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Pleurotus ostreatus as a source of enoate reductase

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ABSTRACT

(*E*)-4-Phenylbut-3-ene-2-one (**2a**) and its derivatives with different substituents at phenyl ring **(2b–e)** have been subjected to biotransformation mediated by whole cells of *Pleurotus ostreatus*. The strain showed enoate reductase activity towards all the substrates tested. Saturated ketones with *p*-isopropyl-, *p*-methoxy- and 2,4-dimethoxyphenyl ring (**3c–e**) were obtained with 100% chemoselectivity. In case of (*E*)-4-phenylbut-3-ene-2-one (**2a**) and (*E*)-4-(benzo[1,3]dioxol-5-yl)but-3-en-2-one (**2b**) C=C bond reduction was slowly followed by C=O bond reduction, which afforded corresponding saturated alcohols **4a,b**, respectively with low or moderate predominance of (*S*)-isomers. The presence of enoate reductase in *P. ostreatus* as well as enantiomerically enriched alcohol **4b** has not been reported so far.

1. Introduction

The fungi of *Pleurotus* genus (oyster mushroom), to which belong about 40 species (Jose and Janardhanan, 2000), is commonly found in nature in different climate conditions, both in tropical and temperate zone. It is one of the most cultivated edible mushroom because of the presence of nutritional and taste components, i.a. carbohydrates, free amino acids and flavour 5′-nucleotides (Yang et al., 2001). The great advantage of oyster mushroom is ease of cultivation on agricultural by-products containing lignocelluloses like rice straw supplemented with slurry manure, the residue after biogas production (Banik and Nandi, 2004), rice and wheat straw (Zhang et al., 2002) or even on waste papers supplemented with peat, chicken manure and husk rice (Baysal et al., 2003). In this way these residues can be converted into human food in the biological recycling process.

Nevertheless, little is known about the ability of *Pleurotus* genera to the biotransformation of organic compounds. The only example is regio- and stereoselective reduction of carbonyl group of 4-methyl-3,5-heptanedione, which yielded (4*S*,5*S*)-5-hydroxy-4-methyl-3-heptanone with purity 96% and ee 86%. This isomer is a possible sex pheromone of lucerne weevil *Sitona discoideus*. The process was performed at 25 °C/120 rpm for 72 h in water suspension of the substrate using thin slices of *P. ostreatus* as the biocatalyst (Bohman and Unelius, 2009). In our present investigations we decided to check the activity of *P. ostreatus* towards more complex substrates i.e. (*E*)-4-phenylbut-3-ene-2-one and its derivatives with various substituents at phenyl ring (**2a–e**). We expected that the *P. ostreatus* may show enoate reductase (EC 1.3.1.31) activity towards the substrates mentioned. This flavine-dependent enzyme belongs to the Old Yellow Enzyme (OYE) family (Williams and Bruce, 2002), ubiquitous enzymes which have been found mainly in yeast (Clososki et al., 2007; Silva et al., 2010; Raimondi and Roncaglia 2010; Luo et al., 2010; Raimondi and Romano, 2011), but also in fungi (Fuganti et al., 1998; Yamazaki et al., 1988; Arnone et al., 1990) bacteria (Shimoda et al., 2004a,b; Sakai et al., 1985) and plant cells (Shimoda et al., 2004a,b; Hirata et al., 2004). In nature they play significant role in the metabolic pathways as they catalyze the stereoselective reduction of C=C bond conjugated to an electronic withdrawing group (EWG) (Stuermer et al., 2007). The examples are enoyl-CoA reductase involved in fatty acid biosynthesis (Mizugaki et al., 1979), morphinone reductase in morphine biosynthesis (Barna et al., 2002) and 12-oxophytodienoic acid reductase in jasmonic biosynthesis (Schaller et al., 2000). Additionally, these enzymes catalyze reductive cleavage nitroesters to the corresponding alcohol and nitrite (Snape et al., 1997), reduction of aromatic nitro groups *via* the nitroso species to the oxime (Nishino and Spain, 1993) and transform electron-deficient nitroaromatics, such as 2,4,6-trinitrotoluene (TNT), to the non-aromatic Meisenheimer complex (Barna et al., 2001). These latter bioactivities are response to oxidative stress and are attributed to the detoxification of xenobiotics.

The first discovered member of the OYE family is the Oye1p produced by *Saccharomyces pastorianus* (Warburg and Christian, 1932). Oye1p is a dimeric protein of around 45 kDa, with a single α/β-barrel domain. The non-covalently bound cofactor flavin

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mononucleotide is the source of electrons that are transferred from the coenzyme NAD(P)H to the substrate. The cofactor and the substrate use the same binding site and react following the Bi Bi Ping Pong mechanism (Raimondi and Romano, 2011).

The catalytic mechanism of C=C bond reduction has proved that the carbonyl oxygen atom of α,β-unsaturated carbonyl substrate forms the hydrogen bonds with His-191 and Asn-194 in OYE, aligning the Cβ of substrate above the flavin N-5 atom (Brown et al., 1998). Hydride from flavin N-5 is stereoselectively transferred to Cβ of substrate, whereas a Tyr-196 residue adds a proton (ultimately derived from the solvent) to Cα from the opposite side (Kohli and Massey, 1998). The process resembles the Michael-type addition and the result of the reaction is the stereoselective *trans*-hydrogenation of the double bond, which is difficult to perform by conventional chemical methods. The substrates for enoate reductase are α,β-unsaturated aldehydes, ketones, carboxylic acids, lactones, acid anhydrides, cyclic amides and nitroalkenes. The process has been exploited commercially in the food industry e.g. to the production of dihydrocarvone (Goretti et al., 2009), (*R*)-levodione (Leuenberger et al., 1976) and raspberry ketone (Fuganti and Zucchi, 1998).

2. Materials and methods

2.1. Analysis

The progress of transformations and the purity of isolated products were checked by TLC on silica gel-coated alumina plates (DC-Alufolien Kieselgel 60F_{254}, Merck) as well as GC analysis performed on Agilent Technologies 6890N instrument using DB-5HT (polyimide-coated fused silica tubing) and hydrogen as the carrier gas. The temperature programme was as follows: injector 220 °C, detector (FID) 330 °C, column temperature: 90–330 °C (rate 20 °C min^{-1}), 330 °C (hold 2 min). After the derivatization with acetic chloride the enantiomeric excesses of alcohols **4a** and **4b** were calculated on CP-cyclodextrin-B-2,3, 6-M-19 column (25 × 0.25 × 0.25 m) at the following conditions: injector 280 °C, detector (FID) 280 °C, column temperature: 60 °C (hold 1 min), 60–200 °C (rate 2 °C min^{-1}), 200 °C (hold 2 min).

Products of transformations were separated and purified by preparative column chromatography on silica gel (Kieselgel 60, 230–400 mesh, Merck).

^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on the Bruker Advance DRX 300 spectrometer. The assignments of ^{13}C chemical shifts were made by means of distortionless enhancement by the polarization transfer method (DEPT 135) and C/H correlation heteronuclear multiple quantum coherence (HMQC). IR spectra were determined using the Mattson IR 300 Thermo Nicolet spectrophotometer.

Optical rotations were measured on the digital polarimeter DIP 1000 (Jasco).

2.2. Chemicals

(*E*)-4-Phenylbut-3-en-2-one (**2a**) was purchased from Fluka AG (Switzerland). Substrates in the synthesis: piperonal, cuminaldehyde, *p*-anisaldehyde and 2,4-dimethoxybenzaldehyde (**1b–e**) were purchased from Aldrich. All the solvents and reagents of analytical grade used in the experiments were purchased from Chempur.

2.3. Synthesis of α,β-unsaturated ketones *2b–e*

Flask (100 mL) containing corresponding aldehyde (**1b–e**) (0.0342 mol), acetone (0.141 mol) and distilled water (5 mL) was placed in the cold water bath and stirred. Then 1 mL of 10% NaOH was added dropwise and the mixture was stirred for six hours in room temperature. Then the mixture was acidified with 1 M HCl to pH 2 and extracted three times with 30 mL of methylene chloride. The organic fractions were combined, washed with brine and dried over anhydrous magnesium sulphate. The solvent was evaporated under reduced pressure and corresponding known (Barltrop et al., 1959; Winter, 1961; Viviano et al., 2011; Dhuru et al., 2011) α,β-unsaturated ketone (**2b–e**) was obtained.

2.4. Microorganism and growing media

The strain *P. ostreatus* PO310783 came from the collection of the Department of Plant Protection, University of Environmental and Life Sciences of Wrocław. It was cultivated at 28 °C on Sabouraud agar slants consisting of 5 g L^{-1} of aminobac, 5 g L^{-1} of peptone K, 40 g L^{-1} of glucose, 15 g L^{-1} of agar and stored in refrigerator at 4 °C. Biotransformations were conducted in the medium containing 3 g of glucose and 1 g of peptobac in water (100 mL), pH 6.8.

The chemicals used for the preparation of the growing media were purchased from BTL in Poland, except glucose which was bought in POCH (Poland).

2.5. General biocatalytic procedure

α,β-Unsaturated ketone (**2b–e**) (120 mg in 12 mL acetone) was added to 12-day cultures of *P. ostreatus* PO310783 cultivated at 25 °C in Erlenmeyer flasks (250 mL) containing the culture medium (50 mL) described earlier. The substrates were incubated on an orbital shaker (210 g). The progress of transformation was monitored by TLC (silica gel, hexane:acetone 4:1) and GC (DB-5HT column). The samples were withdrawn and extracted after 2, 4, 6, 8 and 10 days with methylene chloride. The results are shown in Table 1.

The products were extracted three times with methylene chloride (50 mL). The organic solutions were combined, dried over $MgSO_4$ and evaporated under reduced pressure. The products were purified by column chromatography (silica gel, hexane:acetone 10:1).

3. Results and discussion

3.1. Synthesis

The substrates for biotransformation were α,β-unsaturated ketones containing phenyl ring with different substituent

Table 1
Composition (in % according to GC) of the products mixture during biotransformation of ketones **2a–e** by *Pleurotus ostreatus*.

Entry	Compound	Time of biotransformation [days]				
		2	4	6	8	10
1	**2a**	81	56	0	0	0
	3a	19	44	94	32	2
	4a	0	0	6	68	98
2	**2b**	84	62	0	0	0
	3b	16	38	96	28	3
	4b	0	0	4	72	97
3	**2c**	69	33	7	0	0
	3c	31	67	93	100	100
4	**2d**	67	31	8	0	0
	3d	33	69	90	100	100
5	**2e**	68	33	5	0	0
	3e	32	67	95	100	100