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Production of Antibacterial Triterpene Acids Not Detected in the Native Plant by Cell Suspension Culture of *Tectona grandis*

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The callus culture of *Tectona grandis* was previouly reported by us to produce five antibacterial triterpene acids which occurred only in very small amounts or were not detected in the native plant. This paper shows the production of these antibacterial compounds in much higher yield by cell suspension culture of the plant.

Key words : high productivity, callus culture, lupenoic acid, oleanenoic acid, ursenoic acid

Introduction

Plant cell culture has been considered since the 1970s to be a potential technique for producing natural secondary metabolites *in vitro*. The possibility of using plant cell culture for secondary metabolite production has been investigated for over 25 years, with the potential advantages of producing useful metabolites under environmentally controlled conditions: free from diseases, pests, floods and drought, and irrespective of weather. One of the most important advantages of using cell culture is a potential for producing useful compounds not occurring in the parent plant[1,2]. However, in spite of extensive optimization of culture conditions, cultured cells synthesized and accumulated specific secondary metabolites at mostly lower levels than the native plant. We[3] previously isolated 5 antibacterial triterpene acids from the callus of *Tectona grandis* ('teak'), a tropical tree of the family Verbenaceae, identified these compounds as 2α, 3β-dihydroxy-olean-12-en-28-oic acid (**1**), 2α, 3β-dihydroxy-urs-12-en-28-oic acid (**2**), 2α, 3α-dihydroxy-urs-12-en-28-oic acid (**3**), betulinic acid (**4**), and 2α, 3α, 23-trihydroxy-urs-12-en-28-oic acid (**5**) in decreasing order of antibacterial activity, and reported that these triterpene acids (**1**, **2**, **3**, **5**) except betulinic acid (**4**) occurred only in very small quantities or did not occurr in the parent native plant. In this paper we report the possibility of production of these antibacterial triterpene acids by cell suspension culture.

Materials and Methods

General

HPLC was carried out on a Waters LC Module I equipped with a U6K injector and a UV detector (234 nm) with an Inertsil ODS-3 column (ϕ 4.6 × 250 mm), eluted with H_2O-MeOH (8:92 - 1:99) of 1 ml/min at room temperature.

Induction and subculture of the callus

The callus was induced from young leaves of the plant grown in the botanical garden, Bandung Institute of Technology, Indonesia. The medium for the induction and subculture was the Murashige and Skoog (MS) medium containing 3 % sucrose, 0.2 % Gelrite (San-Ei Gen F.F.I.), 5 m*M*

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NAA and 0.1 m*M* BA. The cultures were kept in the dark at 25 °C and subcultured at 3-week intervals.

Initiation of cell suspension culture and subculture

Cell suspension culture was initiated by transferring 2 g of friable callus into a 100 ml conical flask containing liquid MS medium supplemented by 3 % sucrose, 0.1 m*M* NAA, and 0.1 m*M* BA. The culture was agitated on a rotary shaker (120 rpm) in the dark at 25 °C, and subcultured at 10-day intervals in the fresh medium of the same composition as the initiation medium.

Suspension culture for quantitation of the triterpene acids

The fast growing cells (10 g) from the established cell suspension culture were collected by filtration through a Buchner funnel under slight vacuum and transferred into a 500 ml conical flask containing 200 ml of the same liquid medium as the initiation medium. The culture was agitated on a rotary shaker (120 rpm) in the dark at 25 °C.

Measurement of cell growth

At 2-day intervals, 10 ml of the suspension-cultured cells were harvested, centrifuged, lyophilized, and weighed.

Quantitative analysis of the triterpene acids in suspension cultured cells

For quantitative analysis of the triterpene acids were used the lyophilized cells which were prepared for measuring the weight of cells during the course. The quantitative analysis was conducted as previously reported[3] by HPLC of the benzoyl derivatives of their methyl esters.

Results and Discussion

The growth of cells in the suspension culture during the course of the experiment peaked on the 12th day (Fig. 2). Compounds **1**, **2**, **3**, **4**, and **5** (Fig. 1) were produced with the cell growth (Fig. 2). Compounds **3** and **5** markedly increased after the 10th day and decreased abruptly after

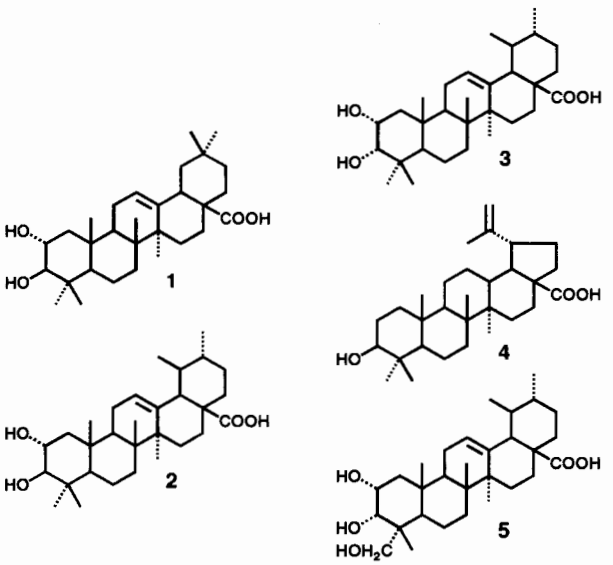

Fig. 1 Chemical structures of triterpene acids

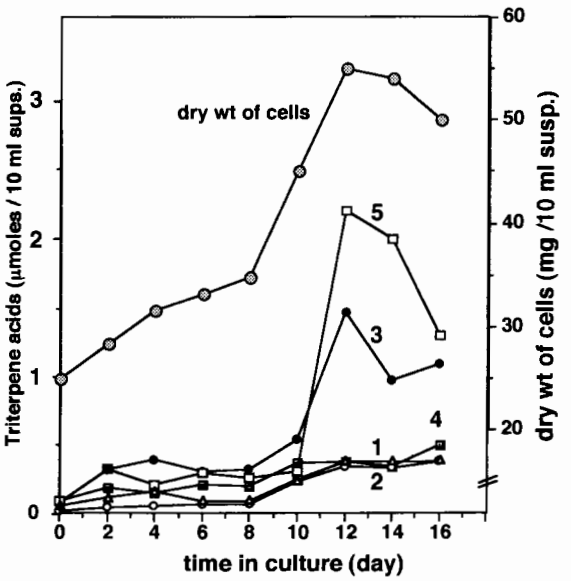

Fig. 2 Growth of *T. grandis* cells and triterpene acid content during the course of the suspension culture.

△ **1** 2α, 3β-dihydroxy-olean-12-en-28-oic acid
○ **2** 2α, 3β-dihydroxy-urs-12-en-28-oic acid
● **3** 2α, 3α-dihydroxy-urs-12-en-28-oic acid
■ **4** 3β-hydroxy-lup-20(29)-en-28-oic acid
□ **5** 2α,3α,23-trihydroxy-urs-12-en-28-oic acid

the 12th day. Compound **5** was produced in the highest yield among the triterpene acids, and **3** placed second. Compound **4** significantly increased after the 14th day. In the cell suspension culture, compound **4** was produced at a level

Table 1 Ratio of triterpene acid productivity of suspension culture to callus culture

Sample	1	2	3	4	5
	Content (μg/g dry wt)				
cell susp.*	1652.0	1935.2	10,300.0	4560.0	13,400.0
callus	23.6	20.0	70.8	387.6	58.5
cell susp./callus	ratio				
	70	97	146	12	229

*: the highest production during the course of cell growth (on the 12th day after initiation of culture)

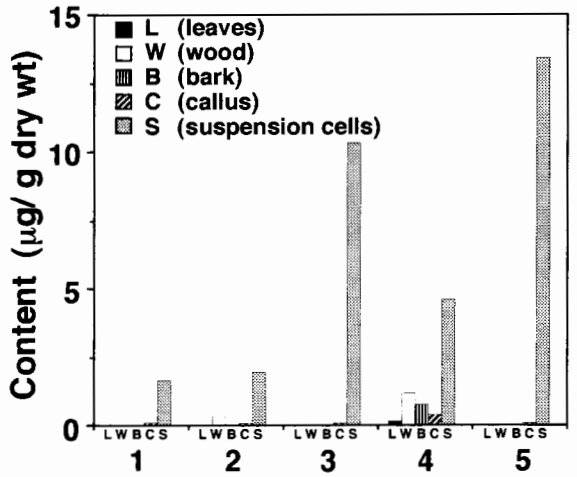

Fig. 3 Maximum yield of triterpene acid in suspension culture compared with that of callus and various parts of the plant.

higher than the most active compounds **1** and **2**. Compound **1** peaked on the 12th day and then drecreased. The content of **2** reached the maximum on the 14th day and then drecreased.

In summary, these antibacterial triterpene acids were produced in the highest yield ca. 12 days after initiation of culture.

Fig. 3 shows that the content of each triterpene acid on the 12th day in the suspension culture was compared with that in various parts of the plants and in the callus, and indicates that the suspension-cultured cells produced these compounds much more than the callus. The ratios of productivity of suspension culture to callus culture are shown in Table 1. Compound **5** as well as the more active compounds **1**, **2**, and **3** is shown to be produced 70 fold or greater by suspension culture.

This result indicates the possibility of producing these antibacterial compounds by cell culture.

Acknowledgments

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Tectona grandis の母植物体には少量しか，あるいは全然存在しない抗菌性トリテルペン酸の同植物細胞懸濁培養による生産

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著者らは以前，*Tectona grandis* のカルスが，母植物には少量しか，あるいは全然存在しない抗菌性トリテルペン酸を相当量生産することを報告した。本報は，確立した細胞懸濁培養系では，これらの抗菌性トリテルペン酸の生産性がカルス培養の100-200倍にも上昇することを述べ，これらの抗菌性化合物を細胞懸濁培養で生産できることを示した。