Plant Biotechnology 23, 329–331 (2006)

Secondary Metabolite Note

Biotransformation of monofluorophenols by cultured cells of *Eucalyptus perriniana*

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Received September 22, 2005; accepted October 24, 2005 (Edited by K. Yazaki)

Abstract On the biotransformation of phenol and monofluorophenols by the cultured cells of *Eucalyptus perriniana*, phenyl and fluorophenyl β-D-glucosides were isolated after a 1-h incubation. Especially, the yield of 2-fluorophenol is higher than those of the other substrates, i.e., phenol, 3- and 4-fluorophenols. These results indicated that *E. perriniana* cells selectively glycosylate the hydroxyl group of 2-fluorophenol discriminating phenol, 3-and 4-fluorophenols.

Key words: Cultured plant cells, *Eucalyptus perriniana*, glycosylation, monofluorophenol.

Halophenols and their derivatives are the primary pollutants of mainly anthropogenic origin. Over several decades, these compounds have been widely used as herbicides, pesticides or building blocks in chemical and pharmaceutical syntheses, and they have caused serious local contamination of the environment. Therefore, soil microorganisms have developed the capacity of utilizing halophenols for their growth by a diverse set of biodegradation pathways (Haggblom 1992). Furthermore, the microbial transformation of monofluorophenols by the whole cells of *Rhodococcus opacus* 1cp was investigated (Finkelstein et al. 2000). However, up to now there are no reports concerning the biotransformation of monofluorophenols by cultured plant cells. To clarify the abilities of cultured plant cells to convert monofluorophenols, we studied the biotransformation of monofluorophenols by eucalyptus cultured cells. We describe that eucalyptus cultured cells selectively glycosylate the hydroxyl groups of the 2- fluorophenol and can discriminate the ortho, meta and para positions of the fluorophenols.

The cultured cells of *Eucalyptus perriniana* were prepared according to the method described in the literature (Furuya et al. 1987), and the feeding and incubation experiments were carried out in a manner similar to that previously reported (Hamada et al. 1997; 2001). Just prior to use for this work, part of the callus tissues (fresh weight 20 g) was transplanted to freshly prepared Murashige and Skoog's medium (100 ml in a 300 ml conical flask, pH 6.2) containing 1 ppm of benzyladenine and 3% sucrose and grown with continuous shaking for three days at 25°C in the dark. Then the phenol (**1**) (0.5 mM/flask, 200 μl EtOH solvent) was added to the cultured cells and the cells were incubated at 25°C for a 1-hour on a rotary shaker (120 rpm) in the dark. After incubation, the cultures were harvested and product **5** was isolated from the extracts of the media and cells. The media were applied to a Diaion HP-20 column. The column was then washed with H_2O followed by elution with MeOH. The cells were extracted ($\times$3) by homogenization with MeOH and the extract was concentrated. The residue was applied to a Diaion HP-20 column which was washed with H_2O followed by elution with MeOH. The MeOH eluates of the media and cells were combined and subjected to HPLC [column: CrestPak C18S, ϕ 4.6$\times$150 mm; solvent: Dioxane : H_2O (1 : 9, v/v); detection: UV (269 nm); flow rate: 1.0 ml/min] and products **5** (17.2%; retention time 5.8 min) was obtained. Product **5** was identified by comparison of NMR spectrum with that of an authentic sample (Matsumoto et al. 1986). The yield of the product was determined by HPLC of the standard carve of the product. No additional conversion products were observed in spite of the careful HPLC analyses (Table 1). Feeding and incubation experiments of **2**–**4** were also carried out in the same manner as described above. The substrates **2**–**4** were converted into **6**–**8**, respectively (Table 1). Products **6** and **7** were new compounds and were identified on the basis of the ^{1}H and ^{13}C NMR, H-H COSY, C-H COSY, HMBC, NOESY and FABMS

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spectra (Yu et al. 2004). Product **8** was identified by comparison of the ^{1}H, ^{13}C NMR, H-H COSY, C-H COSY and FABMS spectra with those in the literature (Arita et al. 1978). The anomeric signals of the products (**5**, **6**, **7** and **8**) were observed at δ 4.90 (1 H, d, J=7.2 Hz, H-1′), δ 4.97 (1 H, d, J=8.0 Hz, H-1′), δ 4.92 (1 H, d, J=7.2 Hz, H-1′) and δ 4.83 (1 H, d, J=7.2 Hz, H-1′) indicating the β-D-glucosides configuration, respectively.

Spectral data of **6**: 2-fluorophenyl β-D-glucoside. White amorphowus powder, FABMS m/z: 297 $[M+Na]^{+}$. ^{1}H NMR (400 MHz, CD_3OD, δ in ppm): 3.43–3.90 (6 H, m, H-2′–H-6′), 4.97 (1 H, d, J=8.0 Hz, H-1′), 6.96–7.01 (1 H, m, H-4), 7.06–7.12 (2 H, m, H-3, H-6), 7.25–7.30 (1 H, ddd, J=1.6 Hz, J=8.4 Hz, H-5). ^{13}C NMR (100 MHz,CD_3OD, δ in ppm): 62.3 (C-6′), 71.1 (C-4′), 74.6 (C-2′), 77.8 (C-3′), 78.0 (C-5′), 102.5 (C-1′), 117.0 (1C, d, $J_{F\text{-}3C}$=18.9 Hz, C-3), 119.1 (C-6), 123.8 (1C, d, $J_{F\text{-}4C}$= 6.6 Hz, C-4), 125.4 (1C, d, $J_{F\text{-}5C}$=4.2 Hz, C-5), 146.3 (1C, d, $J_{F\text{-}C}$=10.8 Hz, C-1), 153.9 (1C, d, $J_{F\text{-}2C}$=244.2 Hz, C-2).

Spectral data of **7**: 3-fluorophenyl β-D-glucoside. White amorphous powder, FABMS m/z: 297 $[M+Na]^{+}$. ^{1}H NMR (400 MHz, CD_3OD, δ in ppm): 3.38–3.91 (6 H, m, H-2′–H-6′), 4.92 (1 H, d, J=7.2 Hz, H-1′), 6.74 (1 H, m, H-4), 6.86 (1H, m, H-2), 6.90 (1 H, dd, J=2.2 Hz, J= 8.0 Hz, H-6), 7.27 (1 H, m, H-5). ^{13}C NMR (100 MHz, CD_3OD, δ in ppm): 62.3 (C-6′), 71.2 (C-4′), 74.7 (C-2′), 77.7 (C-3′), 78.0 (C-5′), 102.1 (C-1′), 105.2 (1C, d, $J_{F\text{-}4C}$ =25.5 Hz, C-4), 109.7 (1C, d, $J_{F\text{-}2C}$=20.6 Hz, C-2), 113.4 (1C, d, $J_{F\text{-}6C}$=2.4 Hz, C-6), 131.3 (1C, d, $J_{F\text{-}5C}$=9.9 Hz, C-5), 160.1 (1C, d, $J_{F\text{-}1C}$=10.5 Hz, C-1), 164.5 (1C, d, $J_{F\text{-}3C}$=241.7 Hz, C-3).

Spectral data of **8**: 4-fluorophenyl β-D-glucoside. White amorphous powder, FABMS m/z: 297 $[M+Na]^{+}$. ^{1}H NMR (400 MHz, CD_3OD, δ in ppm): 3.37–3.91 (6H, m, H-2′–H-6′), 4.83 (1 H, d, J=7.2 Hz, H-1′), 6.96–7.02 (2H, m, H-3, H-5), 7.08–7.12 (2 H, m, H-2, H-6). ^{13}C NMR (100 MHz, CD_3OD, δ in ppm): 62.4 (C-6′), 71.2 (C-4′), 74.7 (C-2′), 77.7 (C-3′), 78.0 (C-5′), 102.9 (C-1′), 116.4 (2C, d, $J_{F\text{-}3,\,5C}$=23.1 Hz, C-3, C-5), 119.3 (2C, d, $J_{F\text{-}2,\,6C}$=8.3 Hz, C-2, C-6), 155.2 (1C, d, $J_{F\text{-}1C}$=2.5 Hz, C-1), 159.4 (1C, d, $J_{F\text{-}4C}$=237.6 Hz, C-4).

The biotransformation scheme of **1**–**4** by the cultured cells of *E. perriniana* is shown in Figure 1. The biotransformation yields of the phenol and monofluorophenols by the cultured cells of *E. perriniana* are shown in Table 1. As show in Table 1, the yields of products **5**, **6**, **7** and **8** were 17.2, 49.2, 19.6 and 28.0%, respectively. The conversion yield of 2-fluorophenol showed the highest value in comparison with that of phenol, 3- and 4-fluorophenols. Especially, 2-fluorophenol was quantitatively converted to the corresponding β-D-glucoside. Furthermore, we studied the time-course experiment of 2-fluorophenol (Figure 2). After a 1-hour incubation, 2-fluorophenol completely disappeared and

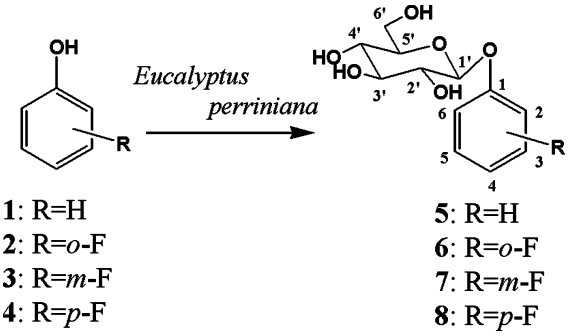

Figure 1. Biotransformation of **1**–**4** by the cultured cells of *E. perriniana*. **1**, phenol; **2**, 2-fluorophenol; **3**, 3-fluorophenol; **4**, 4-fluorophenol; **5**, phenyl β-D-glucoside; **6**, 2-fluorophenyl β-D-glucoside; **7**, 3-fluorophenyl β-D-glucoside; **8**, 4-fluorophenyl β-D-glucoside.

Table 1. The glucosylation of **1**–**4** by the cultured cells of *E. perriniana*.

	Product	Yield (%)[a)]
1	Phenyl β-D-glucoside (5)	17.2
2	2-Fluorophenyl β-D-glucoside (6)	49.2
3	3-Fluorophenyl β-D-glucoside (7)	19.6
4	4-Fluorophenyl β-D-glucoside (8)	28.0

[a)] The yield of the product was determined by HPLC of the standard carve of the product.

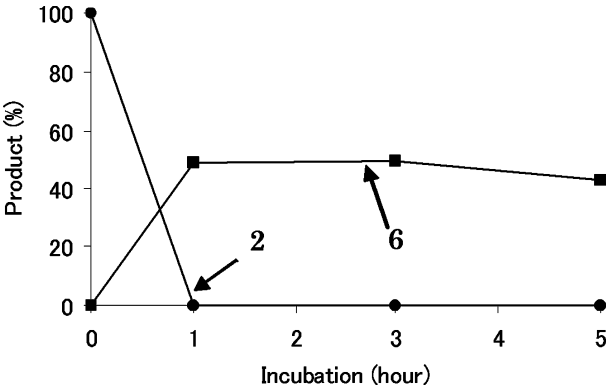

Figure 2. Time-course of biotransformation of **2** by the cultured cells of *E. perriniana*. **2**, 2-fluorophenol; **6**, 2-fluorophenyl β-D-glucoside. After a 1-hour incubation, 2-fluorophenol completely disappeared and then the product, 2-fluorophenyl β-D-glucoside, was quantitatively produced.

then the product, 2-fluorophenyl β-D-glucoside, was quantitatively produced. These demonstrate that the cultured cells of *E. perriniana* selectively glycosylate the hydroxyl group of 2-fluorophenol rather than that of the other substrates, i.e., phenol, 3- and 4-fluorophenols.

Based on the results of this experiment, it was found that the eucalyptus cultured cells were able to glycosylate selectively the hydroxyl group of the 2-fluorophenol and were capable of discriminating the ortho, meta and para positions of the fluorophenols. This plant enzyme should be a valuable addition to the group of biocatalysts used

for the chemical modification of halophenols. Also, this method is of considerable interest in green chemistry. Studies of the biotransformation of other halophenols by the cultured cells of *E. perriniana* are now in progress.

Acknowledgements

The authors are grateful to the Research Instruments Center of the Okayama University of Science for the NMR and FABMS spectra.

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