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Biocatalytic deracemisation of aliphatic β-hydroxy esters: Improving the enantioselectivity by optimisation of reaction parameters

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Abstract Optically pure aliphatic β-hydroxy esters were prepared from their racemates by deracemisation using the biocatalyst *Candida parapsilosis* ATCC 7330. High optical purity (up to >99 %) and good yields (up to 71 %) of the product secondary alcohols were obtained. This study highlights the importance of optimization of reaction conditions using ethyl-3-hydroxybutanoate as the model substrate to improve the enantioselectivity (enantiomeric excess from 9 to 98 %). The present study emphasises the broad substrate scope of the biocatalyst towards deracemisation. This is the first report of *Candida parapsilosis* ATCC 7330-mediated deracemisation of various alkyl-3-hydroxybutanoates to produce either the (*R*)-enantiomers (methyl, ethyl, propyl, butyl, *t*-butyl, allyl-3-hydroxybutanoates) or (*S*)-enantiomers (pentyl, *iso*-amyl and *iso*-propyl-3-hydroxybutanoates).

Keywords Deracemisation · *Candida parapsilosis* ATCC 7330 · Aliphatic β-hydroxy esters · Alkyl-3-hydroxybutanoates · Optimisation studies

Introduction

Optically pure aliphatic β-hydroxy esters, which are an important class of chiral building blocks [1–6] can be prepared in optically pure form using biocatalysts and chiral metal complexes like BINAP-Ru, oxazaborolidine [7, 8]. Biocatalytically, strategies like asymmetric reduction (from prochiral ketones) [9], deracemisation [10] and kinetic resolution [11] are employed. For both kinetic resolution and deracemisation, racemates [12] are used as the starting materials. The advantage of deracemisation over kinetic resolution is the theoretical possibility of quantitative yield (100 %) for a single enantiomer while resolution would give only 50 % [13].

In biotransformations which employ oxidation–reduction reactions, whole cells are generally preferred over purified enzymes because whole cells do not require the addition of expensive cofactors [14]. On an industrial scale, whole cells-mediated reactions are being increasingly adopted [15, 16]. However, in a whole cell system, due to the presence of multiple reducing enzymes of opposite stereopreferences, the product formed can have low enantiomeric excess (ee). This problem is addressed by optimising different reaction parameters like pH, cosolvent, temperature, growth conditions etc. to attain improvement in ee as well as yield [17]. To emphasise the importance of reaction parameters, few examples are cited here. Houng and coworkers reported the optical enrichment of (*S*)-ethyl-4-chloro-3-hydroxybutanoate (82.16–92.3 %) by modifying the composition of the growth medium and reaction conditions like pH etc., in the asymmetric reduction of the prochiral ketone using baker's yeast [18]. Chen et al. [19] studied different reaction parameters to improve the enantioselectivity in *Acetobacter* sp. CCTCC M209061-mediated stereoselective reduction of 4-(trimethylsilyl)-3-butyn-2-one to anti-Prelog (*R*)-4-(trimethylsilyl)-3-butyn-2-ol, a key chiral precursor used in the treatment of Alzheimer's disease. Zhang and coworkers systematically investigated the effects of different reaction parameters like substrate concentration, pH, temperature and cosubstrate concentration on the asymmetric reduction of the same prochiral ketone

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(4-(trimethylsilyl)-3-butyn-2-one) using the whole cells of *Candida parapsilosis* to produce (*S*)-4-(trimethylsilyl)-3-butyn-2-ol with high optical purity (>99 %) and yield (95.2 %) [17]. Most of the biocatalytic reductions with aliphatic ketones and β-ketoesters result in the formation of Prelog products and only few reports are available for the anti-Prelog products [20]. It was recently reported from our lab, that a switch in enantioselectivity can also be achieved in the asymmetric reduction of ethyl-4-chloro-3-oxobutanoate using *C. parapsilosis* ATCC 7330 by carefully altering the carbon sources in the growth medium that resulted in the production of both enantiomers (ee >99 %) [21].

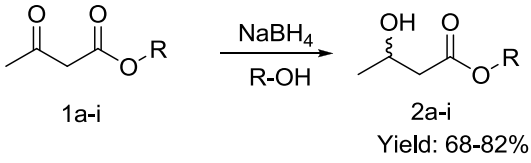
The versatility of *Candida parapsilosis* ATCC 7330 for the deracemisation of different aryl secondary alcohols: α-hydroxy esters [22], β-hydroxy esters [23], allylic alcohols [24], α-hydroxy propargylic esters [25], 1-phenyl ethanols [26] and asymmetric reduction of aryl α-keto esters [27], α-oxoaldehydes [28] α-ketoamides [29] and industrially important aliphatic ethyl-4-chloro-3-oxobutanoate [30] with high optical purity and yields has been established in our lab earlier. Under optimised reaction conditions, the asymmetric reduction of different alkyl-3-oxobutanoates was carried out to produce the corresponding optically enriched (*S*)-hydroxy esters (ee up to >99 %) [31].

Reports on the deracemisation of alkyl secondary alcohols are scarce as compared to deracemisation of aryl secondary alcohols. To mention a few, the deracemisation of 1-cyclohexyl ethanol to its (*R*)-enantiomer (98 % ee, 4 days) [32], optically active (*S*)-1,2-diols (ee up to 98 %, 24–96 h) [33] using different microorganisms, enantioselective oxidation of industrially important 1, 3-butanediol racemate to (*S*)-butane diol (ee 99 %, yield 45 %) using *Kluyveromyces lactis* IFO 1267 [34], stereoinversion of optically active 3-pentyn-2-ol from its racemate to (*R*)-enantiomer (ee >99 %, yield 63 %, 3 days) using different *Nocardia* species [35, 36] among others.

A recent report from our lab on the asymmetric reduction of aliphatic β-keto esters [31] using *C. parapsilosis* ATCC 7330 resulted in the formation of (*S*)-β-hydroxy esters (Prelog products) as already mentioned. The industrial importance of aliphatic (*R*)-β-hydroxy esters [1, 2], further motivated us to explore a strategy to prepare (*R*)-β-hydroxy esters using the same biocatalyst which involved extensive optimization studies. Hence, we report for the first time, the deracemisation of aliphatic β-hydroxy esters using *C. parapsilosis* ATCC 7330 which selectively produced the anti-Prelog products (*R*-enantiomers).

Materials and methods

Methyl, ethyl and *t*-butyl-3-oxobutanoates were purchased from Lancaster and other substrates were synthesised using



R = -CH_3, -C_2H_5, -C_3H_7, -C_4H_9, -C_5H_{11}, -CH-CH=CH_2, -$CH(CH_3)_2$, -$C(CH_3)_3$, -$CH_2CH_2CH(CH_3)_2$

Fig. 1 Synthesis of racemic alkyl-3-hydroxybutanoates [39]

reported procedures [37, 38]. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a Bruker AVANCE III 500 MHz spectrometer operating at 500 and 125 MHz, respectively using TMS as an internal standard. Infrared spectra were recorded on a JASCO FT/IR-4200 instrument. The ee was determined by gas chromatography using Perkin Elmer CLARUS 600 gas chromatograph fitted with FID detector using VARIAN Chirasil Dex CB chiral column (0.25 μm × 25 mm × 25 m). Helium was used as the carrier gas with a (flow rate varied from 1–2 mL/min) split factor of 50:1. Optical rotations were determined on Rudolph, Autopol IV digital polarimeter. TLC was carried out using Kieselgel 60 F254 aluminium sheets (Merck 1.05554).

Synthesis of racemic alkyl-3-hydroxybutanoates

Racemic alkyl-3-hydroxybutanoates were prepared in quantitative yields by reduction of the corresponding alkyl-3-oxobutanoates (1 mmol) with sodium borohydride (0.5 mmol) in their corresponding alcohol (10 mL) at 0 °C to room temperature using a reported procedure [39] (Fig. 1). The reaction was monitored by TLC and after completion, excess alcohol was removed, reaction mixture was hydrolysed with dilute HCl and the mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate, concentrated and purified by column chromatography using hexane:ethyl acetate (90:10). Spectroscopic data were identical with those reported in the literature.

Growth conditions of *Candida parapsilosis* ATCC 7330

Candida parapsilosis ATCC 7330 was purchased from ATCC, Manassas, VA 20108, USA and maintained at 4 °C in yeast malt agar medium (pH of growth medium: 6.2 ± 0.2) that contained 5 g/L peptic digest of animal tissue, 3 g/L malt extract, 3 g/L yeast extract, 10 g/L dextrose and 20 g/L agar. All chemicals used for media preparation were purchased from Himedia.

Candida parapsilosis ATCC 7330 was precultured for 12 h at 25 °C with shaking at 200 rpm in yeast malt broth medium (contains 5 g/L peptic digest of animal tissue,

3 g/L malt extract, 3 g/L yeast extract, 10 g/L dextrose). The precultured broth, 2 mL (4 % v/v) was transferred to a 250 mL Erlenmeyer flask that contained 48 mL of yeast malt broth. The culture was grown on orbital shaker at 25 °C and 200 rpm for 14 h [30]. The cells were harvested by centrifugation at 10,000 rpm for 10 min at 4 °C and subsequently washed thrice with distilled water and the wet cells were used for biotransformation.

Optimisation of reaction conditions

As mentioned earlier, the whole cells of *Candida parapsilosis* ATCC 7330 are expected to contain multiple oxidoreductases with opposite stereochemical preferences. Initially, the deracemisation of ethyl-3-hydroxybutanoate (2b), a model substrate, was carried out using earlier reported reaction conditions [22].

Procedure for deracemisation of ethyl-3-hydroxybutanoate using whole cells of *Candida parapsilosis* ATCC 7330

In a 150 mL Erlenmeyer flask, 2.4 g of wet cells of *Candida parapsilosis* ATCC 7330 was suspended in 9.8 mL of water. The substrate, ethyl-3-hydroxybutanoate (8 mg, 0.06 mmol) which was dissolved in 200 µL (2 % of total volume) of ethanol was added and the reaction was continued up to 24 h. The aliquot was taken every 6 h, extracted with ethyl acetate and the ee of the product thus formed was determined using a chiral GC column using earlier reported condition [31].

The reaction when carried out in water with ethanol as a cosolvent gave an ee of 9 % of the product (*R*)-enantiomer in 24 h. This could be due to the action of multiple enantiocompletmentary enzymes present in the biocatalyst [21]. To improve the ee of the desired product, a study of the different reaction parameters was undertaken.

As a first attempt, the above reaction was repeated by suspending the wet cells in 9.6 mL of water. Acetone (200 µL, 2 % of the total volume) was added to the reaction mixture in addition to ethanol (200 µL, 2 % of the total volume). The reaction was continued up to 24 h, the product thus formed was analysed by GC and interestingly an improvement in ee (16 %) was observed. Hence, with repeated experiments, the volumes of acetone and ethanol were optimised to 4 and 1 % of the total volume respectively, which resulted in an improvement in ee of up to 24 %. Further increasing the volume of acetone did not show any improvement in the ee. Hence, 4 % of acetone was considered optimal and used for further optimisation studies. To study the role of cosolvent in the biotransformation, a non-alcohol cosolvent, acetonitrile (1 %) was used [31] instead of ethanol, in addition to acetone and notably the product formed was found to have an improved ee (39 %).

Effect of pH

As the next step, the racemic ethyl-3-hydroxybutanoate was incubated with *C. parapsilosis* ATCC 7330 (2.4 g of wet biomass) in 9.9 mL of Tris–HCl buffer (pH 8.5, 10 mM) and acetonitrile (100 µL, 1 %) improved the ee to 36 %. The ee further improved significantly up to 69 % when the reaction was repeated in 9.5 mL of the buffer with acetone (400 µL, 4 % of the total volume) in combination with acetonitrile (100 µL, 1 % of the total volume).

Screening of cosolvents

The improvement in the ee with acetonitrile (cosolvent) and acetone led us to study the effect of different co-solvents like tetrahydrofuran, hexane, chloroform, 1,4-dioxane, dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO) on the deracemisation of ethyl-3-hydroxybutanoate. The biocatalyst (2.4 g of wet cells) was suspended in 9.5 mL of Tris–HCl buffer (pH 8.5, 10 mM). To the cell suspension, 4 % of acetone (400 µL) was added followed by the addition of substrate (8 mg, 0.06 mmol) dissolved in 1 % (100 µL) of the cosolvent under study. The reaction was carried out for up to 24 h and the product formed was analysed as described earlier [31]. Of all the solvents tested, DMF and DMSO gave the highest ee (up to 98 %).

Reaction time

The deracemisation reaction with the model substrate ethyl-3-hydroxybutanoate (2b) using acetone (4 %) as the cosubstrate and DMF (1 %) up to 24 h was monitored for ee at 6 h intervals up to 18 h, after which it was monitored every 2 h. The reaction time was optimised to 20 h to produce the anti-Prelog (*R*)-enantiomer with high ee (98 %).

Preparation of (*R*)-ethyl-3-hydroxybutanoate by deracemisation of ethyl-3-hydroxybutanoate using the whole cells of *Candida parapsilosis* ATCC 7330 under optimised reaction conditions.

In a 150 mL Erlenmeyer flask, 15 g of wet cells of *Candida parapsilosis* ATCC 7330 was suspended in 28.5 mL of Tris–HCl buffer (pH 8.5, 10 mM), 1.2 mL of acetone was added to it and incubated at 25 °C and 150 rpm. The substrate, ethyl-3-hydroxybutanoate (98 mg, 0.74 mmol) which was dissolved in 300 µL of DMF was added and the reaction was continued for 20 h. After the reaction time, the crude product was extracted thrice with ethyl acetate, the organic layer was dried over anhydrous sodium sulphate and the solvent was removed by rotary evaporator and the

optically pure ethyl-3-hydroxybutanoate was obtained as a light yellow oil after purification with silica gel column chromatography using hexane:ethyl acetate (90:10) as eluent (ee: 98 %, yield: 62 %).

Using the optimised reaction conditions, the other alkyl-3-hydroxybutanoates (Fig. 2) were deracemised using *Candida parapsilosis* ATCC 7330 to their corresponding optically enriched enantiomers in varying reaction time 18–24 h. The enantiomeric excesses of the optically pure alkyl-3-hydroxybutanoates (3a–i, Table 1) were determined by chiral GC column using earlier reported conditions [31]. The control experiments were done in parallel without the whole cells and also using heat-killed cells under the same conditions.

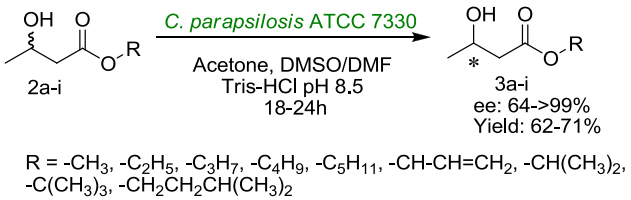


Fig. 2 Deracemisation of alkyl-3-hydroxybutanoates using *Candida parapsilosis* ATCC 7330 (present study)

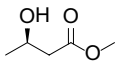
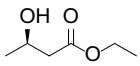
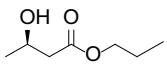
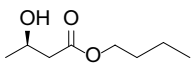
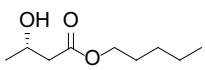
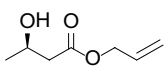
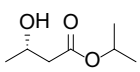
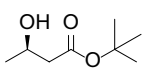
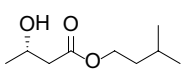
Procedure for cell viability studies of *Candida parapsilosis* ATCC 7330

The study was carried out as reported earlier [44] with the substrate, ethyl-3-hydroxybutanoate (2b) for the deracemisation reaction under optimised conditions i.e., using 2.4 g of wet biomass suspended in 9.5 mL of tris–HCl buffer (pH 8.5) with acetone as cosubstrate (4 %) and DMF (1 %) as cosolvent. An aliquot of the reaction mixture was taken every 6 h (up to 30 h) and was serially diluted up to 10^{-5} dilution fold. Under aseptic conditions, a fixed volume (100 µL) of 10^{-5} dilution was added into the pre-sterilised plates containing yeast malt agar medium and was spread thoroughly using a sterile L-shaped spreader. The plates were then incubated at 25 °C for 72 h and the number of viable colonies appeared in each plate were counted. Control experiments were carried out in parallel under identical conditions in the absence of the substrate.

Statistical analysis

All the experiments in the present study were carried out in triplicate under identical reaction conditions and the standard deviation is calculated. Hence, the variations observed in the experiments are presented with standard deviation (Table 1) and the corresponding error bars (Figs. 3, 5).

Table 1 Deracemisation of alkyl-3-hydroxybutanoates using *Candida parapsilosis* ATCC 7330

Entry	Product	Reaction time (h)	Yield (%)	% ee (configuration), retention time (in min); specific rotation $[\alpha]_D^{24}$ {lit. values, [ref]}[a]	Refs. for spectral data
3a		20	69 ± 2.1	>99 ± 0.2 (*R*), 17.95 (*S*), 18.18 (*R*); −28.1 (c 0.8, $CHCl_3$) {−41.9 (c 1.09, $CHCl_3$) (*R*) [40]}	[41]
3b		20	62 ± 4.7	98 ± 0.6 (*R*), 12.06 (*S*), 12.36 (*R*); −28.4 (c 0.7, $CHCl_3$) {+12.97 (c 1, $CHCl_3$), (*S*) [31]}	[41]
3c		22	66 ± 2.3	79 ± 2.5 (*R*), 14.47 (*S*), 14.86 (*R*); −18.1 (c 0.8, $CHCl_3$) {+24.3 (c 1.2, $CHCl_3$), (*S*), [31]}	[42]
3d		18	64 ± 4.0	>99 ± 0.3 (*R*), 15.06 (*S*), 15.30 (*R*); −20.9 (c 0.9, $CHCl_3$) {+19.36 (c 0.6, $CHCl_3$), (*S*) [31]}	[43]
3e		24	63 ± 3.8	>99 ± 0.2 (*S*), 27.75 (*R*), 27.94 (*S*); +21.2 (c 0.8, $CHCl_3$) {+24.75 (c 1, $CHCl_3$), (*S*) [31]}	[31]
3f		22	68 ± 3.7	>99 ± 0.4 (*R*), 12.93 (*S*), 13.08 (*R*); −35.8 (c 1.0, $CHCl_3$) {+28.2 (c 0.97, $CHCl_3$), (*S*) [41]}	[41]
3g		22	71 ± 3.3	66 ± 3.5 (*S*), 8.97 (*S*), 9.38 (*R*); +26.4 (c 0.7, $CHCl_3$) {+38.89 (c 1, $CHCl_3$) (*S*) [43]}	[43]
3h		22	67 ± 3.5	64 ± 2.1 (*R*), 4.44 (*S*), 4.52 (*R*); −18.4 (c 0.7, $CHCl_3$) {+32.3 (c 1. 03, $CHCl_3$) (*S*) [41]}	[41]
3i		24	64 ± 5.0	81 ± 1.5 (*S*), 11.59 (*R*), 11.91 (*S*); +20.5 (c 0.8, $CHCl_3$) {+23.95 (c 1.2, $CHCl_3$) (*S*) [31]}	[31]

[a] Enantiomeric excess was determined using chiral GC column as given in "Materials and methods"

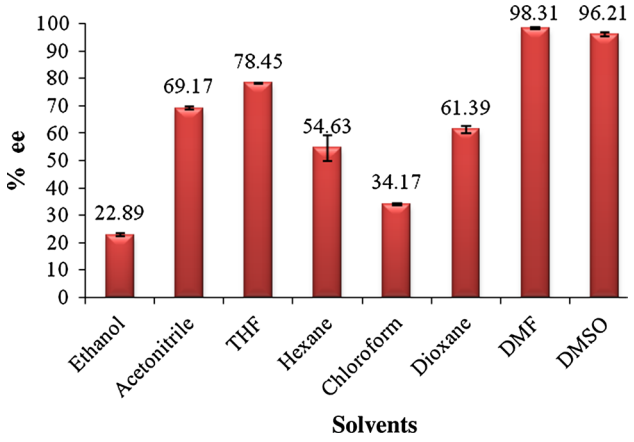


Fig. 3 The % ee of the product (*R*)-ethyl-3-hydroxybutanoate obtained from the deracemisation of racemic ethyl-3-hydroxybutanoate using *C. parapsilosis* ATCC 7330 in different cosolvents; each experiment was done in triplicate and the values shown are mean values with standard deviation

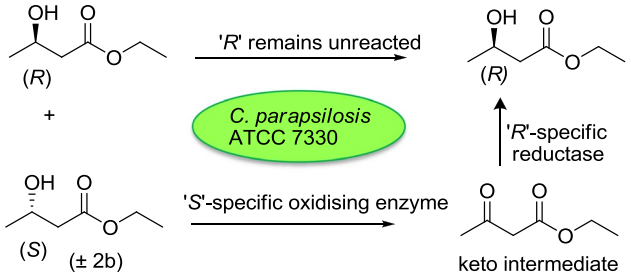


Fig. 4 Plausible mechanism (stereoinversion) for deracemisation of ethyl-3-hydroxybutanoate using *C. parapsilosis* ATCC 7330

Results and discussion

The deracemisation of ethyl-3-hydroxybutanoate (model substrate) using *Candida parapsilosis* ATCC 7330 in water using ethanol as a cosolvent [22] for 24 h resulted in only 9 % ee of the product (*R*)-alcohol, which increased to 24 % on addition of acetone (4 % of total volume) as a cosubstrate to the reaction mixture containing ethanol (1 % of total volume) as cosolvent. Acetone is known to act as a hydrogen acceptor to form 2-propanol [45] which improves cofactor regeneration in various oxidation–reduction reactions [46]. When a non-alcohol cosolvent, acetonitrile was used [31] instead of ethanol in addition to acetone, the product formed showed further improvement in ee (39 %). Alcohols are likely to be competing substrates for dehydrogenases, with possibly opposing enantiospecificities. Acetonitrile, on the other hand, is not a competing substrate and that could possibly explain the improved ee. Further increase of ee to 69 % was seen when instead of water, Tris–HCl buffer of pH 8.5, (10 mM) was used as the reaction medium. It is noteworthy that oxidation (first step of the deracemisation) is more favoured at basic pH i.e. the abstraction of hydride from the substrate [26, 33]. Deracemisation of aryl ethanols at pH 8.5, occurs at 1.02 mmol/h and the ee attained was maximum (>99 %), while at pH 5 the rate was 0.17 mmol/h and ee was 70 % [26].

The non-alcohol cosolvents i.e. DMSO and DMF do not compete for alcohol dehydrogenases. It is probably for this reason and solubility of the substrate that the product (*R*)-ethyl-3-hydroxybutanoate (Fig. 3), is formed in high ee (up to 98 %) when these cosolvents were used. In the case of *t*-butyl-3-hydroxybutanoate acetone and DMF (cosolvent) gave an ee 67 % and DMSO gave 52 % ee for the (*R*)-alcohol. The other alkyl-3-hydroxybutanoates gave higher ee in DMSO as cosolvent (Table 1). The role of cosolvents in improving optical purity in deracemisation of racemic (3*E*, 5*E*)-alkyl-2-hydroxy-6-arylhexa-3, 5-dienoates [47] and in asymmetric reduction [31] reactions was reported by us earlier.

Under optimised reaction conditions, the optically enriched alkyl-3-hydroxybutanoates were produced (Fig. 2; Table 1) in varying reaction times (18–24 h). In general, the deracemisation of aliphatic substrates requires longer reaction times with different biocatalysts (24–96 h) [33, 35] unlike the aryl α- and β- hydroxy esters which are deracemised using *C. parapsilosis* ATCC 7330 in only 1–6 h [22, 23, 26]. The plausible mechanism for deracemisation of *rac*-ethyl-3-hydroxybutanoate follows (Fig. 4) stereoinversion involving the oxidation of (*S*)-enantiomer to the corresponding ketone by a (*S*)-specific oxidising enzyme leaving the (*R*)-enantiomer unreacted. The prochiral ketone thus formed in situ was first observed at 4 h as confirmed by spiking with standard ethyl-3-oxobutanoate (retention time 9.04 min) using GC analysis. This then gets reduced to the (*R*)-enantiomer by a (*R*)-specific reducing enzyme (in Tris HCl buffer pH 8.5). Interestingly, the same ketone when used as a substrate (our previous study [31]) for asymmetric reduction in aqueous medium (pH 6.8) with acetonitrile as cosolvent and glucose as cosubstrate resulted in the formation of its optical counterpart (*S*)-enantiomer (ee 41 %).

Scope of substrates

Deracemisation of methyl-3-hydroxybutanoate (2a) in this study produced the (*R*)-enantiomer (3a, Table 1) (ee >99 %, yield 69 %) in 20 h, while Nakamura et al. [40] reported the same biotransformation using *Geotrichum candidum* IFO 5767 in 24 h (ee 97 %). In the present study, ethyl, propyl, butyl, allyl and *t*-butyl-3-hydroxybutanoates (2b, 2c, 2d, 2f and 2h) are deracemised to their anti-Prelog (*R*)-enantiomers (3b, 3c, 3d, 3f and 3h, Table 1) respectively, with maximum ee (up to >99 %) and good yields (up to 68 %) in 18–22 h.

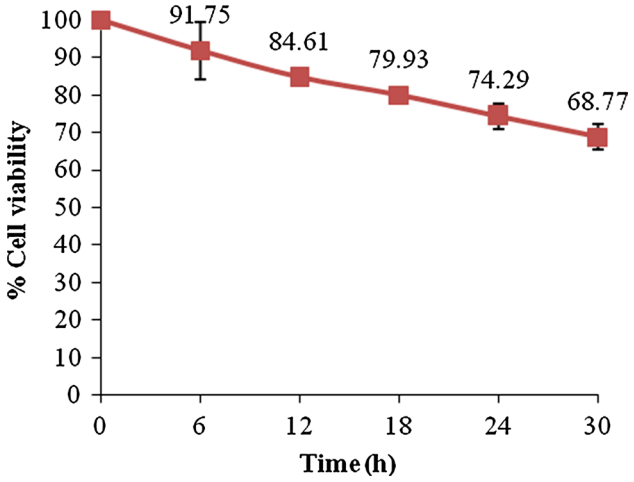


Fig. 5 The % cell viability of *C. parapsilosis* ATCC 7330 used in the deracemisation of ethyl-3-hydroxybutanoate carried out under optimised reaction conditions (mentioned in the "Materials and methods"); each experiment was done in triplicate in comparison with the blank and the values shown are mean values with standard deviation

The selectivity towards (*R*)-enantiomers by the biocatalyst for these substrates is in consensus with the hypothesis mentioned by Keinan and co-workers [48]. Earlier reports on the deracemisation of ethyl-3-hydroxybutanoate which was carried out in 16 h to its (*S*)-enantiomer (>99 % ee) used different biocatalyst systems [49] for oxidation and the subsequent reduction steps. Another trial for deracemisation of the same was carried out using multiple purified enantioselective dehydrogenases to obtain (*R*) or (*S*)-enantiomers using enantio-complementary enzymes [50]. In this case, the external addition of cofactors becomes essential and the subsequent addition of enzymes necessary for recycling these cofactors becomes inevitable. On the other hand, in the present study, the use of whole cells of *Candida parapsilosis* ATCC 7330 resulted in a one-pot two-step deracemization without the requirement of any purified enzymes and involvement of external addition of cofactors.

The deracemisation of pentyl-3-hydroxybutanoate (2e) gave the corresponding (*S*)-enantiomer (Prelog product) with maximum ee (>99 %; 3e, Table 1) while *iso*-propyl (2g) and *iso*-amyl 3-hydroxybutanoates (2i) resulted in the formation of (*S*)-enantiomers (Prelog products) with moderate ee 66 and 81 %, respectively (3g, 3i, Table 1). The substrates very likely follow the mechanism proposed for aryl β-hydroxy esters [51]. In our earlier study, the optically enriched (*S*)-alkyl-3-hydroxybutanoates were obtained by asymmetric reduction of alkyl-3-oxobutanoates using *Candida parapsilosis* ATCC 7330 in water (glucose was used as cosubstrate and acetonitrile as cosolvent) [31] while in the present study, the optically pure (*R*)-alkyl-3-hydroxybutanoates were obtained by deracemisation of the racemic alkyl-3-hydroxybutanoates using the same biocatalyst in Tris–HCl buffer (pH 8.5) medium along with acetone as cosubstrate and DMF/DMSO as cosolvent. It was also observed that the branching in the carbon chain (3g–i, Table 1) on the ester side showed comparatively lower ee (66, 64 and 81 %) than the linear chain bearing substrates (79, >99 and >99 %) (3c–e, Table 1) respectively, which may be attributed to steric factors.

Cell viability studies

The cell viability study was carried out using the reported protocol [44]. As seen in Fig. 5, 74 % of the cells were viable up to 24 h under the optimised reaction conditions stated in the "Materials and methods".

Conclusion

An efficient biocatalytic method for the deracemisation of different alkyl-3-hydroxybutanoates to produce predominantly anti-Prelog products (*R*)-enantiomers was developed using *Candida parapsilosis* ATCC 7330 with good yield (up to 71 %) and excellent ee (>99 %). The study highlights that the same biocatalyst can be used for deracemisation of alkyl-3-hydroxybutanoates as well. It also emphasises that the optical purity of the product can be improved by carrying out the optimization studies using different cosolvents, pH and reaction time using ethyl-3-hydroxybutanoate as the model substrate. Using optimized conditions, the racemic methyl, ethyl, propyl, butyl, *t*-butyl and allyl-3-hydroxybutanoates are deracemised to produce anti-Prelog (*R*)-enantiomers except for pentyl, *iso*-propyl and *iso*-amyl-3-hydroxybutanoates which were obtained as (*S*)-enantiomers (Prelog products). Notably, there was a significant decrease in the optical purity of substrates with branched chain on the ester side (64–81 %) compared to their linear counterparts (79 to >99 %). For the first time in literature, the biocatalytic deracemisation of propyl, butyl, pentyl, allyl, *iso*-propyl, *iso*-amyl and *t*-butyl-3-hydroxybutanoates with high optical purity is reported here.

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