well as the ee, of compound **2a** to compound **2b** at different times using substrate to cell ratio of 1:100 was dependent on the media pH, which was mostly acidic. Thus, a low conversion was observed (39 and 60 %), however, at 20 and 144 h, enantioselectivity was evident with production of the (*R*) enantiomer regardless the pH (98 %). The production of the (*R*) enantiomer in the reduction of compound **2a** was previously reported with different microorganisms.[27,28,33,34]

The low conversion could have been because the regeneration of NAD^+ by lactate dehydrogenase was slow, thus becoming the limiting factor in the reduction of compound **2a** because of the side reaction between compound **2a** and NAD^+; thus being a factor to be examined in later studies when the amount of substrate is considered.[35,36]

In the case of methyl(2-chlorophenyl)(oxo)acetate (**3a**, Fig. S-3), it was reduced quantitatively at the two final pH values (5.80 and 7.30), which produced mainly the alcohol (*R*)-**3b**, although at the lower pH, the enantioselectivity was highest (62 % for pH 5.8 and 22 % for pH 7.30) at 24 h, but at 48 h and pH 5.80, the ee remained constant and at pH 7.30, the enantiomeric excess increased from 22 to 50 %.

The reductase activity and enantioselectivity of the *N. corallina* cells were mainly influenced by the final pH of the culture media, and the structure of the substrate, Fig. 2 and Figs. S-1–S-3. On the one hand, the pH can affect the ionization of the active site of the enzyme as well as the substrate, *e.g.*, as observed in Figs. S-1 and S-2, the pH optimum to achieve maximum reductase activity was approximately at pH 7.62. It should be noticed that in a whole cell catalysis, such as the one studied in this research, there may be other enzymes that could have affinity for the substrate.[35,37] Considering the structure of **1a**, the alkyl group induced the biotransformation with high selectivity and an excellent conversion. In contrast, it was easier for **2a** to generate hydrogen ions, which probably competed with NAD^+ during the regeneration of the oxide reductase making the bioconversion less effective compared to **1a**. Regarding **3a**, the presence of an ester group in the side chain makes the enzyme more active,[38] which is the reason for the complete transformion of **3a** to **3b**.

The results of the biotransformation of **1a** were different from those obtained with **2a** and **3a** at 24 h because in the case of **1a** (Fig. S-1), the conversion and enantioselectivity were less influenced by the final pH. At pH values of 5.36 and 7.62, the conversions were 98 %, and only the enantiomer (*R*)-**1b** was present, the biotransformation **2a** (Fig. S-2) at final pH of 4.74 conversions were 39 %, and again only the enantiomer (*R*)-**2b** was observed. In contrast, the reduction of **3a** (Fig. S-3), the final pH were 5.80 and 7.30 and the transformation was almost quantitative and the enantioselectivity was medium.

Resolution of rac-*alcohols **1b**–**3b** by selective oxidation with* N. corallina

The oxidations of the alcohols **1b**–**3b** to the corresponding ketones **1a**–**3a** were performed using the *N. corallina* cells obtained with uncontrolled pH of the culture medium as the biocatalyst. The biotransformations were performed at different times and final pH values. The results are given in Figs. 3 and 4, and S-4 and S-5 of the Supplementary material.

In Fig. S-4, it could be observed that only (*S*)-**1b** was oxidized to ketone **1a**, while (*R*)-**1b** remained in the enantiopure form if the final pH was 7.62 (24 h), with a slightly variation in the percent ketone was observed on extending the reaction time to 36 h. A competitive reduction may be involved to enrich the (*R*)-alcohol.

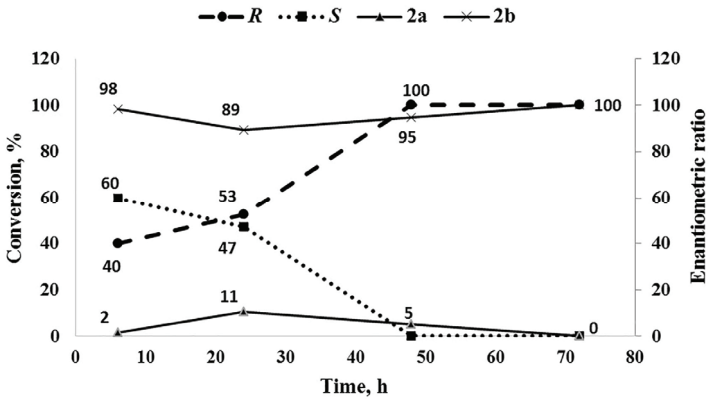


Fig. 3. Biotransformation of **2b** with *N. corallina* biomass, final pH 5.99.

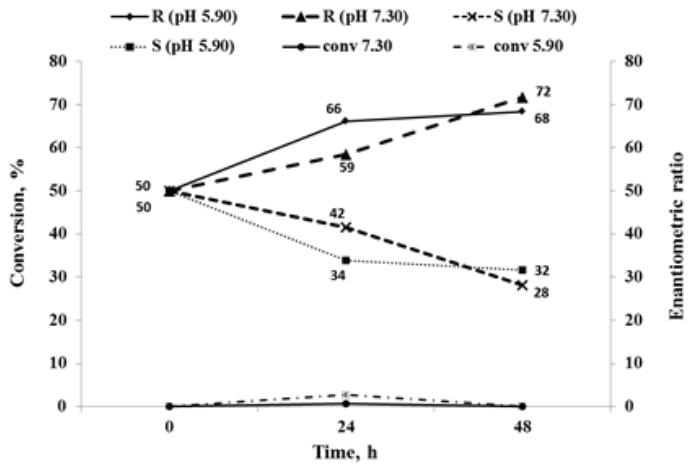


Fig. 4. Biotransformation of **3b** with *N. corallina* biomass obtained with uncontrolled pH of the culture medium (5.90 and 7.30), substrate:cells ratio (1:500).

In Fig. S-5, the conversion, as well as the ee from compound **2b** to compound **2a**, at different final pH values and times using a substrate:cell ratio 1:400 can be seen.

Depending on the media, the final pH after the incubation of the bacteria and different conversions can be observed at 48 h, *i.e.*, from 12 % at pH 6.18 to 42 % at pH 6.8 with a low ee for both pH values. These results may be due to by-product formation, since at pH 6.8 after 96 h, a quantitative conversion was achieved with a higher ee, while after 120 h, the conversion diminished but the highest ee was observed. After 96 h at pH values of 5.99, 6.28 and 6.80, different conversions and ee values can be observed. The highest conversion and ee were obtained at pH 6.28, this could be because at the same time that *N. corallina* oxi-

dizes alcohol **2b**, another enzyme, such as glycerol dehydrogenase, acts on the same substrate.[35]

As could be seen in Fig. 3, at pH 5.99 after 24 h, a stereoinversion process occurs and the ee remains constant after 48 h.

A first attempt to obtain diols and ketones using *N. corallina* whole cells was achieved when it was determined that the conversion is low in acidic pH although deracemization was observed. Observing both reactions, it could be concluded that the pH value is an essential factor because either the substrate can be ionized or the active site can be modified, as was the case of glycerol dehydrogenase and its cofactors, thus affecting the binding of the substrate to the enzyme.

During the expected oxidation of **3b** (Fig. 4) the quantity of ketone was insignificant, but an increase in the quantity of (*R*)-**3b** was observed. Several authors found that the activity of reductases from different microorganisms was higher with *α*-ketoesters than with ketones, but the enantioselectivity was higher with ketones,[39,40] which is similar to the results obtained with *N. corallina*.

This indicates that the oxidation of **3b** is slower than the reduction of **3a**. When the final pH of the culture medium is slightly acidic (pH 5.90), *N. corallina* biotransforms **3b** to the ketone (3 %), with 32 % ee of (*R*) in the remnant alcohol at 24 h. On extending the reaction time to 48 h, the ketone was not observed and only a slight increment to 37 % ee of (*R*) was obtained. At a pH 7.30, 1 % of **3a** was obtained at 24 h but the ketone was not detected at 48 h. In addition, at 24 and 48 h, 17 and 40 % ee of (*R*) were observed. This could be explained by the stereoinversion of the alcohol (*S*) to (*R*) *via* the ketone. It was also observed that *N. corallina* can perform the bio-oxidation of *rac*-**3b** to **3a** followed by reduction of **3a** to **3b**, leading to an enantio-enrichment of (*R*)-**3b**, with a substrate:cells ratio of 1:500, during 48 h. These results fully demonstrated the redox ability of *N. corallina* to obtain enantiopure alcohols.

Increasing the substrate:cells ratio and the time to 1:1000 and at 120 h, at pH 7.57, the conversion to ketone (**3a**) was 92 % and residual **3b** was obtained with an ee of 10 %. Thus under these conditions, *N. corallina* oxidizes *rac*-**3b**, inhibiting the inverse reaction.

The substituents of the carbonyl (**1a**–**3a**) exerted an important influence over the biocatalytic activity of the *N. corallina* enzymes, due to steric hindrance and electronic effects.[41] In the case of the aromatic ketone **2a** substituted in the side chain by –OH, the behaviour of the redox system was different, because the reduction was enantioselective independently of the final pH of the culture medium, but the oxidation of *rac*-**2b** was not a favourable reaction. Of the three compounds of pharmaceutical importance, **3a** was the most interesting compound due to the ester group (*α*-ketoester) in the side chain. With **1a** and **2a**, the reduction was enantioselective, but with **3a** the reduction was almost quantitative but with

low ee; besides, during the expected oxidation of **3b** the quantity of ketone was insignificant, but an increase in the quantity of (*R*)-**3b** was observed.

CONCLUSIONS

The biocatalytic activity of the redox system of *N. corallina*, reductases and oxidases, was dependent of the course of the growth of the microorganism, such as the final pH value, time of reaction and structure of the substrate. *N. corallina* was able to reduce ketones **1a**–**3a**, oxidize the alcohols (*S*)-**1b**, (*S*)-**2b** and **3b** to the corresponding ketones. This allows for the enrichment of (*R*)-alcohols by biocatalytic synthesis with *N. corallina* biomass, obtained with uncontrolled pH of the culture media during the growth.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of the synthesized compounds are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgement. The authors would like to thank the Department of Biological Systems of the Metropolitan Autonomous University, Xochimilco Unit, for the support.

ИЗВОД

ПРИМЕНА РЕДОКС-СИСТЕМА *Nocardia corallina* B-276 У ЕНАНТИОСЕЛЕКТИВНОЈ БИОТРАНСФОРМАЦИЈИ КЕТОНА И АЛКОХОЛА

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Циљ овог рада је био испитивање редокс-система *Nocardia corallina* B-276 у реакцији биотрансформације 1-фенил-1-пропанона (**1a**), 2-хидрокси-1-фенилетанона (**2a**) и метил-(2-хлорфенил)(оксо)ацетата (**3a**) у 1-пропан-1-ол (**1b**), 1-фенил-1,2-етандиол (**2b**) и метил (2-хлорфенил)(хидрокси)ацетат (**3b**). Биомаса *N. corallina* добијена у воденом раствору при почетном pH 8,50 се мењала током 96 h и коначан pH раствора је био између 4,74 и 7,62. Биомаса *N. corallina* врши биокатализу енантиоселективне редукције **1a**–**3a** до одговарајућих алкохола. Такође, током реакције рацемских алкохола **1b**–**3b,** код **1b** енантиоселективно је оксидован један од енантиомера, оксидација **2b** није селективна, док се **3b** углавном преводи у (*R*)-**3b**. Добијени резултати указују да *N. corallina* производи редуктазе и оксидазе, док је биокаталитичка активност под утицајем крајњег pH медија, реакционог времена и структуре супстрата.

(Примљено 6. августа 2018, ревидирано 15. јула 2019, прихваћено 19. августа 2019)

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