Notes Plant Tissue Culture Letters, **10** (2), 191-193 (1993)

Effects of Basal Media on Growth and Polyacetylene Production of *Lobelia inflata* Hairy Roots

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Recently, we reported the production of an alkaloid[1] (lobeline) and polyacetylenes [lobetyol (**1**)[2], lobetyolin (**2**)[2,3] and lobetyolinin (**3**)[4]] in *Agrobacterium rhizogenes* transformed roots (hairy roots) of *Lobelia inflata* L. (Campanulaceae). For the determination of the optimal condition for the production of these polyacetylenes (**1-3**), the effects of five basal media on the growth and polyacetylene production of the hairy roots were investigated.

About 0.2 g (fw) of two clones of the hairy roots (Li-A and Li-B)[1] were separately inoculated into five hormone-free media [Murashige-Skoog[5] (MS) (containing 30 g/*l* sucrose), 1/2 MS, Gamborg B5[6] (B5) (containing 20 g/*l* sucrose), Woody Plant[7] (WP) (containing 20 g/*l* sucrose) and Root Culture[8] (RC) (contaning 15 g/*l* sucrose)] (50 m*l* medium per 100 m*l* flask) and cultured on a rotary shaker (100 rpm) in the dark at 25 °C. The growth rate was determined periodically (**Fig. 1**).

Li-A started to proliferate at the early stage of the culture in MS, B5 and WP media (**Fig. 1-a**). Both in MS and WP media, Li-A showed the highest fresh weight at week 6 (MS : 8.32 g, WP : 6.76 g per flask). In B5 medium, the growth continued until the end of the culture (week 7 ; 7.43 g per flask). In 1/2 MS medium, Li-A rapidly grew, after a long lag phase (4 weeks), to 7.79 g per flask at week 7. Similarly, Li-B continued to grow in the four media (MS, 1/2 MS, B5 and WP) throughout the culture time (**Fig. 1-b**). Clearly its final weight was heavier than Li-A ; *e. g.* in 1/2 MS medium Li-B reached to 13.00 g per flask. In sharp contrast to the cultures in these four media, both Li-A and Li-B in RC medium did not grow well. Therefore, RC medium was unsuitable for the growth of *L. inflata* hairy roots.

The production of polyacetylenes in these hairy roots were determined by HPLC analysis.

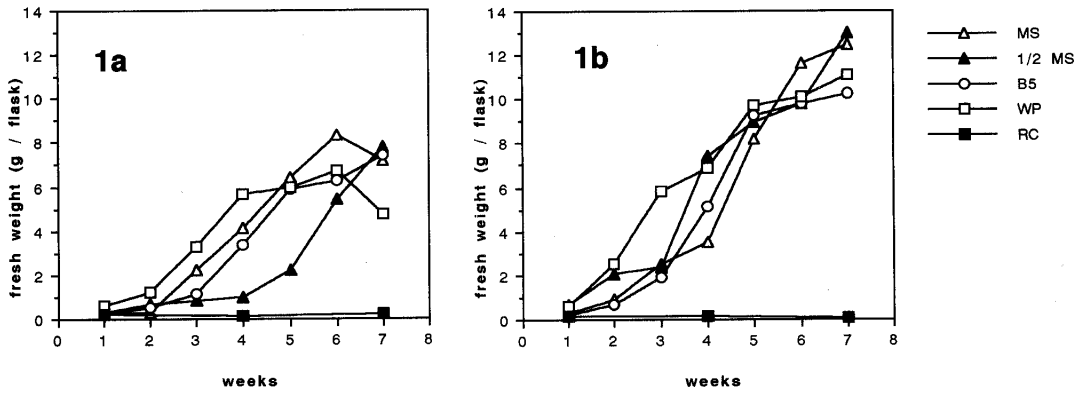

Fig. 1 Growth of *Lobelia inflata* hairy roots in five basal liquid media.
1a : Li-A, 1b : Li-B

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Lyophilized hairy roots (20-30 mg) were mashed by pestle and extracted with MeOH (2 m*l*) for 15 hr at room temp. The extract, after filtration with Milipore filter, was injected (3 μl) to HPLC: column, Inertsil ODS (4.6 mm *i. d.* ×250 mm) ; mobile phase, MeCN-H_2O (1 : 4 → 9 : 1, linear gradient in 30 min.) ; flow rate, 0.68 m*l*/min. ; detection, 270 nm (UV) ; column temp., 40 °C ; R_t (min.) : **3** (15.9), **2** (19.8) and **1** (23.9).

Figs. 2 and **3** show the production of polyacetylenes (**1**-**3**) in Li-A and Li-B hairy root cultures. In MS, 1/2 MS and B5 media, the content (mg per flask) of glycosylated polyacetylenes (**2** and **3**) roughly paralleled the growth of the hairy roots (**Figs. 2-a～c ; 3-a～c**). Generally, the amount of monoglucoside (**2**) exceeded that of diglucoside (**3**), and the content of the free polyacetylene (**1**) was the lowest of the three (with minor exceptions : *e. g.* excess of **3** at week 7 of Li-A in B5 culture, **Fig. 2-c ;** comparable amounts of **1** and **3** in Li-B in MS culture, **Fig. 3-a**). The highest levels of both **2** and **3** among these cultures were observed at week 6 of Li-A in MS culture (**2**, 14.0 mg ; **3**, 13.2 mg per flask). Throughout the culture period, Li-B produced smaller amounts of polyacetylenes than Li-A.

In WP medium, the pattern of polyacetylene production did not coincide with that of growth in the stages of the culture (**Figs. 2-d** and **3-d ;** *cf.* **Fig. 1**). Both Li-A and Li-B showed the decline in polyacetylene accumulation after week 4, and only Li-B restored the productivity after week 6. Nevertheless a very high content of polyacetylenes was observed with this medium (**1**, 5.1 mg ; **2**, 17.6 mg ; **3**, 12.1 mg per flask in Li-A at week 4 : **2**, 14.5 mg per flask in Li-B at week 7 ; **3**, 9.3 mg per flask in Li-B at week 4).

As expected from the growth data, neither Li-A nor Li-B in RC medium produced appreciable amounts of any of the polyacetylenes (**Figs. 2-e** and **3-e**).

In conclusion, Li-A and Li-B showed both the good growth and polyacetylene production in four

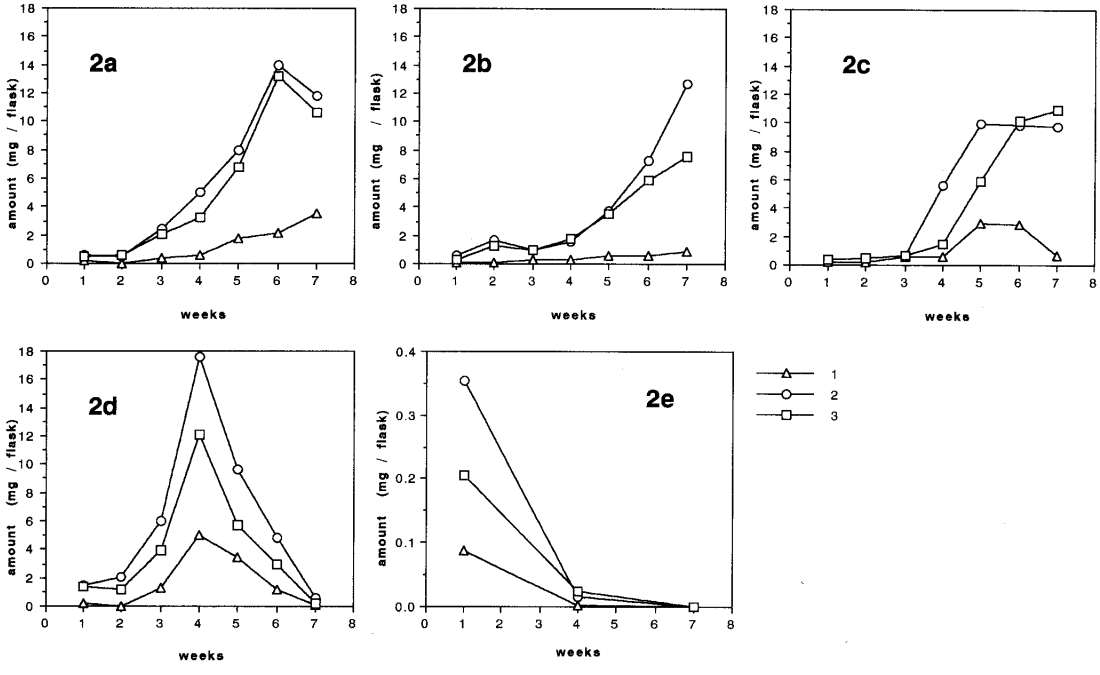

Fig. 2 Polyacetylene production in Li-A.
2a : in MS medium, 2b : in 1/2 MS medium, 2c : in B5 medium, 2d : in WP medium, 2e : in RC medium (note the vertical scale)

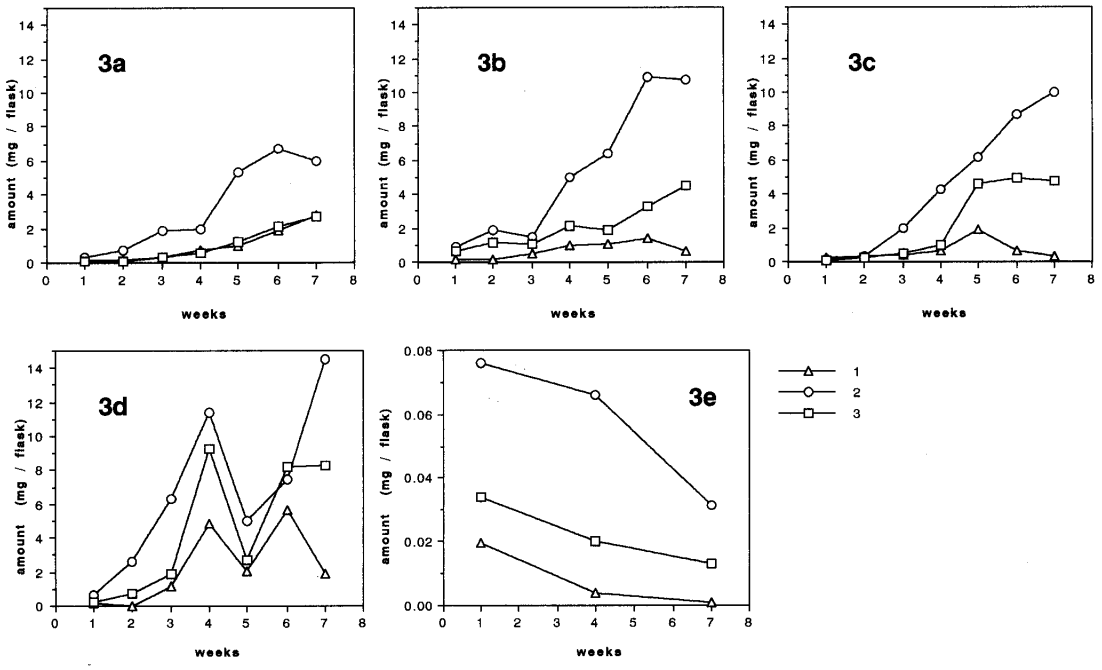

Fig. 3 Polyacetylene production in Li-B.
3a : in MS medium, 3b : in 1/2 MS medium, 3c : in B5 medium, 3d : in WP medium, 3e : in RC medium (note the vertical scale)

basal liquid media (MS, 1/2 MS, B5 and WP) indicating their usefulness as a source of these polyacetylenes. Although the growth of Li-A was poorer than of Li-B, the production of polyacetylenes [especially **3** (gentiobioside) in MS, 1/2 MS and B5 media] in Li-A was superior to that of Li-B, suggesting the strong glycosylation ability of Li-A. Our results indicate the importance (and necessity) of the selection of clones and the optimal culture media in the use of hairy roots for the production of secondary metabolites.

Acknowledgement

This work was supported by Ministry of Health and Welfare, Science Research Fund Subsidy granted to Japan Health Science Foundation.

(Accepted January 27, 1993)

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