Biotransformation of estragole by the plant cultured cells of Caragana chamlagu

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Abstract Estragole (EG) is biosynthesized in herbs including anise, basil, bay, tarragon, fennel and marjoram, and is thought to be a useful biomass for the food and health industries. Moreover, the metabolites from estragole are useful intermediates in organic synthetic chemistry. However, estragole has been transformed only by chemical methods, and no biocatalysts have been reported. In this paper, we report the biotransformation of estragole using the plant cells of Caragana chamlagu gave 4-methoxycinnamaldehyde (MCAL), 4-methoxycinnamyl alcohol (MCA) and 4-methoxybenzaldehyde (MBAL). In addition, we propose a reaction mechanism in the biotransformation of estragole using Caragana chamlagu. Since estragole generates malignant liver tumors in the rat, it is necessary to reduce exposure. The present study reveals the transformation of harmful estragole. Furthermore, we succeeded in biotransforming estragole as biomass using plant cells into useful compounds.

Key words: Biotransformation, Caragana chamlagu, estragole, plant cultured cells.
Tasmanian lavender, and these cultures were grown in MS solid medium (Murashige and Skoog 1962) containing 3 g l⁻¹ sucrose and 1 mg l⁻¹ 2,4-dichlorophenoxycetic acid (2,4-D) at 25°C for about 21 days in the dark. In this reaction, the callus tissues (5 g wet weight) were transferred to an MS liquid medium (10 ml) containing 30 g l⁻¹ sucrose and 1 mg l⁻¹ 2,4-D, and were grown with shaking at 120 rpm at 25°C for 24 h in the dark. EG (15 mg) in ethanol (1 ml) was added to the suspension. The mixture was shaken at 120 rpm at 25°C in the dark, and the callus tissues (0.2 g dry weight) were filtered to separate and washed by EtOAc (2 x 10 ml). The filtrate was dissolved in EtOAc (2 x 15 ml), and washed with sat. aq. NaCl (2 x 15 ml), and were filtered to separate and washed by EtOAc (2 x 10 ml). The combined organic phase was dried over Na₂SO₄ and concentrated in vacuo. The results of the conversions from the gas chromatographic analyses, which were performed using a Shimadzu GC-2014 equipped with a GC-column (DB-1, 60 m), are summarized in Table 1. The chemical yields were identified by 1H-NMR, 13C-NMR, or MS. GC-MS spectra were recorded using a JOEL JNM-GX400 spectrometer with tetramethylsilane as the internal standard. MCAL was identified by NMR and GC as compared with the reference (Su and Takaishi 1999) and the purchased standard sample: 1H-NMR (CDCl₃) δ=9.66 (d, J=7.8 Hz, 1H), 7.53 (d, J=9.2 Hz, 2H), 7.43 (d, J=15.6 Hz, 1H), 6.95 (d, J=8.7 Hz, 2H), 6.62 (dd, J=16.0, 7.8 Hz, 1H), 3.87 (s, 3H). 13C-NMR (CDCl₃) δ=193.8, 162.2, 152.8, 130.3, 126.8, 126.5, 114.5, 55.4; EI-MS m/z 162 [M⁺]⁺ (100), 161 (60), 147 (20), 121 (38), 119 (34), 108 (37), 91 (45), 77 (35). And MCA: 1H-NMR (CDCl₃) δ=7.33 (d, J=8.7 Hz, 2H), 6.86 (d, J=8.7 Hz, 2H), 6.56 (d, J=16.0 Hz, 1H), 6.20–6.27 (m, 1H), 4.30 (d, J=6.0 Hz, 2H), 3.80 (s, 3H), 1.57 (brs, 1H); 13C-NMR (CDCl₃) δ=159.1, 131.1, 129.4, 127.8, 126.3, 114.1, 64.1, 55.4; EI-MS m/z 164 [M⁺]⁺ (44), 162 (16), 147 (9), 131 (22), 121 (100), 108 (39), 91 (30), 77 (25); HRMS (TOF-Cl) caleed for C₉H₁₃O₂ (MH⁺): 165.0915 (Found: 165.0919). MBAL was identified by NMR and GC as compared with the purchased standard: 1H-NMR (CDCl₃) δ=9.89 (s, 1H), 7.85 (d, J=9.0 Hz, 2H), 7.01 (d, J=8.5 Hz, 2H), 3.90 (s, 3H); 13C-NMR (CDCl₃) δ=190.8, 164.6, 132.0, 129.9, 114.3, 55.6; EI-MS m/z 136 [M⁺]⁺ (78), 135 (100), 107 (13), 92 (13), 77 (21). Surprisingly, all the biocatalysts used here can easily convert estragole. However, in many cases, only a few products were detected, perhaps because of the facile biodegradation of the products. Among them, C. chamlagu gave considerable amounts of the products. When EG (15 mg) was added to the suspension of C. chamlagu (5 g wet weight) and shaken at 120 rpm at 25°C in the dark for 2 days, MCAL, MCA and MBAL were obtained in 19.5 and 2% yields, respectively.

The time course for the reaction using C. chamlagu is shown in Figure 2. The results show that more than 60% of EG was rapidly consumed in the first 2 days and then the decrease of EG was stopped for the following 4 days and then EG was decreased gradually. Corresponding to the decrease of EG, the yield of MCAL, the oxidation product, was obtained slowly up to 10% in 20 days. The other products, MCA and MBAL were increased gradually up to 7–10% in 14–20 days.

In order to elucidate the mechanism of this reaction

![Figure 1. Biotransformation of estragole (EG) by some plant cultured cells.](Image)

Table 1. Biotransformation of estragole by some plant cultured cells

<table>
<thead>
<tr>
<th>Runa</th>
<th>Plant cultured cells</th>
<th>Product (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EG</td>
<td>MCAL</td>
</tr>
<tr>
<td>1</td>
<td>Caragana chamlagu</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>Ocimum basilicum</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>O. basilicum cv. Purpurascens</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>Corchorus olitorius</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>Perilla frutescens var. crispa</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>Lavandula angustifolia</td>
<td>16</td>
</tr>
</tbody>
</table>

*Reaction time: 2 days.

EG, estragole; MCAL, 4-methoxycinnamaldehyde; MCA, 4-methoxycinnamyl alcohol; MBAL, 4-methoxybenzaldehyde

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pathway, MCAL was reacted with C. chamlagu and the results are shown in Figure 3. Then, MCAL (7.5 mg) was added to the suspension of C. chamlagu (3 g wet weight) and the resulting mixture was shaken at 120 rpm at 25°C in the dark. The starting material MCAL was decreased rapidly in the first 24 h and corresponding to the decrease of MCAL, the allyl alcohol, MCA was obtained (nearly 50–60% yield) and then the yield of MCA was decreased after 3 days and the yield of the starting material, unsaturated aldehyde, MCAL was increased again. From the results, MCAL was firstly reduced into MCA by C. chamlagu (nearly 60% yield in 3 days), and the product MCA was converted to give a mixture of MCAL, MCA and MBAL.

In addition, MCA was reacted with C. chamlagu and the results are shown in Figure 4. MCA (7.5 mg) was added to the suspension of C. chamlagu (3 g wet weight) and the resulting mixture was shaken at 120 rpm at 25°C in the dark. MCA was decreased rapidly in the first 12 h and corresponding to the decrease of MCA, MCAL was obtained (about 50%). Then the yield of MCAL was decreased after 12 h and MCA was slowly and slightly increased (about 10% in 10 days). From the results, the starting material MCA was oxidized into MCAL by C. chamlagu, and the product MCAL was afforded to a mixture of MCAL, MCA and MBAL (6, 9 and 3% yields, respectively).

From these results, the reaction pathway of the biotransformation of EG by C. chamlagu is explained as shown in Figure 5. At first, EG was oxidized into MCAL...
and (or) MCA by \textit{C. chamlagu}. Next, the degradative product, 4-methoxybenzaldehyde, MBAL was obtained from MCAL or MCA. MCAL was reduced into MCA, and MCA was oxidized into MCAL by \textit{C. chamlagu}. Since these oxidative and reductive reactions were attained to equilibrium between MCAL and MCA, the yields of MCAL and MCA were afforded to constant. The other pathway, the formation of MBAL via 4-methoxycinnamic acid (MCAc) was excluded. (In this reaction, MCAc and 4-methoxybenzoic acid as the products were not yielded and MCAc was not reacted in the reaction system.) Total yield (the yields of EG, MCAL, MCA and MBAL) was gradually reduced; it seems that MBAL was further degraded.

Tsai and co-workers reported the metabolic pathway of EG into MCA in animals (Tsai et al. 1994). Metabolic oxidation of the allyl side chain of EG may proceed into benzylic and terminal hydroxylation via radical intermediates. Moreover, Hatanaka and co-workers reported that trans-2, cis-6-nonadienal and trans-2, cis-6-nonadienol in the biooxidation of linolenic acid by plants are achieved to equilibrium state (Hatanaka et al. 1975). Therefore, we proposed the reaction mechanism in the formation of MCAL and MCA from EG (Figure 6). EG was immediately converted into MCAL and MCA via radical intermediates by \textit{C. chamlagu} and redox equilibrium between MCAL and MCA was achieved.

The production of MBAL was explained by the biooxidation of MCAL or MCA by a similar mechanism to that Ishikawa and co-workers reported in the biooxidation of coniferaldehyde and (or) ferulic acid by fungi where vanillin was obtained (Ishikawa et al. 1963). Therefore, it seems that MBAL was formed by the biooxidation of MCAL by \textit{C. chamlagu}.

Since EG generates malignant liver tumors in the rat, it is necessary to restrict and to reduce its exposure (European Commission 2001; Rietjens et al. 2005; Smith et al. 2002). The present study reveals the transformation of harmful EG. Furthermore, it was shown that MCAL, which can easily be purchased, was transferred into MCA as an available compound by \textit{C. chamlagu}.


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