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Hairy Root from Pak-bung for Peroxidase Production

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Plant roots contain various useful materials, such as pharmaceuticals and pigments, and the effective utilization of such materials have received much attention. In 1934, White[1] established tomato root cultures capable of unlimited growth in medium containing macro- and micronutrients, sucrose and yeast extract. However, the root culture is not suitable for large scale production of secondary metabolites because of their relatively slow growth *in vitro*. Although the techniques of plant cell culture with callus have been expected to be realized for industrial production of such various useful materials, few processes based on callus culture have been developed on the industrial scale except for production of shikonin derivatives,[2] because there are several problems which involve low level of content and unstable production of desired metabolites.[3]

Recently, plant hairy roots have become of interest because of their indefinite and active proliferation in phytohormone-free medium and capacity to produce valuable materials synthesized and accumulated at comparable level to the original plant root.[4] The hairy root affecting a wide range of dicotyledonous species is caused by a soil bacterium, *Agrobacterium rhizogenes*. The induction mechanism of hairy root and expression of the diseased phenotype were studied by many investigators. In our previous works,[5] we showed that the horseradish hairy root was cultivated efficiently in an air-lift column bioreactor with the immobilized horseradish cells and 110 g-fresh root/*l* was obtained for 31 days culture. We also reported that polypepton addition could emphasize the growth and peroxidase production of hairy root significantly.[6]

In this paper, we report induction of hairy root from Pak-bung and the production of peroxidase (POD, E.C. 1.11.1.7), which is widely used as an important reagent for clinical diagnosis and microanalytical immunoassay.

Agrobacterium rhizogenes A 4 and 15834 were used in order to induce hairy roots. Seeds of Pak-bung, *Ipomoea aquatica*, were purchased from a local market. After seeding and growth for about one month at room temperature, fresh leaves were provided for hairy root induction. *A. rhizogenes* was cultivated in modified L-broth medium containing 10 g/*l* Bacto-tryptone, 5 g/*l* Bacto-yeast extract, 10 g/*l* sucrose and, if necessary, 20 g/*l* agar. Fresh leaves sterilized by 5% NaClO were placed in the overnight culture of *A. rhizogenes* in order to induce hairy root by the leaf disk method. The hairy root was maintained on Murashige-Skoog (MS) medium containing 30 g/*l* sucrose, 10 g/*l* agar and no phytohormone, and subcultured every one month. The hairy root culture was carried out described previously.[5] POD activity was measured according to the *o*-aminophenol method reported by Yamada *et al.*[7] Dry cell weight was gravimetrically measured after drying the roots at 60°C for 24 hr as previously reported.[6]

After Pak-bung was infected with *A. rhizogenes* by the leaf-disk method, some filamentous roots were induced from sections of plant leaf. As shown in **Fig. 1**, the root was a white hairy organ. The roots could elongate and showed active proliferation maintaining a filamentous shape, when they

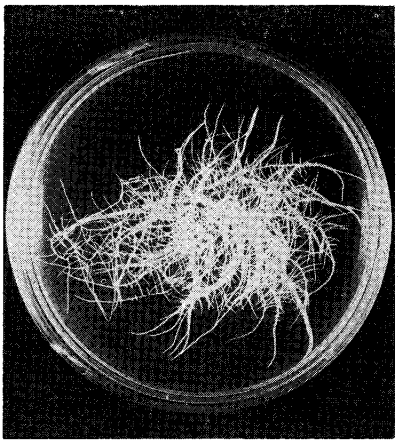

Fig. 1. Hairy root from Pak-bung. The root was cultivated on MS agar medium for 8 days at 25℃ in the dark.

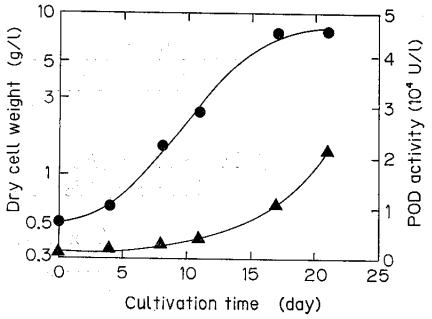

Fig. 2. Time course of hairy root culture. Hairy root was cultivated in phytohormone-free MS medium for 23 days at 100 rpm, 25℃ in the dark. ● : growth of hairy root, ▲ : activity of peroxidase.

Table 1. Production of peroxidase from original plant or hairy root.

	POD activity (U/g-dry cells)
Original plant root	620
stem	480
leaf	510
Hairy root (A 4)	2300
(15834)	2500

were isolated and transferred on phytohormone-free MS agar medium. The opine present in the hairy root clone was analyzed by paper electrophoresis. The extract from hairy root clone (A 4) contained the compound showing the same mobility as authentic mannopine and also reduced the silver nitrate reagent, while opine was absent in original Pak-bung root or stem. Therefore, we regarded the root as a so-called hairy root.

After the hairy roots were well grown in MS liquid medium, the activity of peroxidase was assayed. As shown in **Table 1**, the specific activity of POD in leaf, root or stem of the original plant was 500–600 U/g-dry cells. On the other hand, that of hairy root induced from *A. rhizogenes* A 4 or 15834 showed five times higher level of POD activity. The reason why hairy root clones showed higher content of POD is not yet clear. Mano *et al.*[8] also reported that hairy root clones of *Scopolia japonica* accumulated hyoscyamine at higher level than the original plant.

Time course of hairy root culture in the liquid medium in 100 ml-Erlenmyer flask is shown in **Fig. 2**. The hairy root (0.2 g-fresh weight) from *A. rhizogenes* A 4 was inoculated in 40 ml of MS medium and 7.6 g-dry cell weight/*l* was obtained after 17 days. At the same time, the POD activity reached at the level of 21,000 U/*l*-medium. When 0.2 g of seeds of original plant, Pak-bung, were germinated and grown for 17 days, 1.3 g-fresh weight of plants containing about 70 units of POD were obtained. Since hairy root could produce 840 units of POD at the same cultivation time, the productivity of POD synthesized in hairy root was about 12 times higher than that by original plant.

Mano *et al.*[8] established 29 hairy root clones of *Scopolia japonica*, and showed the great variability in alkaloid production among the clones. Two highly productive clones were isolated; the clone S 1 accumulated scopolamine to a level of 0.5% dry weight and the clone S 22 made hyoscyamine at a

level of 1.3% dry weight. Rhodes *et al.*[9] reported the production of nicotine by hairy root cultures of *Nicotiana rustica* in a two-stage batch/continuous-flow system. Their cultures showed a rapid growth rate and released a major portion of nicotine to the culture medium. We also reported previously the hairy root culture in various bioreactors and concluded that 10 g/*l* of horseradish hairy root was attained efficiently after 30 days.[10] These results suggest that the hairy root culture established by us can also improve the productivity of peroxidase by the selection of hairy root clone and the development of suitable bioreactor or culture system. Since the large scale culture have been also attempted for hairy root,[4] Pak-bung hairy root may become the attractive source for peroxidase.

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パックブンからの毛状根の誘導とパーオキシダーゼ生産

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Agrobacterium rhizogenes を用いてパックブンからの毛状根誘導を試みた．得られた毛状根を MS 培地中で培養したところ，17日後に 7.6 g/*l* に達し，21,000 U/*l* のパーオキシダーゼが得られた．パーオキシダーゼ比活性は親植物よりも約5倍高い値を示した．