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Baicalin and Baicalein Productions of Cultured *Scutellaria baicalensis* Cells

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Three kinds of callus tissues(S.b.p, S.b.c and S.b.r), derived from petiole, cotyledon, and root of a young plant of *Scutellaria baicalensis* were assayed for their biologically active flavonoid productions. The baicalin and baicalein contents of the extracts of S.b.p, S.b.c and S.b.r cultured cells were measured by HPLC. The baicalin contents in fresh S.b.p, S.b. c and S.b.r were 8.5, 7.1 and 0.6 % g/dw, respectively and the baicalein contents of the same extracts 0.3, 1.2 and 0.2% g/dw, respectively. After drying at 60°C, the baicalin content of S. b. c decreased to 0.3% g/dw with an increase in the baicalein content to 2.9 % g/dw, but the baicalin and baicalein contents of S.b.p did not change after drying. On the other hand, in S.b.r both, baicalin and baicalein almost disappeared. It is of interest that the three kinds of callus tissues obtained from the same original plant have different productivity for baicalin and baicalein.

Keywords----*Scutellaria baicalensis* ; Labiatae; callus culture; baicalin; baicalein; baicalinase; baicalein 7-*O*-glucuronosyltransferase

Scutellaria Radix, dried root of *Scutellaria baicalensis* is a well known drug in traditional Chinese medicine used for treatment of bronchitis, hepatitis and diarrhoea.[1,2]

Recent papers have reported that the flavonoids, such as baicalein and baicalin from the roots of these species have many biological activities such as an inhibitory effect on the human immunodeficiency virus(HIV-1),[3] human T cell leukemia virus type I(HTLV-I)[4] and mouse skin tumour promotion.[5] In this context, the preparation of bioactive flavonoids by plant tissue culture seems to be an important and promising scheme for production of medicinal sources. For that purpose, we established three kinds of callus tissues, S.b.p, S.b.c and S.b.r, derived from petiole, cotyledon and root of a young plant of *S. baicalensis*. Many studies on calli of *S. baicalensis* have been reported, but the fact that these three kinds of calli have different productivities of baicalin and baicalein has not been reported.[6-13]

In this paper, we report the establishment of three kinds of callus cultures of *S. baicalensis* and their productivities of baicalin and baicalein. Furthermore, the activities of baicalinase and baicalein 7-*O*-glucuronosyltransferase of S.b.p and S.b.c were examined.

MATERIALS AND METHODS

Culture methods Three original strains of callus tissues(S.b.p, S.b.c and S.b.r) were derived from petiole, cotyledon and root of a sterile young plant of *Scutellaria baicalensis* in April 1991. They were subcultured every 3 weeks in a Murashige & Skoog's medium[14] containing 3 mg/l naphtaleneacetic acid, 0.1mg/l kinetin and 3% sucrose. Callus was cultured on a 50ml solid medium/100 ml flask at 25°C in the dark and suspension culture was achieved in a 250ml medium /l flask or a 125 ml /500 ml flask at 25°C in the dark at 80 strokes/min.

Extraction and analyses of baicalin and baicalein After 3 week culture, fresh callus was refluxed with absolute EtOH for 30 min. Then the residue was refluxed with 50% EtOH, absolute EtOH and H_2O, successively. Dried callus prepared in an oven at 60 °C for 7 days was refluxed with 50% EtOH for 30 min. Then the residue was refluxed with absolute EtOH and H_2O, successively. The EtOH and H_2O solutions were combined and evaporated to dryness.

The extract was dissolved in 50% EtOH and used for HPLC analysis. HPLC conditions are shown below.

Detection of baicalinase and baicalein 7-*O*-glucuronosyltransferase(UBGAT) The cultured cells(3g) were homogenized in 6ml of 0.1M phosphate buffer at pH 7.0 and the homogenate was centrifuged at 20,000 x g for 15 min. The supernatant was subjected to gel filtration using Sephadex G-25 column (Pharmacia PD-10), and the eluate was used as crude enzyme. The standard baicalinase assay mixture consisted of 0.17mM baicalin dissolved in 200μl of 17mM citrate buffer pH 5.0, and 100μl of the crude enzyme solution. The reaction mixture was incubated for 30min at 30°C. The reaction was stopped by the addition of 1 ml MeOH. The aglycone, baicalein, was identified and quantified by HPLC, the conditions of which are described below. UBGAT assay was carried out as follows. Baicalein and UDP-glucuronate as substrates were reacted with the crude enzyme solution in a citrate-phosphate buffer pH 6.5 at 37 °C for 5 min and then the reaction was stopped by addition of 100μl MeOH. The enzyme activity was determined by measuring the amount of baicalin by HPLC.

Conditions for HPLC The HPLC system used (Waters, Japan) was composed of a model 510 pump and a SPD-2A spectrophothometric detector equipped with a Hibar Purospher PR-18 column (4.6mm i. d., 150mm long; Cica-Merck) for the component analysis and a Mightysil PR-18 column(4.6mm i. d., 150mm long; Kanto Chemical) for the detection of the enzyme reaction products. Two solvent systems were used: MeOH-60mM phosphoric acid (55:45) ; flow rate of 0.8ml/min for baicalin(Rt 18 min) and baicalein(Rt 32 min); CH_3CN-60 mM phosphoric acid(21:79, V/V); flow rate of 1.1 ml/min for baicalin(Rt 4.6 min) and baicalein(Rt 17.4 min). The effluent was monitored by absorption at 274 nm. The peak intensity was determined with a C-R3A Chromatopac(Shimadzu, Kyoto, Japan). The amount of component from callus extracts and the enzyme reaction products were estimated from the standard curves obtained with authentic samples.

RESULTS and DISCUSSION

The three kinds of calli, S.b.p, S.b.c and S.b.r used for the experiments were a slightly friable, a moist and nodular calli, respectively.

The HPLC patterns of the extracts of fresh and dried calli are shown in Fig. 1. The retention times of baicalin and baicalein were about 18 and 32 min. respectively, under the HPLC conditions.

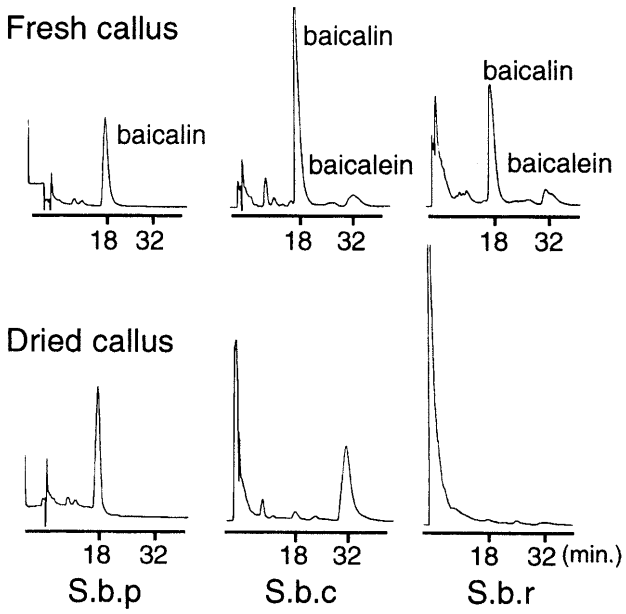

Fig. 1. HPLC patterns of the extracts from fresh and dried calli (Stains S.b.p, S.b.c and S.b.r).

In both fresh and dried S.b.p calli, the major peak was baicalin with no detectable baicalein peak. In fresh S.b.c callus, peaks for baicalin and baicalein were detected but after drying, the peak for baicalin almost disappeared and the peak of baicalein increased markedly. In fresh S.b.r callus, both baicalin and baicalein were detected but after drying, both baicalin and baicalein peaks almost disappeared. They were apparently converted into compounds which could not be identified by the present assay.

Then, the baicalin and baicalein contents in each of the fresh and dried callus tissues were examined. The baicalin contents in fresh S.b.p and S.b.c calli were 8.5 and 7.1 % g/dw, respectively, but that of S.b.r callus was only 0.6 % g/dw. The baicalein contents of these three extracts were 0.3, 1.2 and 0.2% g/dw, respectively. After drying at 60 °C, the baicalin content of strain S.b.c decreased markedly from 7.1% to 0.3% g/dw, causing a corresponding increase in the baicalein content from 1.2% to 2.9 % g/dw. However, as demonstrated by HPLC profiles, in strain S.b.p, the baicalin content did not change after drying, and the baicalin and baicalein contents of S.b.r strain were very low compared with those of S.b.p and S.b.c strains.

The time courses of baicalin and baicalein productions by S.b.p and S.b.c strains, by using cell suspension cultures of S.b.p and S.b.c strains are shown in Figs. 2 and 3. On Murashige & Skoog's [14] medium, the growth reached its maximum on day 15.

The baicalin content increased markedly from day 10, reached its maximum on day 17, and then, decreased sharply after day 21. On the other hand, baicalein was

detected only on day 19 and 21.

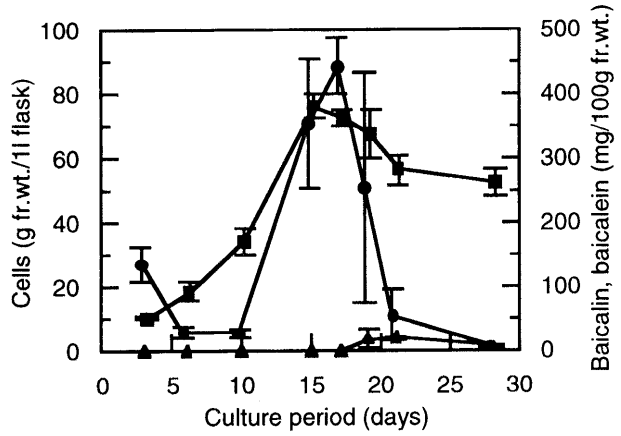

Fig. 2. Time courses of flavonoid production by cultured cells of the S.b.p strain.
-■- growth; -●- baicalin; -▲-baicalein
Each point and bar shows average and standard deviation from three different cultures.

In the case of S.b.c strain, although, the cell growth pattern was similar to that of S.b.p strain, the baicalin content did not change markedly, from day 13 to day 24. A small amount of baicalein was also detected from day 3 to day 24(Fig. 3).

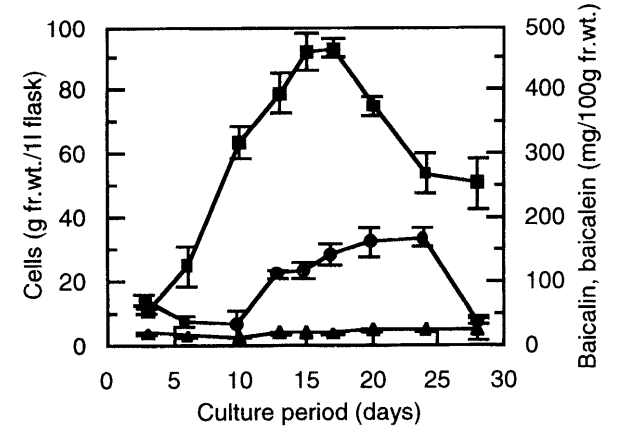

Fig. 3. Time courses of flavonoid production by cultured cells of the S.b.c strain.
-■- growth; -●- baicalin; -▲-baicalein
Each point and bar shows average and standard deviation from three different cultures.

The time course experiments using S.b.p and S.b.c strains demonstrated that these strains have different productivities for baicalin and baicalein. The activities of baicalinase(baicalin β-glucuronidase) and baicalein 7-*O*-glucuronosyltransferase (UBGAT) of 6-, 14- and 20-day old cell cultures of S.b.p and S. b.c are shown in Fig. 4. The baicalinase activity in the

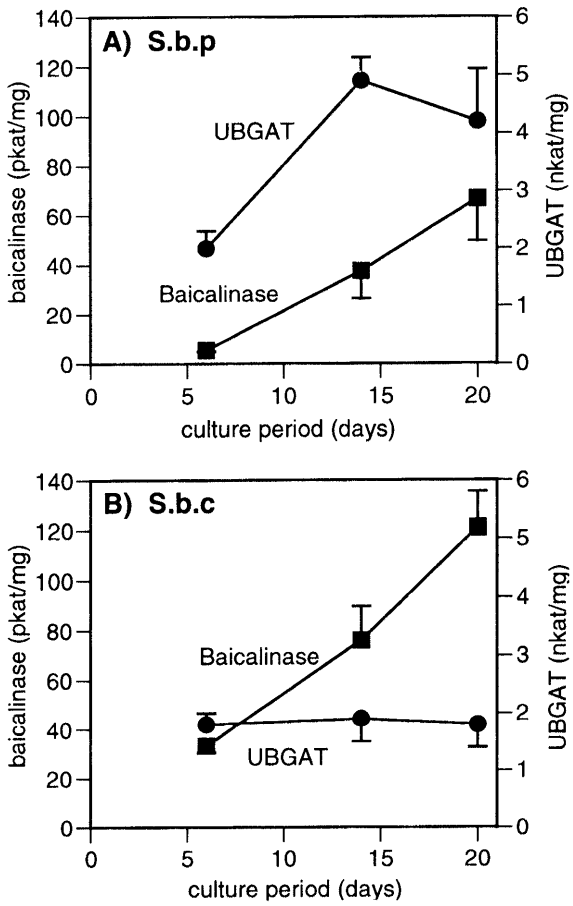

Fig. 4. Activities of baicalinase and baicalein 7-*O*-glucuronosyltransferase in *S. baicalensis* cultured strains S.b.p and S.b.c.
Each point and bar shows average and standard deviation from three different cultures.

S.b.c strain was twelve times that of S.b.p strain on day 6 but it was two times that of S.b.p on days 14 and 20. The UBGAT activity in the strain S. b. c remained constant during this period(Fig. 4. A, B).

These enzyme activities may account for the time coureses of baicalin and baicalein contents in these strains, partly. However, these data do not explain the causes of rapid increase and decrease of the baicalin contents in S. b. p strain.

The structure of baicalein is shown in Fig. 5 which is known to be glycosylated to baicalin by UBGAT characterized by us[15]. It is of interest that the three types of calli induced from the same original plant have different productivity for baicalin and baicalein.

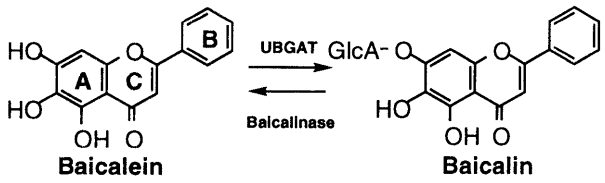

Fig. 5. Baicalin is produced via a baicalein by baicalein 7-*O*-glucuronosyltransferase.

It is likely that in S.b. c and S.b.p strains, the different baicalinase and UGBAT activities is one of the important factor of the difference in baicalein content. It was demonstrated for the first time that baicalin can be biosynthesized from baicalein using a crude enzyme solution.

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