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Biocatalysis using Rajma - a green process for the synthesis of chiral carbinols

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Summary

A simple biocatalytic reduction of acetophenone and its derivatives was investigated in aqueous medium using soaked Rajma. The bioreduction process exhibited anti-Prelog specificity and furnished (*R*)-carbinols. This non-conventional bioreduction process showed a broad substrate specificity and chiral selectivity and could therefore provide a practical utility to traditional synthetic chemists for the preparation of chiral carbinols in terms of simplicity, and in an environmentally benign way. Being an easy and handy protocol, it also represents the possibility of scaling up the process to be used in organic synthesis.

Keywords: biocatalysis, chirality, rajma, anti-Prelog specificity

Introduction

Asymmetric reduction of prochiral ketones is one of the most fundamental and straightforward approach for producing chiral alcohols. It is an essential transformation in organic synthesis, both in the laboratory and in the industry. Chiral alcohols occupy a central place, as they are intermediates in the synthesis of pharmaceuticals, pheromones, pesticides, flavours, fragrances and advanced materials like liquid crystals (Zheng and Xu, 2011; Lakshmi et al., 2011; Faber, 2004; Wu and Xiao, 2007; Patel, 2002; Nakamura et al., 2003; Chattrain et al., 2001). Moreover, they can be easily transformed into other functional group(s) without racemisation.

Various metal-mediated chemical reductions are associated with harsh conditions, require hazardous metals, use of expensive chiral ligands, complicated synthetic steps, and often raise ethical issues about the environmental impact. Biocatalysts offer an alternative tool for the easy access of chiral alcohols by the enantioselective reduction of the corresponding ketones. The main advantages of biocatalytic methods are commercial availability of biocatalysts at low cost, use of aqueous medium, mild reaction conditions, acceptance of xenobiotic substrates from a broad range, easy recovery of the product, high stereoselectivity and a disposal of the spent biological materials, as they are biodegradable. The isolated oxido-reductase produce high optical and theoretical yields of chiral alcohols, but these enzymes require expensive co-factors. On the other hand, whole-cell biocatalysts ensure recycling of co-factors, and also avoid a number of downstream processes required for the isolation and purification of enzymes which are stable within the cells.

Even though microbial whole-cell systems have an economical advantage due to the inherent cofactors and cofactor regeneration system, these cultures are not easily accessible to the organic chemists, who are not familiar with the cell cultivation and maintenance, except for the baker's yeast. Plant cell cultures represent a unique class of chiral reagents in the modern organic synthesis and have the ability to carry out certain chemical reactions (Bordón et al., 2015; Suárez-Franco et al., 2010; Chang et al., 2010; Orden et al., 2009; Matsuo et al., 2008; Machado et al., 2008; Yang et al., 2008; Ishihara et al., 2003; Bruni et al., 2002). Among these plant cell systems, carrots (*Daucus carota* L.) (Ravia et al., 2006; Blanchard and Weghe, 2006; Caron et al., 2005; Comasseto et al., 2004; Mączka and Mironowicz, 2004; Yadav et al., 2002; Baldssare et al., 2000; Akakabe et al., 1995), tobacco (*Nicotiana tabacum* L.) (Takemoto et al., 1995; Hamada et al., 1988), soaked mung beans (*Phaseolus aureus* L.) (Kumaraswamy and Ramesh, 2003), *Vigna unguiculata* L. (Bizerra et al., 2010), Adzuki beans (Xie et al., 2009), sprouted *Pisum sativa* L. (Yadav et al., 2009) etc. are used effectively for the stereospecific reduction of ketones to the corresponding alcohols with moderate to good enantioselectivities and yields.

The whole-plant cells as biocatalyts have many merits over microbial cells. A large number of taxonomically different plants are cultivated all over the world throughout the year. They are commercially easily available from local markets at very low cost and can be preserved for prolonged periods. Many of them are edible and they occupy an important place in the human diet, and are therefore more suitable as green catalysts in the food and pharmaceutical industries. Both growing and intact

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cells can be used as biocatalysts. Vegetable seeds as a whole-cell system also ensure cofactor recycling. Moreover, they can be easily separated from the reaction mixture just by filtration, and could be disposed of, as they are biodegradable or reused as manure (Kumaraswamy and Ramesh, 2003). As a part of the project on the application of biocatalysts for the synthesis of chiral carbinols, the ability of various vegetable seeds as reducing agents has been studied. The obtained results have been discussed in this paper.

Materials and methods

Chemicals

All substrates were products from Fluka and Lancanster, and were used as received. (*R*)-(+)-α-Methoxy-α-trifluoromethylphenylacetic acid (MTPA) was purchased from Fluka. All other reagents and solvents were of the AR grade from Sigma-Aldrich-Fluka. All seeds were obtained commercially from the Chembur local market, Mumbai, India.

Instruments

The IR spectra were scanned as films on the Jasco model A-202 FT-IR spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with the BrukerAC-200 spectrometer (200 MHz), and the Varian 500 MHz. Optical rotations were recorded with the Jasco DIP 360 digital polarimeter.

General bioreduction process

The surface sterilization of the commercially available seeds was done by rinsing with tap water, with 1% sodium hypochlorite solution, and then with sterilized water. The treated seeds (10 g) were then soaked in 100 mL sterile, distilled H_2O in a 500 mL Erlenmeyer flask, which was closed with a cotton plug for 24 h at room temperature. Next, 5% glucose was added, followed by the substrate (100 mg / mL ethanol), and the reaction mixture was then incubated on an orbital shaker (100 rpm) at room temp (24-25 °C) for 3 days. The substrate and the seed controls were also run simultaneously. The suspension was then filtered through muslin cloth, and seeds were washed with water. This filtrate was then extracted with chloroform (3 x 50 mL), washed with water (2 x 20 mL), and the organic layer was dried on the sodium sulphate, filtered and concentrated under vacuum. The controls were also extracted in a similar way. The transformed product and the unchanged substrate were isolated from the filtrate extracts and purified by preparative TLC (silica gel G, EtOAc / Pet. Ether). The structure of the produced alcohols was corroborated by ^{1}H and ^{13}C NMR spectroscopy, and was found to be in agreement with the literature data (Salvi and Chattopadhyay, 2016; 2008; 2001). For the determination of the % of ees, the chiral carbinols were converted into the corresponding MTPA esters with the (*R*)-MTPA (Dale and Mosher, 1973).
The % of ees was then assayed by the ^{1}H NMR analyses of the respective esters.

Results and discussion

Screening of vegetable seeds

In order to choose the best and most suitable biocatalysts for the reduction of acetophenone, and an interesting xenobiotic, initially we screened various locally available edible seeds. The reaction of acetophenone (1) to chiral 1-phenylethanol has been widely studied as a model reaction for the bioreduction, because of its low cost and easy accessibility. It's both enantiomers are important natural aromas. The (*S*)-1-Phenylethanol is characterized by a mild hyacinth, gardenia aroma with strawberry nuances (Farbood et al., 2004), while the (*R*)-1-phenylethanol has a floral, earthy-green, honeysuckle odour (Leffingwell, 2010). Both enantiomers are also used as building blocks for the synthesis of bioactive compounds, such as pharmaceuticals, agrochemicals and natural products (Mukaiyama et al., 2005; Schoemaker et al., 2003; Zaks, 2001; Wandrey et al., 2000; Nakamura and Matsuda, 1998). Most alcohol dehydrogenases like *Rhizopus arrhizus* (Salvi and Chattopadhyay, 2016; 2008; 2001), *Hansenula capsulata* cells (Hasegawa et al., 1998), *Rhodotorula* glutinis (Kurbanoglu et al., 2010), *Candida utilis* (Cheng and Ma, 1996), *Geotrichum candidum* (Matsuda et al., 2008), catalyze the reduction of acetophenone to (*S*)-1-phenylethanol as per Prelog's rule (Prelog, 1964). The anti-Prelog reduction of acetophenone to (*R*)-1-phenylethanol was observed with the alcohol dehydrogenases from several bacterial strains, for example, *Lactobacillus kefir* (Hummel, 1990), *Lactobacillus brevis* (Schlieben et al., 2005), *Thermoanaerobacter ethanolicus* (Protsko et al., 2010), and *Pichia capsulata* (Homola et al., 2015).
As shown in Table 1, the bioreduction with various seeds occurred in a well-defined fashion. Among these plant seeds, mung beans gave better yields and optical purities, as shown by Kumaraswamy and Ramesh (2003). Most of the tested seeds furnished (*S*)-alcohols, which is in perfect agreement with Prelog's rule. In contrast, Rajma exhibited anti-

Prelog specificity and produced (*R*)-1-phenylethanol, which is rather rare. Because of the equal importance of both enantiomers, there is a demand for the carbonyl reductase with both Prelog and anti-Prelog stereopreference, for the production of (*S*) and (*R*)-configured alcohols. Considering the observed carbonyl reductase activity from Rajma, the reduction of various prochiral arylalkanones was investigated. The generosity and efficiency of these plant cell enzymes was assayed toward various acetophenone derivatives to study the mechanistic aspects of their reduction process and optical purities of the produced alcohols. The relationship between the nature of the substrates and the extent of their bioreduction was studied without optimizing the reaction conditions for each substrate.

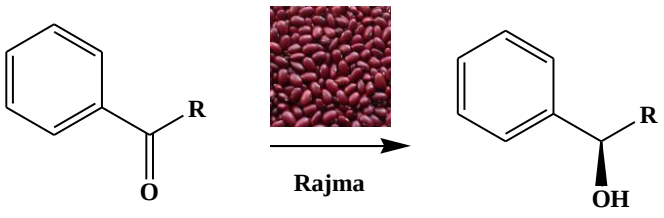
Bioreduction of homologous series of acetophenone using Rajma

First we targeted a homologous series of acetophenone (Fig. 1, 1 - 15) to see the effect of size of the alkyl groups on the reactivity and the enantioselectivity of the produced alcohols. It is evident from Table 2 that the carbonyl reductase from Rajma effectively catalyzed the reduction of arylalkyl ketones having a number of diverse alkyl groups. The reactivity decreased as the alkyl chain length increased (1 → 5). The Rajma mediated reduction of acetophenone to valerophenone (1 - 4) generated (*R*)-isomers as the major product. The anti-Prelog selectivity from Rajma mediated bioreduction was found to be complementary to that of Prelog's specificity. This feature confers an added versatility and attractiveness of this asymmetric bioreduction process.

Table 1. Bioreduction of acetophenone using various vegetable seeds

Entry	Seeds	Botanical names	% Yield[a]	% e.e.[b]	Config.[c]
1	Mung beans (green gram)	*Vigna radiata*	48	84	*S*
2	Moth beans	*Vigna aconitifolia*	31	14	*S*
3	Black gram (urad)	*Vigna mungo*	50	55	*S*
4	Masoor	*Len culinaris*	31	13	*S*
5	Cow beans	*Vigna sinensis*	44	41	*S*
6	Rajma (red kidney beans)	*Phaseolus vulgaris*	38	82	*R*
7	Wheat	*Triticum asetivum*	29	75	*S*
8	Rice	*Oryza sativa*	n. r.	n. r.	n. r.
9	Jawar	*Sorghum bicolour*	17	28	*S*
10	Bajra	*Pennisetum typhoides*	16	78	*S*
11	Ragi	*Eleusine coracava*	7	79	*S*

Surface sterilized seeds (10 g / 100 mL sterile distilled water), 24 h soaked, 5% glucose, acetophenone (100 mg / mL ethanol), 3d stirring on the orbital shaker (100 rpm), [a]isolated yields, [b]based on comparison of optical rotation values from known literature values, [c]from analogy with reported data, duplicate. n. r. (no reaction)



1 – 15 **(*R*) – 1a – 15a**

Ketones		Alcohols
1: R = CH_3	6: R = $(CH_2)_5CH_3$	11: R = $CH(CH_3)_2$
2: R = CH_2CH_3	7: R = $(CH_2)_6CH_3$	12: R = $CH_2CH(CH_3)_2$
3: R = $CH_2CH_2CH_3$	8: R = $(CH_2)_7CH_3$	13: R = $C(CH_3)_3$
4: R = $(CH_2)_3CH_3$	9: R = $(CH_2)_8CH_3$	14: R = Cyclopropane
5: R = $(CH_2)_4CH_3$	10: R = $(CH_2)_{10}CH_3$	15: R = Cyclohexane

Fig. 1. Bioreduction of homologous series of acetophenone using Rajma

The absolute configuration of the produced alcohol of the substrate 5 is switched from (*R*) to (*S*) when the length of the linear alkyl chain increases from 4 to 5 carbon atoms. This could be due to the change in the substituent's priority according to the CIP rule. The interaction of the reductase present in the biocatalyst with both the faces of the carbonyl group may form two competing *R* and *S* transitions states, out of which one is more favored. The sterically demanding alkyl group forces the substrates to approach from the opposite face, compared to less bulky alkyl group. This indicated the dependency of stereochemistry of the produced alcohol on the size of phenyl and the alkyl group. However, the enzyme activity from Rajma diminished when the alkyl chain length was further increased (6 - 10), both in cyclopropyl and cyclohexyl ketones (14, 15). Consequently, as the bulkiness of the alkyl group increased, the reactivity decreased drastically indicating the substrate dependency on the performance of the enzymatic system. This could be due to the poor solubility of the substrates in the reaction mixture, and / or their inability to penetrate through the cell wall due to the size incompatibility.

Notably, ketones with sterically bulky branched alkyl substituents, such as *iso*-propyl, *iso*-butyl and *tert*-butyl (11, 12 and 13) were enzymatically reduced to the corresponding chiral alcohols, and the enantiopreference remained unchanged. This may be due to the structural properties of the ketones themselves. Unexpectedly, higher enantioselectivity was obtained towards the reduction of the substrate 13, having the *tert*-butyl group than those found for *iso*-propyl and *iso*-butyl groups (11 and 12), and its linear counterpart (4). The substrate 13 has sterically demanding bulky *tert*-butyl group with a less free rotation and more rigidity, which enables the enzyme to maximize the selectivity of the produced alcohols, 13a. While the substrates 11 and 12, having one and two alkyl group separating phenyl and branched alkyl chain respectively, have more free rotation. Since both are less rigid, the reduction process is less selective. The order of enantiomeric excess is 13a > 11a > 12a. Thus, an interesting alkyl chain-induced enantiopreference flip-flop was observed in this biotransformation studies (Zhu and Hua, 2010; 2006).

Bioreduction of mono-substituted acetophenones using Rajma

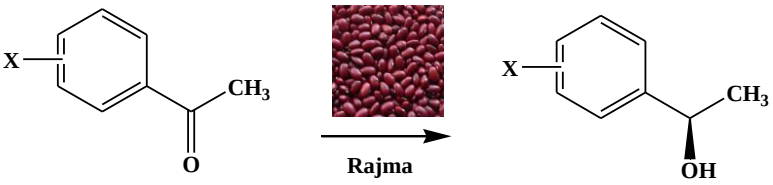
The ability and the scope of Rajma biocatalysis was evaluated for the reduction of mono-substituted acetophenones, 16 – 33 (Figure 2). The effect of substituents on the formation of the produced alcohols, and its enantioselectivity was studied, and the obtained results are summarised in Table 3. It was observed that most of these substrates acquiescent to Rajma mediated bioreduction to furnish chiral alcohols. The process showed favourable interactions between substrates and the enzymes present in the seeds. The change of the substituent group on the phenyl ring did not remarkably affect the stereoselectivity. It could be easily distinguished between small and large groups, in an enantiomeric way *via* transfer of hydrogen, either from *Re*- or *Si*-face. The reductase enzyme from Rajma exclusively produced *R*-alcohols, although in different yields and e.es.

Rajma mediated bioreduction of all halo substituted acetophenones showed similar conversion of the produced alcohols. This enzyme showed a clear preference for fluoro substituted acetophenones, less sterically hindered electron withdrawing group. (*R*)-alcohol 16a, 17a and 18a generated as products furnished best optical purities indicating negligible influence of the position of the substituent. The order of e.e. was *o* > *m* > *p*. But the behaviour of chloro- and bromo- substituted acetophenones was not so predictable. The *meta*- and *para*- chloro- and bromo-acetophenones produced (*R*)-alcohols (20a, 21a, 23a, 24a) with better optical purities compared to the *ortho*-substituted acetophenones (19, 22). The influence of two electron donor methyl and methoxy substituents, at *ortho*-, *meta*- and *para*-positions of acetophenone was also investigated in Rajma mediated bioreduction.

Table 2. Bioreduction of homologous series of acetophenone using Rajma

Substrates	Alcohol	% Yield[a]	$[\alpha]_D^{26}$ (c,$CHCl_3$)	% e.e.[b]	(Confg[c])
1	1a	38	+38.243 (0.774)	82	(*R*)
2	2a	37	+29.975 (1.608)	75	(*R*)
3	3a	35	+1.395 (1.434)	36	(*R*)
4	4a	18	+4.932 (0.730)	17	(*R*)
5	5b	8	-11.976 (0.334)	45	(*S*)
11	11a	29	+23.858 (1.182)	77	(*R*)
12	12a	17	+2.319 (0.690)	31	(*R*)
13	13a	21	+20.561 (0.856)	89	(*R*)

Surface sterilized beans (10 g / 100 mL DW), 24 h soaked, 5% glucose, substrate (100 mg / mL ethanol) [a]isolated yields. [b]based on ^{1}H NMR spectra of the corresponding (*R*)-MTPA esters, [c]from analogy with reported data.



16 – 33 **(*R*) – 161a – 33a**

Ketones		Alcohols
16: X = *o*-F-Ph	22: X = *o*-Br-Ph	28: X = *o*-OMe-Ph
17: X = *m*-F-Ph	23: X = *m*-Br-Ph	29: X = *m*-OMe-
18: X = *p*-F-Ph	24: X = *p*-Br-Ph	30: X = *p*-OMe-Ph
19: X = *o*-Cl-Ph	25: X = *o*-Me-Ph	31: X = *o*-NO_2-Ph
20: X = *m*-Cl-Ph	26: X = *m*-Me-Ph	32: X = *m*-NO_2-Ph
21: X = *p*-Cl-Ph	27: X = *p*-Me-Ph	33: X = *p*-NO_2-Ph

Fig. 2. Bioreduction of mono-substituted acetophenones using Rajma

With regard to methyl derivatives, all ketones furnished the corresponding (*R*)-alcohols (25a, 26a, 27a) in excellent enantioselectivities, and their order is *o* > *m* > *p*. Notably, the *meta*-methoxyacetophenone (29) furnished corresponding (*R*)-alcohol (29a) with an excellent enantioselectivity, whereas o*rtho*-methoxy acetophenone (28) was reduced to (*R*)-alcohol (28a) with better yields and good optical purities. The presence of the methoxy group at *para*-position (30) hindered the reduction.

The electron withdrawing nitro group at the *ortho*-position, being less reactive, hindered the reduction process significantly with no conversion. This is possible because the steric hindrance between the NO_2 and the C=O group hampers the reduction (Contente, et al., 2016). Both *meta*- and *para*-substituted nitro acetophenones (32, 33) furnished chiral alcohols (32a, 33a) with good optical purities, without the reduction of the nitro goup. Between these two, *meta*-nitro acetophenone (32) showed better conversion than that of *para*-nitro acetophenone (33).

Table 3. Bioreduction of arylalkanones using soaked Rajma

Substrates	Alcohol	% Yield[a]	$[\alpha]_D^{26}$ (c, $CHCl_3$)	% e.e.[b]	(Confg[c])
16	16a	37	+36.170 (0.752),	98	(*R*)
17	17a	36	+27.824 (0.726),	94	(*R*)
18	18a	34	+34.694 (0.686),	91	(*R*)
19	19a	40	+19.059 (0.808),	61	(*R*)
20	20a	26	+21.132 (0.530),	84	(*R*)
21	21a	30	+32.353 (0..612),	87	(*R*)
22	22a	39	+19.697 (0.792),	67	(*R*)
23	23a	29	+21.017 (0.590),	84	(*R*)
24	24a	32	+17.925 (0.636),	82	(*R*)
25	25a	57	+56.326 (1.154),	98	(*R*)
26	26a	29	+34.602 (0.578),	96	(*R*)
27	27a	24	+38.333 (0.480),	91	(*R*)
28	28a	57	+14.339 (1.411),	77	(*R*)
29	29a	40	+34.286 (0.560),	94	(*R*)
32	32a	58	+31.508 (0.317),	84	(*R*)
33	33a	21	+19.498 (0.207),	82	(*R*)

Sterilized beans (10 g/100 mL DW), 24 h soaked, 5% glucose, substrate (100 mg/mL ethanol), [a]Isolated yields. [b]Based on ^{1}H NMR spectra of the corresponding (R)-MTPA esters, [c]From analogy with reported data.

Thus, the study demonstrated the applicability of the simple whole-cell system of Rajma biocatalysis of a broad spectrum of substituted acetophenone derivatives to chiral carbinols. The % of optical purities of these chiral alcohols were assayed by the ^{1}H NMR analyses of the respective MTPA esters. In ^{1}H NMR spectra, the methoxy resonance of MTPA ester appeared as two singlets, one at ~ δ 3.58 for *S*-isomers, and other at ~ δ 3.46 for *R*-isomers. The up field ^{1}H NMR signal appeared as major in case of alcohols obtained from Rajma mediated bioreductions, and endorsed as (*R*)-carbinols, anti-Prelog alcohols. It was found that Rajma can be utilized for the bioreduction of broad substrate spectrum since it was found to be a promising biocatalyst for the preparation of anti-Prelog chiral alcohols.

The low yields of the obtained alcohols can be explained on the basis of the complicated extraction procedure. During this process, the emulsions formed, that were difficult to break (Bordón et al., 2015), which might have hampered the isolation procedure. At the end, the recovered Rajma was used to test the feasibility of recycling of the biocatalyst. After the completion of the first reduction cycle for acetophenone, the reduction process was repeated for two times in the batch process. It was observed that the yield of the produced alcohol reduced to 20% in the second cycle, and declined significantly thereafter. After this, the seeds can be used as manure, thus minimizing waste (Kumaraswamy and Ramesh, 2003).

Preparative scale reduction

The preparative scale production of the chiral compound is one of the most important issues to be addressed in the asymmetric synthesis. The experimental simplicity, low cost and easy availability of the biocatalysts offer the possibility of scaling up this protocol. To demonstrate the viability of this protocol as industrial feasibility, 10 times scale up bioreduction of acetophenone was carried out. Surface sterilized with 100 g of Rajma was soaked in 1 L of sterile distilled water into a 5 L conical flask for 24 h. The 5% glucose was added followed by 1 g acetophenone in ethanol and the reaction mixture was incubated on an orbital shaker (100 rpm) at room temp (24-25 $^{\circ}$C) for 3 days. The reaction mixture was then filtered to remove seeds and washed with water. The combined filtrate and washings were extracted with chloroform (3x100 mL), washed with water (2x50 mL) and organic layer was dried on the sodium sulphate, filtered and concentrated under vacuum. The produced alcohol and unchanged substrate were isolated and purified by the preparative TLC (silica gel G, EtOAc/Pet. Ether). (*R*)-(+)-1-Phenylethanol (1a) was obtained in 40.5% yield (412.2 mg), with 80.2% e.e. ($[\alpha]_D^{25}$ + 37.850 (1.562, $CHCl_3$)). As shown in Fig.1a the ^{1} H NMR of MTPA ester showed major peak at δ 3.486 for (*R*)-isomer, and minor at δ 3.571 for (S)-isomer (^{1} H NMR of MTPA ester of (S)-1-phenylethanol is given for comparison, Fig. 1b). This easy and handy system represented the possibility of scaling up the process to be used in organic synthesis.

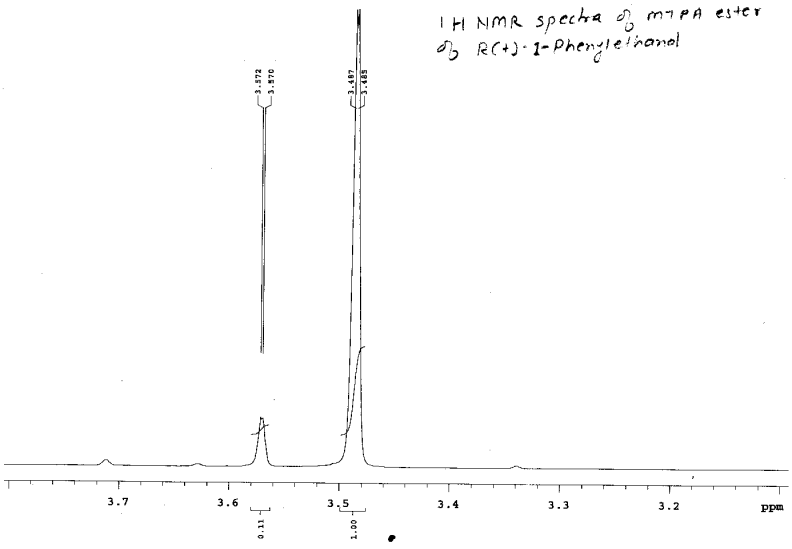


Fig. 1a. ^{1}H NMR spectra of MTPA esters of (*R*)-1-phenylethanol

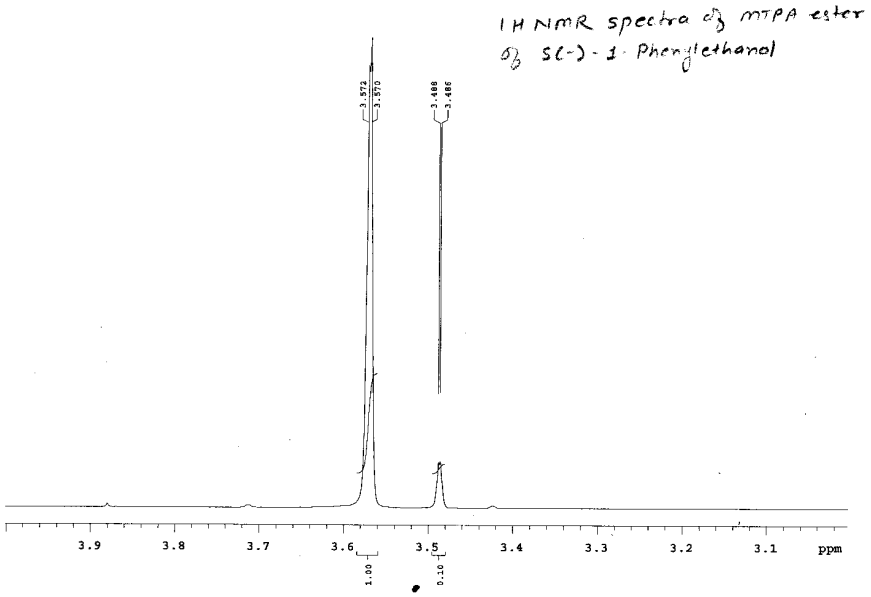


Fig. 1b. [1]H NMR spectra of MTPA esters of (*S*)-1-phenylethanol

Conclusions

Rajma could be one of the few seeds to afford the (*R*)-arylalkanols by anti-Prelog reduction of arylalkanones in an environmentally benign way. This whole-cell biocatalyst showed broad substrate specificity and chiral selectivity to the produced (*R*)-alcohols. This could be a critical factor from a biocatalytic evaluation perspective given the equal importance of both the enantiomers. This aqueous phase biocatalysis provided a simple and attractive alternative for carrying out reduction in the safe, economical and 'green' environment. It does not require particular expertise and specific laboratory facilities. This process can be recommended to the traditional synthetic chemists as practical, cheap and readily available biocatalyst (without addition of external coenzyme). This eco-friendly biotransformation provides opportunities for further modification and development of a variety of enzymatic systems for different substrates with improvement in conversion and stereoselectivity e.g. i) Small particulates of Adzuki beans (Xie et al., 2009) showed better selectivity (≥ 99% ee) in bioreduction of acetophenone than the whole grain (89% ee). Furthermore, the acetone powder of its crude enzyme system significantly improved the efficiency of the bioreduction (44.4% to 97.1% yield). ii) Bioreduction of acetophenone using *Vigna unguiculata* L. (Bizerra et al., 2010) in water produced 34% of (*S*)-alcohol with 92% ee, whereas in presence of Tris-HCl buffer at pH 7.5, the enzyme activity decreased slightly. But in presence of 2% PrOH (v/v), improvement in the % of ee (≥ 99%) was noticed. iii) Matsuo et al. (2008) chose radish sprout (germinated seeds) for the reduction of o-chloroacetophenone, and afforded the corresponding (*S*)-alcohol with > 99% of ee. It is noteworthy that the addition of the sucrose at the cultivation time influenced the growth of the plant, as well as bioconversion of the aromatic ketones.

References

Akakabe, Y., Takahashi, M., Kamezawa, M., Kikuchi, K., Tachibana, H., Ohtani, T., Naoshima, Y. (1995): Biocatalytic preparation of chiral alcohols by enantioselective reduction with immobilized cells of carrot. *J. Chem. Soc. Perkin Trans. I* 1295-1298. https://doi.org/10.1039/P19950001295.

Baldassarre, F., Bertoni, G., Chiappe, C., Marioni, F. (2000): Preparative synthesis of chiral alcohols by enantioselective reduction with Daucus carota root as biocatalyst. *J. Mol. Catal. B: Enzym.* 11, 55-58. https://doi.org/10.1016/S1381-1177(00)00189-2.

Bizerra, A. M. C., de Gonzalo, G., Lavandera, I., Gotor-Fernández, V., de Mattos, M. C., da Conceicão, M., de Oliveira, F., Lemos, T. L. G., Gotor, V. (2010): Reduction processes biocatalyzed by *Vigna unguiculata*, *Tetrahedron: Asymmetry*. 21, 566-570. https://doi.org/10.1016/j.tetasy.2010.03.005.

Blanchard N, van de Weghe P. (2006): Daucus carota L. mediated bioreduction of prochiral ketones. *Org. biomol. chem.* 4, 2348-2353. https://doi.org/10.1039/b605233a.

Bordón, D. L., Villalba, L. D., Aimar, M. L., Cantero, J. J., Vázquez, A. M., Formica, S. M., Krapacher, C.

R., Rossi, L. I. (2015): Weeds as biocatalysts in the stereoselective synthesis of chiral phenylethanols used as key intermediates for pharmaceuticals. *Biocatalysis and Agricultural Biotechnology*. 4, 493–499. (2015:BABD1500123) https://doi.org/10.1016/j.bcab.2015.08.001.

Bruni, R., Fantin, G., Medici, A., Pedrini, P., Sacchetti, G. (2002): Plants in organic synthesis: an alternative to baker's yeast. *Tetrahedron Lett.* 43, 3377-3379. https://doi.org/10.1016/S0040-4039(02)00514-2.

Caron, D., Coughlan, A. P., Simard, M., Bernier, J., Piché, Y., Chênevert, R. (2005): Stereoselective reduction of ketones by Daucus carota hairy root cultures. *Biotechnol. Lett.* 27, 713-716. https://doi.org/10.1007/s10529-005-5187-y.

Chang, X., Yang, Z., Zeng, R., Yang, G., Yan, J. (2010): Production of Chiral Aromatic Alcohol by Asymmetric Reduction with Vegetable Catalyst. *Chinese J. Chem. Engineer*. 18, 1029-1033. https://doi.org/10.1016/S1004-9541(09)60164-6.

Chattrain, M., Greasham, R., Moore, J., Reider, P., Robinson, D., Buckland, B. (2001): Asymmetric bioreductions: application to the synthesis of pharmaceuticals, *J. Mol. Catal. B: Enzyme*. 11, 503-512. https://doi.org/10.1016/S1381-1177(00)00170-3.

Cheng, C., Ma, J-H. (1996): Enantioselective synthesis of S-(−)-1-phenylethanol in *Candida utilis* semi-fed-batch cultures. *Process Biochem.* 31, 119-124. https://doi.org/10.1016/0032-9592(94)00022-0.

Comasseto, J. B., Assis, L. F., Andrade, L. H., Schoenlein-Crusius, I. H., Porto, A. L. M. (2006): Biotransformations of ortho-, meta- and para-aromatic nitrocompounds by strains of *Aspergillus terreus*: Reduction of ketones and deracemization of alcohols. *J. Mol. Catal. B: Enzyme*. 39, 24-30. https://doi.org/10.1016/j.molcatb.2006.01.022.

Comasseto, J. V., Omori, A. T., Porto, A. L. M., Andrade, L. H. (2004): Preparation of chiral organochalcogeno-α-methylbenzyl alcohols via biocatalysis, The role of Daucus carota root. *Tetrahedron Lett.* 45, 473-476. https://doi.org/10.1016/j.tetlet.2003.11.011.

Contente, M. L., Serra, I., Palazzolo, L., Parravicini, C., Gianazza, E., Eberini, I., Pinto, A., Guidi, B., Molinari, F., Romano, D. (2016): Enzymatic reduction of acetophenone derivatives with a benzil reductase from *Pichia glucozyma* (KRED1-Pglu): electronic and steric effects on activity and enantioselectivity. *Org. Biomol. Chem.* 14, 3404-3408. https://doi.org/10.1039/C6OB00047A.

Dale, J. A., Mosher, H. S. (1973): Nuclear magnetic resonance enantiomer regents. Configurational correlations via nuclear magnetic resonance chemical shifts of diastereomeric mandelate, O-methylmandelate, and α-methoxy-α-trifluoromethylphenylacetate (MTPA) esters. *J. Am. Chem. Soc.* 95, 512-519. https://doi.org/10.1021/ja00783a034.

Faber, K. (2004): Biotransformations in Organic Chemistry, Springer Verlag, Berlin. https://doi.org/10.1007/978-3-642-18537-3.

Farbood, M., Blocker, R., Arvizzigno, J., Muralidhara, R., (2004): Mixtures of optical isomers of styralyl alcohol or styralyl acetate, processes for preparing same and organoleptic uses thereof, U.S. patent 20040126471 A1.

Hamada, H., Nakamura, N., Ito, S., Kawabe, S., Funamoto, T. (1988): Enantioselectivity in the reduction of α-dicarvelone by cell suspension cultures of *Nicotiana tabacum*. *Phytochemistry* 27, 3807-3808. https://doi.org/10.1016/0031-9422(88)83021-8.

Hasegawa, Y., Adachi, S., Matsuno, R. (1998): Asymmetric reduction of acetophenone by immobilized *Hansenula capsulata* cells. *J. Ferment. Bioeng*. 85, 322-327. https://doi.org/10.1016/S0922-338X(97)85683-8.

Homola, P., Kurák, T., Illeová, V., Polakovi, M. (2015): Cultivation of *Pichia capsulata* as a whole-cell biocatalyst with NADH-dependent alcohol dehydrogenase activity for *R*-1-phenylethanol production. *Food and bioproducts processing* 96, 126-132. https://doi.org/10.1016/j.fbp.2015.07.007.

Hummel, W. (1990): Enzyme-catalyzed synthesis of optically pure R(+)-phenylethanol. *Biotechnol. Lett.* 12, 403-408. https://doi.org/org/10.1007/bf01024393.

Ishihara, K., Hamada, H., Hirata, T., Nakajima, N. (2003): Biotransformation using plant cultured cells. *J. Mol. Cat. B: Enzym.* 23, 145-170. https://doi.org/10.1016/S1381-1177(03)00080-8.

Kumaraswamy, G., Ramesh, S. (2003): Soaked *Phaseolus aureus* L: an efficient biocatalyst for asymmetric reduction of prochiral aromatic ketones. *Green Chem.* 5, 306-308. https://doi.org/10.1039/B300746D.

Kurbanoglu, E. B., Zilbeyaz, K., Ozdal, M., Taskin, M., Kurbanoglu, N. I. (2010): Asymmetric reduction of substituted acetophenones using once immobilized *Rhodotorula glutinis* cells. *Bioresour. Technol.* 101, 3825-3829. https://doi.org/10.1016/j.tetasy.2010.03.005.

Lakshmi, C. S., Reddy, G. R., Rao, A. B. (2011): Asymmetric Reduction of Heteroaryl Methyl Ketones Using Daucus carota. *Green Sustai. Chem.* 1, 117-122. https://doi.org/10.4236/gsc.2011.14019.

Leffingwell, J. C. (2010): The Styrallyl Alcohols. http://http://www.leffingwell.com/chirality/styrallylalcohol.htm.

Machado, L. L., Monte, F. J. Q., de Oliveira, M. C. F., de Mattos, M. C., Lemos, T. L. G., Fernández, V. G., de Gonzalo, G., Gotor, V. (2008): Bioreduction of aromatic aldehydes and ketones by fruits' barks of *Passiflora edulis*. *J. Mol. Cat. B: Enzym.* 54, 130-133. https://doi.org/org/10.1016/j.molcatb.2007.12.008.

Mączka, W. K., Mironowicz, A. (2004): Enantioselective reduction of bromo- and methoxy-acetophenone derivatives using carrot and celeriac enzymatic

system. *Tetrahedron: Asymmetry* 15, 1965-1967. https://doi.org/10.1016/j.tetasy.2004.05.033.

Matsuda, T., Marukado, R., Mukouyama, M., Harada, T., Nakamura, K. (2008): Asymmetric reduction of ketones by *Geotrichum candidum*: immobilization and application to reactions using supercritical carbon dioxide. *Tetrahedron: Asymmetry*. 19, 2272-2275. https://doi.org/10.1016/j.tetasy.2008.09.018.

Matsuo, K. Kawabe, S., Tokuda, Y., Eguchi, T., Yamanaka, R., Nakamura, K. (2008): Asymmetric reduction of ketones with a germinated plant. *Tetrahedron: Asymmetry* 19, 157-159. https://doi.org/10.1016/j.tetasy.2007.12.015.

Mukaiyama, T., Nagata, Y., Ikegai, K. (2005): Letter - Stereoselective Carbon-Carbon Bond Forming Reactions between Various Chiral Alkyl Aryl Carbinois and Triethyl Methanetricar-boxylate by Oxidation-Reduction Condensation Using Alkyl Diphenylphosphinites. *Chemistry letters* 34, 1676-1677. https://doi.org/10.1246/cl.2005.1676.

Nakamura, K., Matsuda, T. (1998): Asymmetric reduction of ketones by the acetone powder of *Geotrichum candidum*. *J. Org. Chem.* 63, 8957-8964. https://doi.org/10.1021/jo9812779.

Nakamura, K., Yamanaka, R., Matsuda, T., Harada, T. (2003): Recent developments in asymmetric reduction of ketones with biocatalysts. *Tetrahedron: Asymmetry* 14, 2659-2681. https://doi.org/10.1016/S0957-4166(03)00526-3.

Orden, A. A., Magallanes-Nogueraa, C. Agostini, E., Kurina-Sanza, M. (2009): Anti-Prelog reduction of ketones by hairy root cultures. *J. Mole. Cat. B: Enzymatic* 61, 216-220. https://doi.org/10.1016/j.molcatb.2009.07.007.

Patel, R. N. (2002): Microbial/enzymatic synthesis of chiral intermediates for pharmaceuticals. *Enzyme. Microb. Technol.* 31, 804-826. https://doi.org/10.1016/S0141-0229(02)00186-2.

Prelog, V. (1964): Specification of the stereospecificity of some oxido-reductases by diamond lattice sections. *Pur. Appl. Chem.* 9, 119-130. https://doi.org/10.1351/pac196409010119.

Protsko, C., Vieille, C., Laivenieks, M., Prasad, L., Sanders, D. A. R., Delbaere, L. T. J. (2010): Crystallization and preliminary X-ray diffraction analysis of the *Thermoanaerobacter ethanolicus* secondary alcohol dehydrogenase I86A mutant. *Acta Crystallogr. Sect. F. Struct. Biol. Cryst. Commun.* 66, 831-833. https://doi.org/10.1107/s1744309110018981.

Ravia, S., Gamenara, D., Schapiro, V., Bellomo, A., Adum, J., Seoane, G., Gonzalez, D. (2006): In the Laboratory - Green Chemistry - Enantioselective Reduction by Crude Plant Parts: Reduction of Benzofuran-2-yl Methyl Ketone with Carrot (Daucus carota) Bits. *J Chem. Edu*. 83, 1049-1051. https://doi.org/10.1021/ed083p1049.

Salvi, N. A., Chattopadhyay, S. (2001): Studies on *Rhizopus arrhizus* mediated enantioselective reduction of arylalkanones. *Tetrahedron* 57, 2833-2839. https://doi.org/S0040-4020(01)00134-X.

Salvi, N. A., Chattopadhyay, S. (2008): Asymmetric reduction of halo-substituted arylalkanones with *Rhizopus arrhizus*. *Tetrahedron: Asymmetry* 19, 1992-1997. https://doi.org/10.1016/j.tetasy.2008.07.035.

Salvi, N. A., Chattopadhyay, S. (2016): Laboratory scale-up synthesis of chiral carbinols using *Rhizopus arrhizus*. *Tetrahedron: Asymmetry* 27, 188-192. https://doi.org/10.1016/j.tetasy.2016.01.008.

Schlieben, N. H., Niefind, K., Müller, J., Riebel, B., Hummel, W., Schomburg, D. (2005): Atomic resolution structures of R-specific alcohol dehydrogenase from *Lactobacillus brevis* provide the structural bases of its substrate and cosubstrate specificity. *J. Mol. Biol.* 349, 801-813. https://doi.org/10.1016/j.jmb.2005.04.029.

Schoemaker, H. E., Mink, D., Wubbolts, M. G. (2003): Dispelling the myths--biocatalysis in industrial synthesis. *Science* 299, 1694-1697. https://doi.org/10.1126/science.1079237.

Suárez-Franco, G., Hernández-Quiroz, T., Navarro-Ocaña, A., Oliart-Ros, R. M., Valerio-Alfaro, G. (2010): Plants as Green Alternative for Alcohol Preparation from Aromatic Aldehydes. *Biotechnol. Bioproc. Eng.* 15, 441-445. https://doi.org/10.1007/s12257-009-0207-8.

Takemoto, M., Moriyasu, Y., Achiwa, K. (1995): Synthesis of Optically Active α-Phenylpyridylmethanols with Cell Cultures of *Nicotiana tabacum*. *Chem, Pharm. Bull*. 43, 1458-1461. https://doi.org/10.1248/cpb.43.1458.

Wandrey, C., Liese. A., Kihumbu, D. (2000): Industrial biocatalysis: Past, present, and future. *Org. Proc. Res. Dev.* 4, 286-290. https://doi.org/10.1021/op990101l.

Wu, X., Xiao, J. (2007): Aqueous-phase asymmetric transfer hydrogenation of ketones – a greener approach to chiral alcohols. *Chem. Commun.* 2449-2466. https://doi.org/10.1039/B618340A.

Xie, Y., Xu, J-H., Lu, W-Y., Lin, G-Q. (2009): Adzuki bean: a new resource of biocatalyst for asymmetric reduction of aromatic ketones with high stereoselectivity and substrate tolerance. *Bioresource Technol.* 100, 2463-2468. https://doi.org/10.1016/j.biortech.2008.11.054.

Yadav, J. S., Nanda, S., Thirupathi Reddy, P., Bhaskar Rao, A. (2002): Efficient enantioselectivity reduction of ketones with Daucus carota root. *J. Org. Chem.* 67, 3900-3903. https://doi.org/10.1021/jo010399p.

Yadav, J. S., Subba Reddy, B. V., Sreelakshmi, C., Bhaskar Rao, A. (2009): Enantioselective reduction of prochiral ketones employing sprouted *Pisum sativa* as biocatalyst. *Synthesis* 11, 1881-1885. https://doi.org/10.1055/s-0028-1088047.

Yang, Z.-H., Zeng, R., Yang, G., Wang, Y., Li, Li.-Z., Lv, Z.-S., Yao, M., Lai, B. (2008): Assymetric reduction of prochiral ketones to chiral alcohols catalyzed by plants tissue. *J. Ind. Microbiol.*

Biotechnol. 35, 1047-1051. https://doi.org/10.1007/s10295-008-0381-2.

Zaks, A. (2001): Industrial biocatalysis. *Curr. Opin. Chem. Biol.* 5, 130-136. https://doi.org/10.1016/S1367-5931(00)00181-2.

Zheng, G.-W., Xu, J.-H. (2011) New opportunities for biocatalysis: driving the synthesis of chiral chemicals. *Curr. Opin. Biotechn.* 22, 784-792. https://doi.org/10.1016/j.copbio.2011.07.002.

Zhu, D., Hua, L. (2006): Enantioselective Enzymatic Reductions of Sterically Bulky Aryl Alkyl Ketones Catalyzed by a NADPH-Dependent Carbonyl Reductase. *J. Org. Chem.* 71, 9484-9486. https://doi.org/10.1021/jo061571y.

Zhu, D., Hua, L. (2010): How carbonyl reductases control stereoselectivity: Approaching the goal of rational design. *Pure Appl. Chem.* 82, 117-128. https://doi.org/10.1351/PAC-CON-09-01-03.

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