Journal of Bioscience and Bioengineering
VOL. 121 No. 3, 265–267, 2016

www.elsevier.com/locate/jbiosc

Improved conversion of cinnamaldehyde derivatives to diol compounds via a pyruvate decarboxylase-dependent mechanism in budding yeast

Shunichi Miyakoshi,* Yukari Negishi, Yusuke Sekiya, and Satoshi Nakajima

National Institute of Technology, Gunma College, 580 Toriba-machi, Maebashi, Gunma, 371-8530, Japan

Received 17 January 2015; accepted 22 June 2015
Available online 27 July 2015

Cinnamaldehyde is stereospecifically converted to (2*S*,3*R*) 5-phenylpent-4-ene-2,3-diol, an important starting material for the synthesis of biologically active compounds, by the budding yeast *Saccharomyces cerevisiae*. Immobilization of the yeast in calcium alginate capsules suppressed the formation of by-products and increased accumulation of the diol compounds. The mechanism of cinnamaldehyde conversion was investigated by using recombinant strains of *Escherichia coli* and *S. cerevisiae* carrying the pyruvate decarboxylase gene *PDC1*. As a result, condensation of the substrate with acetaldehyde was enhanced by PDC and flow to the diol product was altered.

[**Key words:** Bioconversion; Cinnamaldehyde; Diol; Immobilized yeast; *Saccharomyces cerevisiae*; Pyruvate decarboxylase]

Budding yeast (*Saccharomyces cerevisiae*) is used as the catalyst for microbial conversions, mainly for reduction of compounds. A few stereospecific C–C compounds are known to be formed by budding yeast. Benzaldehyde and cinnamaldehyde were reported to be stereospecifically converted to 1-phenyl-1,2-propanediol and 5-phenyl-4-pentene-2,3 diol, respectively (1,2). It has been reported that cinnamaldehyde **1** is stereospecifically converted to (2*S*,3*R*) 5-phenylpent-4-ene-2,3-diol **4** (Fig. 1), but the conversion rate is low unless pyruvate or thiamine is added to the reaction mixture (3). This conversion process is reported to be catalyzed by the same mechanism as acyloin condensation. An improved method for performing this conversion using pyruvate decarboxylase as the catalyst has also been proposed (3). The converted products are diol compounds, which are important starting materials for the synthesis of medicines and pheromones such as daunomicin and frontalin (4,5). However, the conversion rate of cinnamaldehyde to (2*S*,3*R*) 5-phenylpent-4-ene-2,3-diol **4** is only 5–20% under both the original and modified conditions (1,3). It is thought that formation of reduced by-products may dominate conversion to diol compounds, rendering the process less efficient.

Here we describe an improved method for conversion of cinnamaldehyde and its derivatives to diol compounds using immobilized yeast, in which the mechanism of condensation is dependent on pyruvate decarboxylase.

MATERIALS AND METHODS

Microbial strain and vector DNA The commercially available *S. cerevisiae* Kodama strain and *Escherichia coli* JM109 were used. The shuttle expression vector pAUR123 and other materials for molecular biological procedures were purchased from Takara Bio Inc. (Shiga, Japan).

Culture conditions and microbial transformation Biotransformation of cinnamaldehyde was performed by the method for microbial conversion of flavonols with some modifications (6). 1/2 YPD medium (80 ml) containing 0.5% yeast extract (Bacto), 1% Polypepton (Nihon Seiyaku), and 2% glucose (pH 5.8) was dispensed into a 500-ml Erlenmeyer flask and autoclaved. One loop of yeast cells was inoculated into the medium and culture was performed at 28°C and 210 rpm for 2–3 days. After the growth of mycelia, 80 mg of cinnamaldehyde (or other related compounds) dissolved in a small amount of dimethyl sulfoxide was added to the culture and incubation was continued for 3–24 h under the same conditions as before. *E. coli* and its transformant were inoculated into 100-ml Erlenmeyer flask containing 20 ml of Luria-Bertani medium and cultured at 37°C, 210 rpm for 12 h, followed by addition of 0.1% of cinnamaldehyde and further culture for 24 h. After completion of culture, the reaction mixture was extracted twice with the same volume of ethyl acetate. Then the extract was evaporated to dryness, and converted metabolites were separated by silica gel column chromatography (4 mm × 100 mm) and silica gel thin-layer chromatography (150 mm × 150 mm). Finally, *n*-hexane-ethyl acetate solvent mixtures were used as the eluent to separate the substrate, by-product, intermediate metabolite, and product. The substrate was eluted with *n*-hexane-ethyl acetate (9:1), the by-product and the intermediate metabolite were eluted with *n*-hexane-ethyl acetate (5:5), and the product was eluted with *n*-hexane-ethyl acetate (1:9).

Yeast cells grown in 1/2 YPD medium were collected by centrifugation (1500 ×*g*, 10 min) and resuspended in 2 volumes of 1.5% sodium alginate solution, after which the suspension was dispensed into 5% $CaCl_2$ solution to form calcium alginate capsules. Then the capsules containing immobilized yeast cells were collected and used for microbial conversion.

The effect of acetaldehyde was investigated by adding 0–0.3% acetaldehyde to the conversion culture with 0.05% cinnamaldehyde. After products were extracted with ethyl acetate, the extract was evaporated and resuspended in methanol, and the conversion rate was determined by HPLC.

Analysis of conversion products and intermediates Conversion products and substrates were analyzed by HPLC (Hitachi 500 series) using a TSKgel ODS-120T column (Tosoh, 4.6 mm × 150 mm) and 35% acetonitrile as the mobile phase with a flow rate of 1.0 ml/min. Detection was performed with a UV detector at 254 nm.

One of the yeast pyruvate decarboxylase genes (*PDC1*) was amplified by PCR (25 cycles of 94°C for 30 s, 66°C for 30 s, and 72°C for 60 s) using yeast genomic DNA, TaKaRa Ex Taq polymerase, and a pair of primers (CACTCGAGATGTCTG AAATTACTTTGGGTAAA and AATCTAGATTGTTCAACCAAGTTTTGTGGAGCA). Amplified DNA was digested with the XhoI and XbaI restriction enzymes, and was ligated to pAUR123 digested with the same enzymes. *E. coli* JM109 was transformed with the recombinant plasmid to obtain *E. coli* pAUR-PDC1, which was cultured in 80 ml

* Corresponding author. Tel.: +81 27 254 9122; fax: +81 27 254 9115.
E-mail address: miyako@nat.gunma-ct.ac.jp (S. Miyakoshi).

1389-1723/$ – see front matter
http://dx.doi.org/10.1016/j.jbiosc.2015.06.013

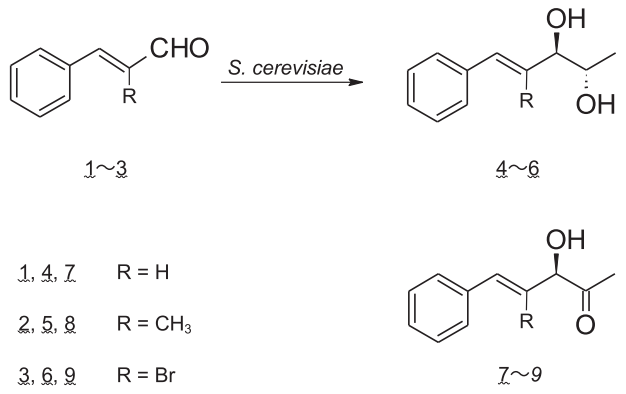

FIG. 1. Conversion of cinnamaldehyde **1** and its derivatives **2** and **3** to chiral diol compounds **4**–**6** and structure of the intermediate metabolites, 3-hydroxy-5-phenyl-4-penten-2-one and related compounds **7**–**9**.

of LB medium at 37°C for 18 h. Then 0.1% cinnamaldehyde was added and culture was continued for another 24 h.

RESULTS AND DISCUSSION

In preliminary experiments, the conditions for immobilization of the yeast were investigated by using ethyl 3-oxobutanate as the substrate and detecting 3-hydroxybutyrate as the reduced product. By altering the concentration of sodium alginate (from 0.5% to 2.0%), the ratio of sodium alginate solution to the culture medium

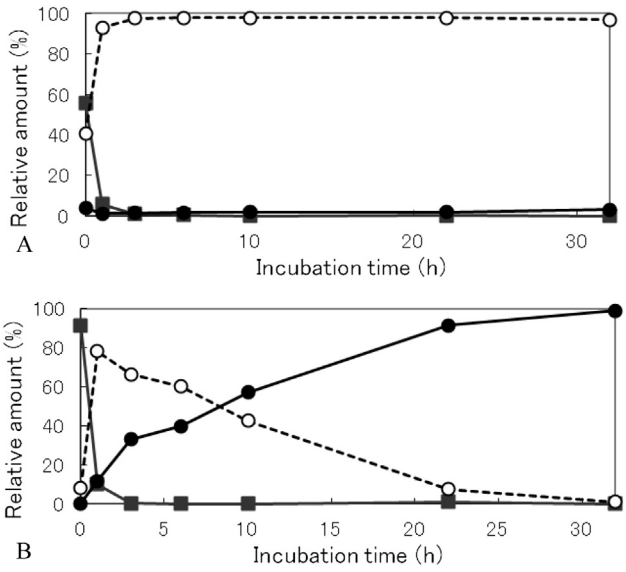

FIG. 2. Improved conversion of cinnamaldehyde to (2*S*,3*R*)-5-phenyl-4-penten-2,3-diol by immobilization of *S. cerevisiae*. (A) Conversion by free yeast cells; (B) conversion by immobilized cells. Symbols: closed squares, substrate compound **1**; closed circles, product **4**; open circles, by-product **10**.

containing yeast cells (2:1, 1:1, or 1:2), the temperature (24°C or 28°C) (data not shown), and the optimum conditions for immobilization were determined as described in Materials and methods (1.5 g of yeast cells, 1.5 ml of medium, and 6 ml of calcium alginate solution).

The optimum concentration of cinnamaldehyde was determined by incubating unimmobilized yeast with various concentrations of cinnamaldehyde. Conversion products were extracted with ethyl acetate and were redissolved in methanol for analysis by quantitative HPLC. Addition of cinnamaldehyde at a concentration of 0.05–0.1% resulted in efficient conversion of the substrate, whereas cinnamaldehyde concentrations exceeding 0.1% were associated with a decrease of the conversion rate (Fig. S1). By varying the pH of the medium from 5.3 to 7.8 (Fig. S2), the optimum pH was determined to be 5.8.

Under the optimum condition, the concentration of the product obtained after conversion by immobilized yeast was analyzed with HPLC and the result was compared with that obtained after conversion by unimmobilized (free) yeast. As shown in Figs. 2 and S3, cinnamaldehyde was converted to the product at quantifiable levels by immobilized yeast cells. Without addition of acetaldehyde, the second substrate, the efficiency of conversion was improved from the rate of 5–20% previously reported for unimmobilized yeast to become almost equimolar. Under the same conditions, a small amount of the product and an unidentified metabolite accumulated after conversion by unimmobilized (free) yeast. The products obtained with immobilized yeast were purified by preparative silica gel TLC and the structure of the compound was determined to be 5-phenyl-4-pentene2,3-diol **4** by ^{1}H NMR spectrometry (^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.20 (d, 3H, J = 6.4 Hz, CH_3), 2.02 (br, 2H, OH), 3.97 (dd, 1H, J = 3.7, 6.4 Hz, =CH–CH(OH)–*CH*(OH)–CH_3), 4.25 (dd, 1H, J = 3.7, 7.0 Hz, =CH–*CH*(OH)–CH(OH)–CH_3), 6.27 (dd, 1H, J = 7.0, 16.0 Hz, Ph-CH=*CH*–), 6.66 (d, 1H, J = 16.0 Hz, Ph-*CH*=CH–), 7.2–7.4 (m, 5H, Ar)). Specific rotation of this compound had a value of $[a]^{20}_{\alpha}$: + 16.4°(*c* 1.05 for ethanol), which was equal to the reported data and showed that its stereoselectivity was unchanged by immobilization. Addition of cinnamaldehyde to the suspension of immobilized cells three times at intervals of 48 h resulted in a constant conversion rate ranging from 73% to 83%.

The substrate specificity of this conversion process and the effect of immobilization of the yeast on the reaction profile were examined (Table 1). Immobilized yeast catalyzed the reaction of cinnamaldehyde and its derivatives. However, the effect of immobilization on diol formation was not as efficient as in the case of cinnamaldehyde **1**. To determine the structure of the intermediate compound, α-methylcinnamaldehyde **2** was used as the substrate because this led to greater accumulation of the intermediate compound. In addition, α-methylcinnamaldehyde **2** was added to unimmobilized (free) yeast and incubated as described above, after which the resulting compound was extracted and purified with preparative silica-gel TLC. The structure of the purified intermediate was determined to be 3-hydroxy-4-methyl-5-phenyl-4-penten-2-one **8** (Fig. 1) by ^{1}H NMR spectroscopy (^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.73 (s, 3H, α-CH_3), 2.09 (br, 1H, OH), 2.25 (s, 3H, $COCH_3$), 4.69 (s, 1H, C*H*(OH)), 6.72 (s, 1H, –CH=), 7.2–7.4 (m, 5H, Ar)).

TABLE 1. Effect of immobilization on the substrate specificitiy of cinnamaldehyde conversion to diol compounds.

R	Unimmobilized (free)				Immobilized			
	Substrate consumption	Accumulation of			Substrate consumption	Accumulation of		
		diol (product)	intermediate	by-product		diol (product)	intermediate	by-product
H	+++	+	+	+++	++	+++	+	++
CH_3	++	++	++	+++	++	++	+	++
Br	+	++	+	+++	++	+	–	++

R represents the substituent at the 2-position of cinnamaldehyde (see Fig. 1).