Berberine Production by Cultured Coptis japonica Cells in a One-stage Culture Using Medium with a High Copper Concentration

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Berberine is an important phytochemical known to have antibacterial, stomachic and anti-inflammatory activities. The rhizome of a typical source plant, Coptis japonica, has a low content of berberine and more than 5 years growth is necessary before the rhizomes can be harvested. Yamada and Sato succeeded in establishing a method for berberine production by cell cultures of Coptis japonica. We have undertaken to develop a method for the industrial production of berberine using their cell line.

Since Zenk et al. showed that a large amount of indole alkaloids could be produced in a two-stage culture of Catharanthus cells, in which cell growth and metabolite production took place in separate media, and since Fujita et al. established a method for the commercial production of a secondary metabolite, shikonin, involving a large scale two-stage culture of Lithospermum erythrorhizon cells, many other researchers have tried to use two-stage cultures to enhance the metabolite production from various plant species.

We investigated the optimum media for cell growth and berberine production using a highly productive cell line for berberine, of which the berberine productivity was enhanced by repeated colony selection from cell aggregates of about 1 mm in diameter. This line was originally induced from small fragments of rootlets derived from the leaves and petioles of Coptis japonica Makino var. dissecta (Yatabe) and selected by Yamada and Sato. The cells were maintained in Linsmaier-Skoog (LS) liquid medium supplemented with $10^{-5}$ M x-naphthaleneacetic acid and $10^{-8}$ M 6-benzylaminopurine by the method mentioned in a previous paper. After 14 days culture, the harvested cells were dried overnight at 40°C and then weighed for calculation of the dry cell yield. Alkaloids were repeatedly extracted from these cells by ultrasonic treatment in 90% methanol until all the yellow pigment had been removed. The concentration of berberine in the extract was determined by HPLC. The amount of berberine produced from the cells was calculated as anhydrous berberine chloride.

We followed the time courses of cell growth and berberine production in C. japonica cell cultures to ascertain the usefulness of a two-stage culture. In our Coptis cell cultures, the cells proliferated initially and then berberine was produced. The cell yield increased from day 7 to 10; whereas, the berberine content rose only after the tenth day. This delay in berberine production suggested that a two-stage culture might be useful for enhancing the production of berberine as in the case of shikonin production.

We investigated the optimum concentrations of all the components of LS medium in order to develop growth and berberine production media. Only sucrose and copper (CuSO$_4$) caused increases in the berberine yield. The optimum concentration of sucrose for cell growth was 4% and that for berberine production (berberine content of cells) 2%. Above 3% sucrose, the berberine yield (mg/l), however, remained constant. Accordingly, a two-stage culture in which the sucrose concentration is varied (4% for cell growth and then 3% for berberine production) can be used; but the effect would be slight.

When the copper concentration was increased to 10 times (1.0 μM) that in the LS formula, the berberine yield was 20~30% higher than that with the LS medium (Fig. 1). A change in the copper concentration, however, had almost no effect on cell growth. Therefore, a single medium with a high concentration of copper can be used...
to subculture *C. japonica* cells; a two-stage culture is unnecessary. In fact, when we subcultured *Coptis* cells in a high copper medium (named 'M-101 medium'), they produced stable yields of berberine in 14-day cultures.

For all the other components, the concentrations used in the LS formula were optimal for both cell growth and berberine production. Because, as to the economics of industrial production, it is important that the berberine yield (not the berberine content) be maximum, the effect of a two-stage culture in which the concentrations of these components are changed would be negligible for commercial berberine production.

There have been other reports of an increase in metabolite production with a high concentration of copper. Bligny and Douce suggested that the amount of copper in the medium affects mitochondrial respiration via copper incorporation into cytochromes in suspension-cultured sycamore cells. Therefore, the effect of copper on secondary metabolite production may be based on its participation in the establishment of the mitochondrial electron transport system.

Researchers intending to produce secondary metabolites usually have a choice of culture methods (a one- or two-stage culture) early in their studies. On the basis of the results reported here and those reported for shikonin production, we propose that the culture method to be used should not be determined by judgement as to whether metabolite production is growth associated or not. Researchers should choose the method on the basis of those factors that initiate or stimulate the production of the metabolite in question. If factors that cause a reduction in cell growth, such as removal of ammonium, or a decrease in the nitrogen or phosphate concentration in the medium (as observed in shikonin production), stimulate metabolite production, then a two-stage culture is preferable; but, if the key factor does not affect cell growth, as with copper in berberine production, then a one-stage culture would be suitable for metabolite production. The appropriate choice of a culture method which is in accord with the characteristics of the cell species should result in enhanced production of metabolites which is commercially feasible.

REFERENCES