Biotransformation of acetophenone to 1-phenylethanol by fungi

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The fungi *Botrytis cinerea* and *Colletotrichum acutatum* were used to convert acetophenone to 1-phenylethanol. *B. cinerea* produced a 98.5% yield of 1-phenylethanol with an enantiomeric excess of 93.8%. Conversely, addition of 1-phenylethanol did not change the reaction rate or product composition. In contrast, the reaction catalyzed by *C. acutatum* was markedly slower, commencing only after an approximately three-day delay. The concentration of substrate decreased gradually over approximately 14 days, coinciding with increased production of 1-phenylethanol. Phenol was produced after 18 days, and the final yields of 1-phenylethanol and phenol were 38.1% and 61.5%, respectively.

Key words: biotransformation, *Botrytis cinerea*, *Colletotrichum acutatum*, acetophenone, chiral compound

1. INTRODUCTION

Because chemical reactions using biocatalysts proceed under “mild” conditions such as atmospheric pressure, room temperature, neutral pH, and in an aqueous solution, they typically do not harm the environment. Cultured microorganisms are therefore frequently used to catalyze reactions such as hydrolysis [1-4], addition [5], substitution [6-9], oxidation [10-13], reduction [14-16], prenylation [17-21], and glycosylation [22-25]. Biotransformations are particularly advantageous compared to organic chemical syntheses because optically active molecules are produced from prochiral substrates, and chiral alcohols are important products of the pharmaceutical industry [26-29].

We previously studied the biotransformation of the prenyl alcohols using cultured pumpkin cells [20]. Several studies were unsuccessful; in one, we evaluated the oxidation of aromatic compounds using apple callus. However, contamination by bacteria occurred in the flask, and the reaction occurred from the middle to the opposite direction. It has since returned to raw material. Inspired by this finding, we became interested in determining if

the various alcohols could be produced from acids or ketones using a more advantageous system. We reasoned that redox potential might be controlled using a green chemistry target, and after reviewing possible biocatalysts, we found that yeasts are used to synthesize chiral compounds as well as to catalyze various biotransformations. We ultimately decided to use plant pathogens that do not infect humans or animals and are used by a small number of researchers [21, 22]. For this purpose, we consulted Professor Yukio Harada, a specialist in plant pathology at the Faculty of Agriculture and Life Science, Hirosaki University, who provided samples of *Botrytis cinerea* and *Colletotrichum acutatum* and instructions for implementing cultivation methods. In particular, *B. cinerea* is indispensable for the manufacture of *la pourriture noble*, or noble rot [30-32]. Here we assessed the abilities of these two fungal species to catalyze the biotransformation of acetophenone. Incidentally, the research of biotransformation of acetophenone using these fungi as biocatalysts is now for the first time.

2. EXPERIMENTAL

2.1. Analysis

Reaction products were extracted with ether and measured using high-performance liquid chromatography (HPLC). We used a LichroCART column (Merck-Japan, Tokyo, Japan) eluted with hexane:2-propanol (40:1). To determine the chirality of the products, we used a chiral column (CHIRALPAK AD-H; Daicel Chemical Industries, Ltd., Tokyo, Japan) eluted with hexane:2-propanol (99:1). Gas-chromatography/mass spectrometric (GC-MS) analysis was used to identify the reaction products. The analytical system consisted of a JEOL Q 1000GC Md II Mass Spectrometer (Japan Electron Optics Laboratory Co., Ltd., Tokyo, Japan) equipped with an HP-5 column (0.32 mm $\times$ 30 m $\times$ 0.25 μ m). Helium was delivered at 1 mL/min. The temperature gradient started with a 4.7-min hold at 50 °C, increased by 15 °C/min for 25 min, and ended with a 2-min hold at 250 °C. Injector and GC-transfer-line temperatures were 200 °C.

2.2 Chemicals

Acetophenone (1st grade) and 1-phenylethanol (special grade) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). NADPH was purchased from Orient Yeast Co., Ltd. (Tokyo, Japan); 2-propanol and dimethylsulfoxide from Kanto Chemical Co., Inc. (Tokyo, Japan); and hexane (special grade) from Nacalai Tesque, Inc. (Kyoto, Japan). To determine enantiomeric excess (% ee), (S)- and (R)-1-phenylethanol were purchased from Sigma-Aldrich, Tokyo, Japan.

2.3. Culture conditions

Fungi were cultured using potato-dextrose-agar (PDA) E-MF21 (Eiken Chemical Co., Ltd., Tokyo, Japan) or potato-sucrose-agar (PSA) culture medium. Stocks of *B. cinerea* and *C. acutatum* (Fig. 1 and 2) were prepared by removing a 5-mm² section of agar using a platinum wire and inoculating the section into fresh culture medium. Stocks were stored at 20 °C.

Fig. 1. *B. cinerea* Fig. 2. *C. acutatum*

2.4. Starter cultures of fungi and biotransformation of acetophenone

Sterile techniques and media were used. The surfaces (hyphae) of colonies of *B. cinerea* and *C. acutatum* were harvested using a platinum wire and transferred to sterile potato-sucrose medium (50 mL) in an Erlenmeyer flask (100 mL). After the cultures were shaken in the dark at 20 °C for 72 h, acetophenone or 1-phenylethanol was added, and

the flasks were rotated (90 rotations/min) using a Taitec NR3 shaker (Taitec, Saitama, Japan).

2.5. Time course of acetophenone production

Samples (1 mL) of cultures were harvested from 0 to 24 h, extracted with ether, and analyzed using HPLC and GC-MS. We determined the effects of adding NADPH (0.50×10^{-3} mmol/L) to the culture medium.

3. RESULTS AND DISCUSSION

3.1. Biotransformation of acetophenone and (rac)-1-phenylethanol by *B. cinerea*

HPLC analysis of culture supernatants of *B. cinerea* detected peaks eluting at 9.4 min (acetophenone) and 19.5 min. The latter peak was identified as 1-phenylethanol, as expected, compared with the preparation. In contrast, 1-phenylethanol did not detectably react. Although numerous reports have used various biocatalysts to synthesize acetophenone, none has used *B. cinerea* as the biocatalyst [33-42].

3.2. Time course of the biotransformation of acetophenone by *B. cinerea* in the presence of NADPH

We investigated the time course of biotransformation of acetophenone using *B. cinerea*. (rac)-1-Phenylethanol was produced in yields of 69.8%, 92.9%, and 97.9% after 24, 48, and 72 h, respectively (Fig. 3).

Fig. 3. Time course of biotransformation of acetophenone in the presence of NADPH.

By the measurement of HPLC with chiral column, it was confirmed that acetophenone is converted to efficient (S)-1-phenylethanol (93.8% ee) (Fig. 4). Although the chirality of the product is influenced by the type of biocatalyst [33-37], the (S)-derivative was the main product detected here.

Fig. 4. Biotransformation of acetophenone by *B. cinerea*.

3.3. Time course of biotransformation of acetophenone by *C. acutatum*

Compared with *B. cinerea*, biotransformation of acetophenone by cultures of *C. acutatum* started slowly. The acetophenone peak that eluted at 9.4 min from the HPLC column began to decrease gradually from 3 to 12 days, while the 1-phenylethanol peak that eluted at 19.5 min began to increase. However, after 18 days, a new peak appeared that eluted at 14.3 min, increasing to 56.8% of the area of the acetophenone peak, and only the 1-phenylethanol and new peak were detectable. Two peaks eluted from the GC column at 6.5 and 8.1 min, and the latter was identified as 1-phenylethanol. The mass spectrum of the new product (GC, 6.5 min) showed a molecular ion [M] at m/z 94 (base peak), corresponding to C_6H_6O , together with characteristic fragments at m/z 66 [$M - 28$]⁺ (relative intensity, 28.3) and 65. We verified that this compound was phenol. The coincidence rate of the GC-MS library data was 92.2%. The time course of the biotransformation of acetophenone by *C. acutatum* is shown in Fig. 5.

Fig. 5. Time course of the biotransformation of acetophenone by *C. acutatum*

These findings indicate that acetophenone was converted to phenyl acetate through Baeyer-Villiger oxidation [38] (Fig. 6), which occurs frequently during biotransformations [35-44]. The reaction is as shown in Fig. 7.

Fig. 6. Predicted biosynthetic pathway of acetophenone.

Fig. 7. Biotransformation of acetophenone by *C. acutatum*.

4. CONCLUSIONS

B. cinerea converted acetophenone to 1-phenylethanol with a yield of 98.5% (93.8% ee). We conclude that the reaction was catalyzed by an enzyme such as oxidoreductase that required NADPH as its coenzyme and that the reaction generated the hydride of the nicotinamide group of NADPH that attacked the *re*-face of the prochiral surface of the substrate (Fig. 8).

In contrast, the biotransformation of acetophenone by *C. acutatum* produced phenol from 1-phenylethanol via phenyl acetate through Baeyer-Villiger oxidation (Fig. 6). The present findings may further improve the yields of beneficial compounds generated using green chemistry.

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Fig. 8. Transfer of H^- (*proR*-H) from NADPH to acetophenone.

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