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Characterization of Yeast Strains with Ketoreductase Activity for Bioreduction of Ketones

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Abstract

This study describes six yeast strains for stereoselective ketone reductions. The reaction conditions for the yeast strains (*Pichia carsonii*, *Lodderomyces elongisporus*, *Candida norvegica*, *Candida guillermondi*, *Debaromyces fabryi* and *Candida parapsilosis*) were optimized in a design of experiments for three ketones of different properties. The pH tolerance, temperature stability and productivity of the bioreductions with lyophilized cells of the yeast strains were characterized. In several cases, the optimized bioreductions resulted in enantiopure alcohols (*ee* > 99 %) with conversions ranging from moderate to excellent.

Keywords

ketoreductase, yeast, whole-cell bioreduction, cofactor regeneration, Design of Experiments (DOE)

1 Introduction

Whole-cell biocatalysts are easily applicable biomaterials which can be used in various fields such as food industry, agriculture, and fine chemical industry including the pharmaceutical industry. Whole-cell biocatalysts have several advantages over isolated enzymes [1]. Since whole cells have several sets of metabolic enzymes, they can be utilized in various types of reactions with different substrates. It is also possible to use the same whole-cell biocatalyst to produce different products, and by-products can be back-converted into main product with properly chosen reaction conditions. The production process with whole-cells is robust and beneficial because it does not require costly processes like enzyme purification steps [2].

The application of whole-cell biocatalysts in stereoselective biotransformations have been increasing for the past decades. The growing need of chiral Active Pharmaceutical Ingredients (APIs) creates a demand for novel and promising biocatalysts, such as yeasts [3–5]. There are plenty of APIs that possess chiral secondary hydroxyl groups, such as ezetimibe [6], fluoxetine [7], aprepitant [8] and atorvastatin [9]. The ketoreductase enzyme family is extensively researched and utilized as biocatalysts to create enantiopure alcohols from the corresponding prochiral ketones [5].

Yeasts are important industrial microorganisms because their production is simpler than that of recombinant bacterial cells. Furthermore, wild-type yeasts contain several metabolic pathways involving enzymes which can be applied to produce enantiopure compounds. Yeasts have already been applied successfully in acyloin condensation [10], bioreductions and amine production [11], as well as acylation of alcohols and amines [12]. Most yeasts harbor several ketoreductases (KREDs) with different properties. Because of the presence of multiple KRED activities, fine tuning of the reaction conditions for every employed ketone substrate is needed to achieve ideal productivity and selectivity. The most relevant parameters of optimization are pH, cosubstrate for co-factor regeneration, temperature and cosolvent. Planned Design of Experiments (DOE) are the best choice

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for investigating the significant parameters (effects and their interactions) of the reaction conditions to achieve the optimum for each bioreduction [13, 14].

Efficient cofactor regeneration is an indispensable step to realize successful ketoreduction because KREDs perform the ketone reduction with nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) as external cofactor or the alcohol oxidation with the oxidized form of these cofactors (NAD^+ or $NADP^+$) [15, 16]. In the case of industrial-scaled bioreductions, it is advisable to use small and cheap cosubstrates for regeneration of the external cofactor, such as 2-propanol, glucose, sodium formate or lactose [5].

Several yeast strains have already been published as robust biocatalysts in stereoselective bioreductions resulting in industrially relevant, optically pure alcohols and alcohol derivatives. *Lodderomyces elongisporus* immobilized in sol-gel matrix proved to be a competent and switchable biocatalyst for ketone reduction and acyloin condensation [17]. *Pichia glucozyma* showed high activity with several monosubstituted acetophenone derivatives, and catalyzed the bioreduction of several aromatic ketones such as with pyridyl, furanyl, thienyl aromatic ring and also ketoesters [18–20]. *Rhodotorula mucilaginosa* turned out to be a good candidate for the bioreduction of acetophenone derivatives in the presence of glucose co-substrate as well [21]. *Wickerhamomyces subpelliculosus*–another yeast strain showing efficient biocatalytic properties in the form of wet and lyophilized cells–could transform several simple ketones and a ketone with aryl and cyclopropyl moieties [22]. *Meyerozyma guilliermondii* isolated from marine environment and *Rhodotorula mucilaginosa* showed high molar conversion and good enantioselectivity in the bioreduction of acetophenone derivatives and *β*-ketonitriles [23]. *Yarrowia lipolytica* strains were tested on several mono- or disubstituted acetophenone derivatives performing bioreductions in moderate to good conversions, although with weak enantiomeric excess [24]. *Candida parapsilosis* is a widely studied yeast strain capable of acting on a broad substrate palette as efficient whole-cell biocatalyst [25–27].

Our paper presents optimized conditions of enantiotope selective bioreductions of three ketones **1a-c** (comprising aryl, aralkyl and alkyl substituents) by lyophilized whole-cells of six different yeast strains. To achieve the best conversion and enantiomeric excess for each of the ketones (**1a-c**), several reaction parameters (pH, temperature, cosubstrate amount) were optimized with DOE. This study also demonstrated the usefulness of the novel yeast strains in preparative scale bioreduction of ketones to enantiopure (*S*)-alcohols.

2 Experimental

2.1 Reagents and yeast strains

The substrates [1-phenylpropan-2-one (**1a**), acetophenone (**1b**), and heptan-2-one (**1c**)], the reagents and the solvents were purchased from Merck (Darmstadt, Germany) and Alfa Aesar Europe (Karlsuhe, Germany).

Yeast strains investigated in this study (*Pichia carsonii* (WY1), *Lodderomyces elongisporus* (WY2), *Candida norvegica* (WY4), *Candida guillermondi* (WY7), *Debaromyces fabryi* (WY11) and *Candida parapsilosis* (WY12)) originated from the Witaria strain culture collection. The yeast strains were prepared and lyophilized as described earlier [22, 28].

2.2 Analytical methods

The NMR spectra were recorded in $CDCl_3$ on a Bruker DRX-300 spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C, and signals are given in ppm on the δ scale. Infrared spectra were recorded on a Bruker ALPHA FT-IR spectrometer and wavenumbers of bands are listed in cm^{-1}. Optical rotation was measured on Perkin-Elmer 241 polarimeter at the D-line of sodium. The polarimeter was calibrated with measurements of both enantiomers of menthol.

Gas chromatography was used to determine the conversion and the enantiomeric excess of the bioreductions from ketones **1a-c**. Measurements were performed on 4890 GC equipment, Agilent with FID detector and Hydrodex *β*-6TBDM column [25 m × 0.25 mm × 0.25 µm film with heptakis-(2,3-di-*O*-methyl-6-*O*-*t*-butyldimethylsilyl)-*β*-cyclodextrin; Macherey & Nagel (Düren, Germany)] using H_2 carrier gas (injector: 250 °C, detector: 250 °C, head pressure: 12 psi, split ratio: 50:1). The temperature program and retention times are included in Table 1. Samples taken from the reaction mixture (150 µL for WY2, WY7 and WY11; and 500 µL for WY1, WY4 and WY12) were

Table 1 GC methods and retention times

Substrate	Method	Retention time (min)		
		1	(*S*)-**2**	(*R*)-**2**
1a	80 °C (5 min); 2 °C min^{-1} up to 120 °C	18.3	21.7	22.3
1b	120 °C (10 min)	3.4	6.5	6.9
1c	40 °C (65 min)	19.6	53.6	55.5

extracted with ethyl acetate (1 mL) and the organic layer was dried over sodium sulfate for 10 minutes. The samples in the EtOAc-extract were analyzed directly.

2.3 Reaction conditions and evaluation method

Optimization of the bioreduction conditions for the selected model ketones **1a-c** were planned with the DOE module of Statistica software. The following factors were investigated: lyophilized biocatalyst amount, co-substrate volume, buffer strength, temperature, and pH. The DOEs were designed and evaluated with Statistica 13 software for every strain–substrate combinations. The results were assessed with the Effect Estimates and Fitted Surface visualization. The first run of the optimization was designed in a $2^{(5-2)}$ design with foldover and triplicates in the center points. The curvatures and the significances of effects and interactions were evaluated. The investigated parameters have been considered to have no significant effect on the conversion or enantiomer excess if the *p*-values were above 0.05. Then, a second run of optimization with DOE was designed with 2^2 or 2^3 full factorial model with triplicates in the center points. The values of the significant reaction parameter were adjusted based on the results of the first optimization round. If the curvature check showed significant result ($p < 0.05$) the DOE was completed to a DOE of 3^2. In the quadratic models both the linear and the quadratic effects were evaluated.

Reactions with the strains WY1, WY4 and WY12 were done in 5 mL volume, reactions with the strains WY2, WY7 and WY11 were performed in 1 mL volume. Reactions were carried out at 6 mg mL^{-1} substrate concentration in sodium phosphate buffer with lyophilized yeast cells as biocatalysts in the presence of 2-propanol as cosubstrate. The reaction mixtures were shaken at 300 rpm for 4 h except for the productivity profiling where the samples were taken after 15 min. The lower, center point and higher values of the investigated parameters (determined by a set of preliminary experiments) were as follows: lyophilized biocatalyst concentration: 20; 30 and 40 mg mL^{-1}; cosubstrate concentration: 4; 5 and 6 % v/v; buffer strength: 32; 64 and 96 mM; temperature: 30; 35 and 40 °C and pH: 6; 7 and 8. In the second stage of the optimization, parameters were re-evaluated where needed. Table 2 shows the optimal reaction setups (where the maximal conversions were achieved) for every yeast strain and substrate.

After the optimization, we characterized the biocatalytic properties of the yeast strains in bioreduction of the three ketones **1a-c** by pH tolerance, temperature stability and substrate loading. For the property-profiling tests, the optimal reactions conditions were applied (Table 2). The pH profiles were investigated from pH 2 to 13, the temperature profiles were examined from 25 to 60 °C. The substrate-loading profile was studied between 2 and 20 mg mL^{-1} substrate concentration.

Table 2 Optimal reaction conditions of the bioreductions of ketones **1a-c** with lyophilized whole-cells of various yeasts determined by DOE

Yeast strain[a]	Sub-strate	Reaction parameter				Conver-sion (%)
		Biocatalyst (mg mL^{-1})	Co-substrate amount (% v/v)	*T* (°C)	pH (-)	
WY1	**1a**	40	3	35	7	97
	1b	40	4	30	7	83
	1c	40	4	30	7	84
WY2	**1a**	30	4	30	8	74
	1b	30	2	30	7	38
	1c	30	4	30	8	75
WY4	**1a**	40	8	30	7	81
	1c	40	8	30	7	83
WY7	**1c**	30	4	30	8	26
WY11	**1a**	30	4	27	7	68
	1b	30	2	27	7	35
	1c	30	8	30	7	84
WY12	**1a**	20	3	35	7	97
	1b	20	4	30	7	82
	1c	20	4	35	7	83

[a] Strains: *Pichia carsonii* (WY1), *Lodderomyces elongisporus* (WY2), *Candida norvegica* (WY4), *Candida guillermondi* (WY7), *Debaromyces fabryi* (WY11) and *Candida parapsilosis* (WY12)

2.4 Production of (*S*)-2a and (*S*)-2c on preparative scale

Lyophilized *Debaromyces fabryi* cells (1500 mg) were suspended in a 100 mL Erlenmeyer flask in sodium phosphate buffer (pH 7, 25 mL, 64 mM): To the cell suspension were added the substrate (**1a** or **1c**, 150 mg) and 2-propanol (4 % v/v for **1a**; 8 % v/v for **1c**) and the resulted mixture was shaken at 150 rpm for 24 h (for **1a** at 27 °C, for **1c** at 30 °C). Then the mixture was extracted with EtOAc (3 × 20 mL), the unified organic layers were dried on sodium sulfate and concentrated under reduced pressure. The resulting product was purified by plate chromatography (silica gel; eluent: hexane-EtOAc 10:4) to yield the enantiopure secondary alcohol [(*S*)-**2a** or (*S*)-**2c**].

(*S*)-**2a**: yield: 66 %; conversion: 70 % (determined with GC); *ee*: 99.9 % (by GC); colorless oil; R_f: 0.53; $[\alpha]_D^{25}$ = +38.2 (c 1.0, $CHCl_3$); lit.: $[\alpha]_D^{25}$ = +48.1 (c 1.0, benzene) [29]; IR (film): 3356, 2986, 1453, 1116, 1078, 936, 740, 697 and 504 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm:

1.3 (*d*, *J* = 6.1 Hz, 3H CH_3), 1.8 (s, 1H, OH), 2.7-2.8 (m, 2H, CH_2), 4.1 (m, 1H, CH), 7.3-7.4 (m, 5H, Ar-H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ ppm: 22.8 (CH_3), 45.8 (CH), 98.9 (CH), 126.1 (Ph), 128.59 (Ph), 129.5 (Ph), 130.6 (Ph). ^{1}H and ^{13}C NMR data agreed with the reported spectra [30].

(*S*)-**2c**: yield: 81 %; conversion: 83 % (determined with GC); *ee*: 99.9 % (by GC); colorless liquid; R_f: 0.71; $[\alpha]_D^{25}$ = +11.6 (c 1.0, $CHCl_3$); lit.: $[\alpha]_D^{25}$ = +12.1 (c 0.66, ethanol) [31], $[\alpha]_D^{25}$ = +10.4 (c 0.52, $CHCl_3$) [32]; IR (film): 3332, 2959, 2927, 2859, 1459, 1375, 1112 and 950 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 0.9 (*t*, *J* = 6.4 Hz, CH_3); 1.2 (*d*, *J* = 6.4 Hz, CH_3); 1.3-1.5 (m, 6H, C_3H_6); 1.6 (s, 1H, OH); 3.8 (m, 1H, CH); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ ppm: 14.2 (CH_3), 22.8 (CH_2), 23.6 (CH_3), 25.6 (CH_2), 32.0 (CH_2), 39.5 (CH_2), 69.4 (CH). IR and ^{1}H-NMR data agreed with the reported spectra [33].

3 Results and discussion

The six yeast strains investigated in this study [(*Pichia carsonii* (WY1), *Lodderomyces elongisporus* (WY2), *Candida norvegica* (WY4), *Candida guillermondi* (WY7), *Debaromyces fabryi* (WY11) and *Candida parapsilosis* (WY12)] were chosen from the Witaria strain collection to demonstrate the usability and adaptability of the yeasts as whole-cell biocatalysts in ketoreductions. The lyophilized yeast cells have excellent long-term storage stability which makes them robust biocatalysts [28]. All of the yeast strains were tested in bioreductions of three model ketones with different molecular properties [phenylacetone (**1a**: a ketone with a methyl and an aralkyl substituents), acetophenone (**1b**: a ketone with a methyl and an aryl substituents), and 2-heptanone (**1c**: a ketone with a methyl and a larger alkyl substituents)] (Fig. 1). The lyophilized biocatalysts were tested just after the preparation and after storage for six months at 4 °C. Activity tests with **1a** (except WY7 with **1c**) as substrate revealed less than 5 % decrease of conversion (1 %, 2 %, 2 %, 5 %, 3 %, and 1 % with WY1, WY2, WY4, WY7, WY11, WY12, respectively) of the bioreductions after the half year long storage.

Efficient cofactor regeneration is an essential requirement to perform efficient bioreductions with high conversion (Fig. 1). In this study, the so-called coupled-substrate strategy involving a cheap cosubstrate transformed by the same biocatalyst in opposite reaction direction was applied using 2-propanol as a cheap and easy-to-handle alcohol.

The following reaction parameters were investigated in the optimization of the bioreduction conditions: lyophilized biocatalyst mass, co-substrate volume, buffer

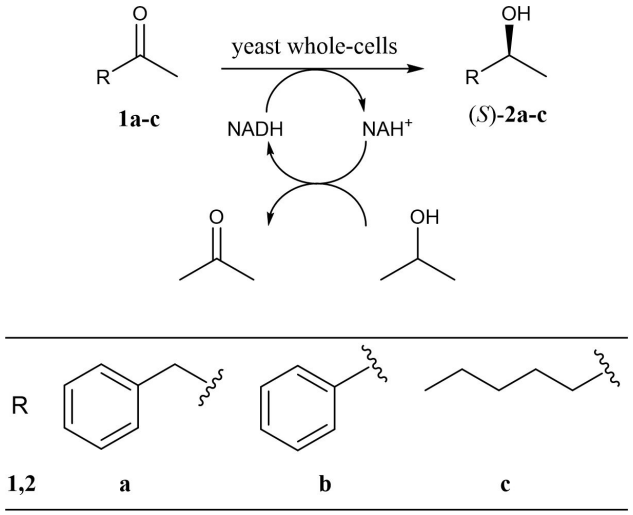

Fig. 1 Bioreduction of ketones **1a-c** into enantiopure alcohols (S)-**2a-c** with 2-propanol-mediated coupled-substrate cofactor regeneration

strength, temperature, and pH. The significance of the investigated parameters was calculated according to the DOE. The initial optimization was made in a $2^{(5-2)}$ DOE with foldover for all yeast strains and substrates. The results showed that the buffer strength was not a significant parameter, but the other parameters had diverse significance in the distinct reaction assemblies. Not all examined biocatalysts could utilize all substrates, WY4 and WY7 did not transform acetophenone **1b** and WY7 did not convert phenylacetone **1a**. The second round of the optimization was performed with 2^2, 2^3 and 3^2 DOE to achieve the maximal conversion for the bioreduction of each substrate. When conversion of the yeast-cell-based bioreductions of ketones **1a-c** exceeded 10 % under the optimal reaction conditions, the corresponding (*S*)-alcohol (*S*)-**2a-c** was produced in high enantiopurity (*ee* > 99.5 %). We have investigated the bioreductions after 4 h and 24 h (when the reactions reached stationary state). Under the optimal reaction parameters, there were no significant differences between conversion data after 4 h and 24 h reaction time.

For industrial application of biocatalysts, the pH-range of operation and the optimum pH are of high importance. The pH profile of each yeast strain with each substrate was examined in the pH-range from 2 to 12 (Fig. 2). The product formation from ketones **1a** and **1c** with the strains *P. carsonii* (WY1) and *C. parapsilosis* (WY12) was comparable in a wide pH-range (between 5 to 10). The bioreduction activity of *L. elongisporus* (WY2) on ketones **1a-c** was optimal between pH 7 and 10 (conversion in the 60–80 % range from **1a** and **1c**, and in the 20–40 % range from **1b**). The optimal pH was 7 for bioreduction of **1a** and **1c** with *C. norvegica* (WY4). The weakest conversion

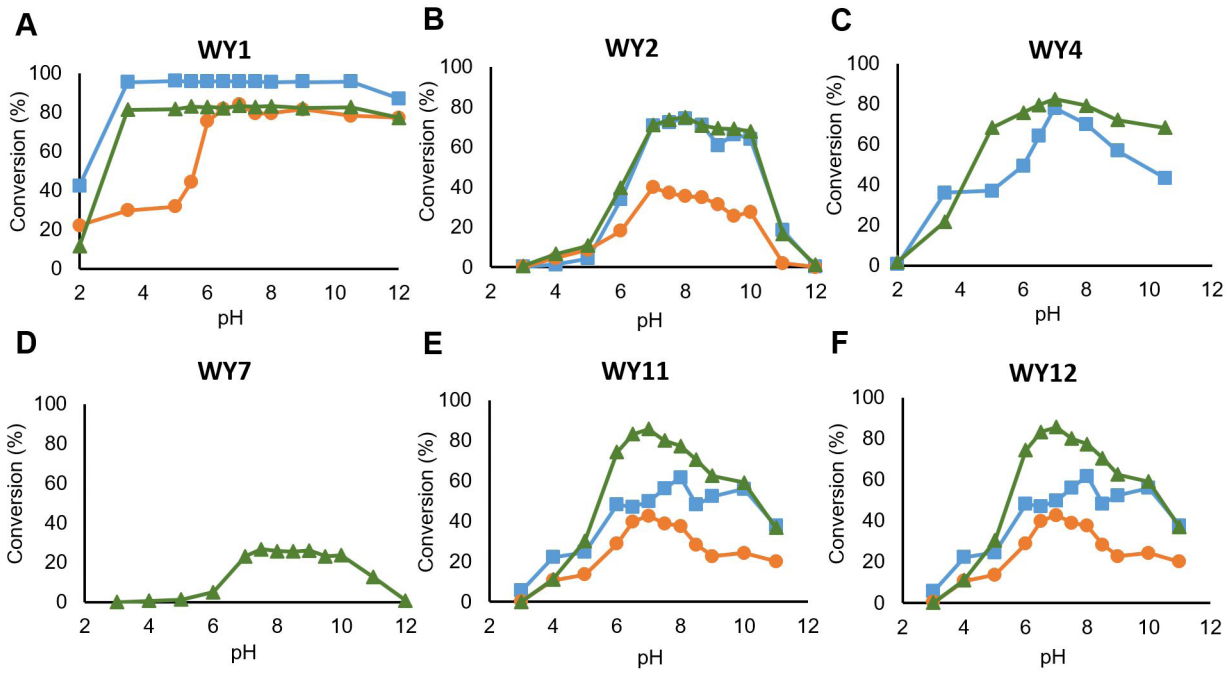

Fig. 2 Dependence of the conversion ketones **1a-c** with yeast strains on the pH [*Pichia carsonii* (WY1), *Lodderomyces elongisporus* (WY2), *Candida norvegica* (WY4), *Candida guillermondi* (WY7), *Debaromyces fabryi* (WY11) and *Candida parapsilosis* (WY12)] in bioreduction of ketones [blue square: phenylacetone (**1a**), orange circle: acetophenone (**1b**), green triangle: 2-heptanone (**1c**)]

(~20 %) from **1c** was observed with *C. guillermondi* (WY7) between pH 7 and 10. *D. fabryi* (WY11) showed optimal biocatalytic activity with **1c** at pH 7 achieving a conversion of 85.7 % which higher than c = 82.6 % with *C. parapsilosis* being one of the most commonly applied yeast strains for biocatalysis. Wild-type form of *Yarrowia lipolytica* was used in the bioconversion of acetophenone **1b** to create (*R*)-**2b** with 91–93 % conversion and with 90 % enantiomeric excess [24]. *Wickerhamomyces subpelliculosus* was used in the synthesis of (*S*)-**2a** with conversion 95–99 % and with enantiomeric excess higher than 99 %. The same yeast strain could catalyze the bioreduction of (*S*)-**1c** with 55 % conversion and 99.5 % enantiomeric excess [22].

Thermal stability of the bioreduction activity of the lyophilized yeast strains was investigated between 25 and 60 °C (Fig. 3). With all yeast strains in this study temperature optima of the bioreduction of ketones **1a-c** were between 27–35 °C. The least thermostable strains were *L. elongisporus* (WY2) and *C. guillermondi* (WY7) as their bioreduction activity decreased rapidly over the optimal 30 °C. The biocatalytic activity of *P. carsonii* (WY1), *C. norvegica* (WY4), and *D. fabryi* (WY11) decreased significantly only above 40 °C. The most thermotolerant was *C. parapsilosis* (WY12) up to 55 °C (conversion of **1a** decreased from 95 % at 35 °C only to 78 % at 55 °C whereas conversion of **1b** and **1c** remained almost constant).

Dependence of the activity on substrate concentration is an important aspect influencing directly the space-time yield achievable with a biocatalyst. In most cases, saturation concentration of the productivity above 5 mM could be observed (Fig. 4).

The bioreduction of phenylacetone **1a** to enantiopure (*S*)-1-phenylpropan-2-ol (*S*)-**2a** with *P. carsonii* (WY1) and *C. norvegica* (WY4) could be carried out with similar maximal productivity (10–15 μmol g^{-1} min^{-1}). A productivity of 28 μmol g^{-1} min^{-1} with *C. parapsilosis* (WY12), and 40 μmol g^{-1} min^{-1} with *L. elongisporus* (WY2) could be achieved. The highest productivity (60 μmol g^{-1} min^{-1}) of the bioreduction from **1a** was realized with *D. fabryi* (WY11) between substrate concentrations of 4 and 8 mM. The decay of bioreduction productivity from **1a** above 10 mM substrate concentration with *L. elongisporus* (WY2) and *D. fabryi* (WY11) indicated significant substrate / product inhibition of these strains. The only strain which could not reduce **1a** was *C. guillermondi* (WY7).

No bioreduction happened from acetophenone **1b** with *C. norvegica* (WY4) and *C. guillermondi* (WY7). The production of enantiopure (*S*)-1-phenylethanol (*S*)-**2b** by reduction of **1b** with *P. carsonii* (WY1) proceeded with low productivity (5 μmol g^{-1} min^{-1}). About twice of this productivity (~10–15 μmol g^{-1} min^{-1}) could be reached with *L. elongisporus* (WY2), *D. fabryi*

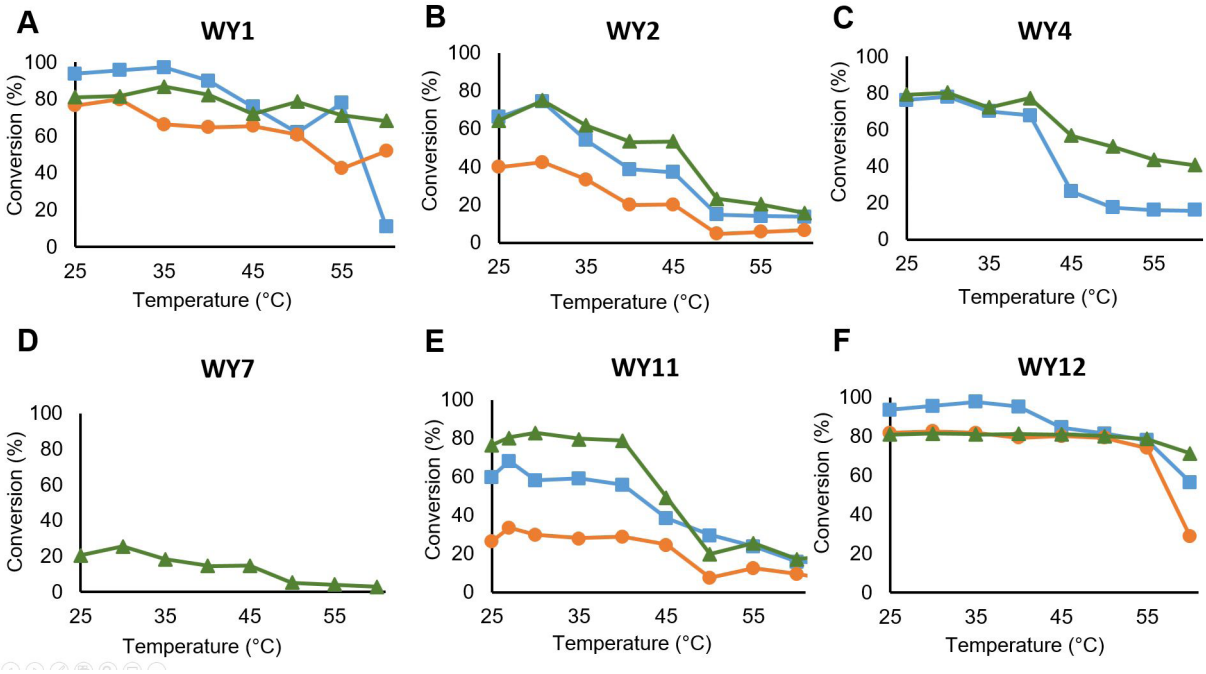

Fig. 3 Temperature tolerance of the yeast strains [*Pichia carsonii* (WY1), *Lodderomyces elongisporus* (WY2), *Candida norvegica* (WY4), *Candida guillermondi* (WY7), *Debaromyces fabryi* (WY11) and *Candida parapsilosis* (WY12)] in bioreduction of ketones [blue square: phenylacetone (**1a**), orange circle: acetophenone (**1b**), green triangle: 2-heptanone (**1c**)]

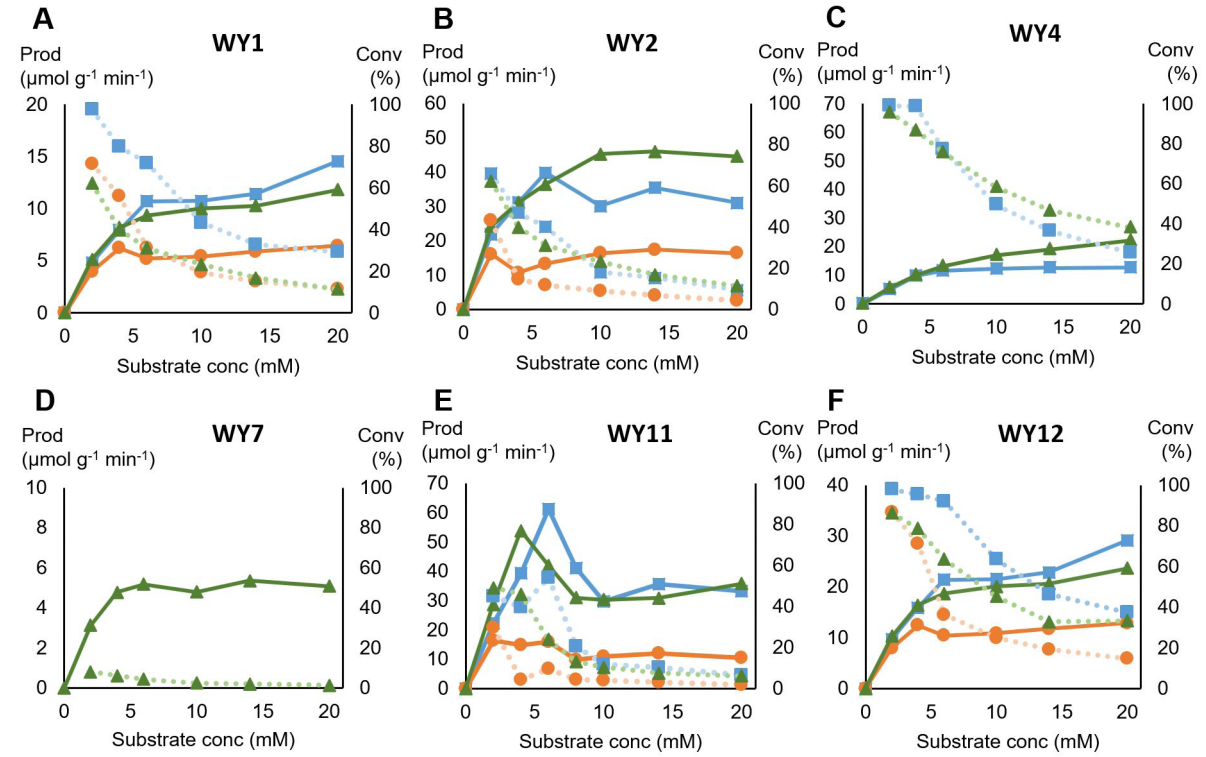

Fig. 4 Dependence of the productivity and conversion in the bioreduction of ketones **1a-c** on the substrate concentration [blue square: phenylacetone (**1a**), orange circle: acetophenone (**1b**), green triangle: 2-heptanone (**1c**); continuous line: productivity; dashed line: conversion] in bioreductions with various yeast strains [*Pichia carsonii* (WY1), *Lodderomyces elongisporus* (WY2), *Candida norvegica* (WY4), *Candida guillermondi* (WY7), *Debaromyces fabryi* (WY11) and *Candida parapsilosis* (WY12)]

(WY11) and *C. parapsilosis* (WY12). All the effective strains indicated substrate / product inhibition of the reduction above 2–4 mM concentration of **1b**.

Production of (*S*)-heptan-2-ol (*S*)-**2c** from the aliphatic ketone **1c** happened with all six investigated yeast strains. Moderate productivity (5–8 μmol g^{-1} min^{-1}) could be reached with *P. carsonii* (WY1) and *C. guillermondi* (WY7). The *C. norvegica* (WY4) and *C. parapsilosis* (WY12) strains could reach higher productivity (~20 μmol g^{-1} min^{-1}) in bioreduction of **1c** without serious substrate / product inhibition. The highest 45–50 μmol g^{-1} min^{-1} productivity of the (*S*)-**2c** formation could be reached with *L. elongisporous* (WY2) and *D. fabryi* (WY11), although at different substrate concentrations (above 10 mM with WY2 but at 4 mM with WY11). Only the strain *D. fabryi* (WY11) exhibited serious substrate / product inhibition in the bioreduction of **1c** above 4 mM substrate concentration.

To demonstrate the real synthetic usefulness of the yeast strains in this study, *D. fabryi* (WY11) was used to prepare (*S*)-**2a** and (*S*)-**2c** on preparative scale under the optimal reaction conditions. In the bioreduction of ketone **1a** using 4 % v/v 2-propanol as cosubstrate 76 % isolated yield of (*S*)-**2a** (*ee* = 99.9 %) was achieved. The ketone **1c** was also transformed to (*S*)-**2c** (*ee* = 99.9 %) in presence of 8 % v/v 2-propanol cosubstrate in 81 % isolated yield.

4 Conclusion

This study indicated that lyophilized yeast cells having excellent long-term storage stability are easy-to-handle biocatalysts for ketoreductions resulting in cost-effective synthesis like with recombinant enzymes. The bioreduction conditions were optimized for three different ketones with six different yeast strains by using extensive Design of Experiments. Our results highlighted that *P. carsonii* exhibited better bioreduction activity on 2-heptanone and phenylacetone in a wide pH range as the well-characterized *C. parapsilosis* strain. Furthermore, the production of (*S*)-1-phenylpropan-2-ol (*S*)-**2a** and (*S*)-heptan-2-ol (*S*)-**2c** by bioreduction with *L. elongisporus* and *D. fabryi* could be achieved with twice of the productivity than that of achievable with *C. parapsilosis*. The lyophilized form of *D. fabryi* proved to be an efficient biocatalyst in the production of enantiopure (*S*)-1-phenylpropan-2-ol (*S*)-**2a** and (*S*)-heptan-2-ol (*S*)-**2c** on preparative scale.

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