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Growth of *Agrobacterium*-mediated Transformed *Lycium chinense* Roots in Bioreactors

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Objectives

Hairy root cultures are characterized by a high growth rate and are able to synthesize secondary metabolites. Hairy roots are unique in the genetic and biosynthetic stability. Several hairy roots have been put to scale up studies in bioreactors. In this study, transgenic hairy roots of *Lycium* chinense were produced via *Agrobacterium*-mediate transformation. We confirmed the hairy root cultures of *Lycium chinense* in bioreactors.

Materials and Methods

1. Plant materials: *Lycium chinense* Mill., roots, stems, leaves
2. *Agrobacterium rhizogenes*: *A. rhizogenes* 15834, R1000, A4
3. Genetic transformation of hairy roots: Plant explants and bacteria were co-culture for 3 days. Hairy roots induced at the site of section were individually isolated and cultured on hormone free MS medium with 300 mg/L cefotaxime.
4. Confirmation of transformation: Plant DNA was isolated from hairy root of *Lycium chinense* and amplified the rol C gene (Size of PCR products is 500bp).
5. Selection of high growth hairy root: Root tips (1 cm) of induced hairy roots were cultured on hormone free MS solid medium for 3 weeks and growth of roots were compared with each hairy roots.
6. Flask and Bioreactor cultures: Hairy roots were cultured in 100 mL flasks containing 30 mL of MS liquid medium supplemented with different concentration of nitrogen and carbon source. Optimal condition for hairy roots was tested in 10 L bioreactor. 0.05, 0.1, 0.2 and 0.3 vvm (volume of air/volume of medium/minute) of air were tested on the growth of hairy roots. After 3 weeks culture, that roots were harvested from bioreactors, fresh and dry weight was measured.

Results and Discussion

1. Induction of hairy roots: Selected roots, stems and leaves were sterilized with 1% sodium hypochlorite solution. This explants were cultured on MS medium with 1% agar, 3% sucrose and 1.0 mg/L 2,4-D for 2 days. After co-cultivation, *A. rhizogenes* strains induced root from the wounded area within 5-10 days of inoculation on explants. To select transformed hairy roots, each explants were transferred onto the medium with 300 mg/L cefotaxime for three weeks and sub-cultured to the same medium.
2. Confirmation of transformation: Putative roots were obtained and examined by PCR analysis. PCR amplification of hairy root DNA with primers specific for rol C gene showed the expected fragment sizes of 500 bp. The control roots did not show amplification with the primers.
3. Growth of hairy roots: Dry weight and growth rate of hairy root increased with the increase of sucrose concentration in the range of 1-6% and low concentration of nitrogen source (1/3 MS). Optimal aeration ratio for root culture was 0.2 vvm of air in 10 L bioreactor.

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