

## HAIRY ROOT CULTURE : APPLICATIONS AND PROSPECTS

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### ABSTRACT

Hairy roots have been investigated as a biological system for the production of valuable compounds from medicinal plants and for studying biochemical pathways for the last 25 years. The whole technique is based on the transfer of *Agrobacterium rhizogenes* T-DNA into the plant genome and thus it has facilitated its increasing use in metabolic engineering. This technique holds immense potential for the pharmaceutical industry because recombinant proteins can be produced from transgenic roots.

**Key words :** *Agrobacterium*, phytochemicals, phytoremediation, inoculum.

### INTRODUCTION

Today more and more individuals worldwide are turning towards herbal medicine and plant derived products. Due to the increasing demand of phytochemicals in pharmaceutical industries efforts have been made to commercialize production of the medicinal plant metabolites from plant cell cultures. Recent technique for enhanced harvesting of the phytochemicals is the technique of hairy root culture. Hairy root cultures are considered a powerful choice for producing active compounds from medicinal plants as this technique has high stability for the production of secondary metabolites. In the past two decades, the production of valuable secondary metabolites through hairy root cultures has received a lot of attention worldwide. The development of novel tools for metabolic engineering now offers new possibilities to improve this technique for the production of useful metabolites. The genetically transformed root culture can produce higher levels of secondary metabolites or amounts comparable to that of intact plants. A better understanding of the molecular mechanism of hairy roots development which is based on the transfer of *Agrobacterium rhizogenes* T-DNA into the plant genome, has facilitated its increasing use in metabolic engineering. The hairy root production holds immense potential for the pharmaceutical industry because recombinant proteins can be produced from the transgenic roots. In addition this technique offers promise for phytoremediation, because of their abundant neoplastic root proliferation and monoclonal

antibody production (Wongsamuth and Doran, 1997).

Hairy root cultures can be established by genetically transforming plant tissues by the pathogenic bacterium *Agrobacterium rhizogenes*. This transformation results in the induction of hairy roots at the site of infection and it is one of the most recent organ culture systems employed for large scale production of secondary metabolites and also for the production of phytochemicals.

## METHODOLOGY

Hairy root cultures are established by inoculating the explant with a suspension of *A. rhizogenes*. This suspension can be generated by growing it in YMB medium for two days at 25°C with gyrator shaking and then pelleting it by centrifugation (5x10<sup>3</sup> rpm, 20 min) and then resuspending this bacterium in Yeast Mannitol Broth (YMB) medium (Hooykaas *et al.*, 1977) to form a fix suspension of approx. 10<sup>10</sup> viable bacteria per ml of suspension.

For inoculation, aseptic plants grown from seeds or explants like leaves, leaf-discs, stem, stalk, shoot tip, cotyledons, storage roots, or tubers, petioles can be used to induce hairy roots (Giri *et al.*, 2001; Krolicka *et al.*, 2001; Azlan *et al.*, 2002). After sterilizing the explant with 10 % (v/v) Domestos (Lever Bros.) for 20 minutes, the midrib of the leaf or the stem of the plantlet is scratched with a sterile needle of a hypodermic syringe containing the thick bacterial suspension which allows the inoculation with small (about 5-10 ml) droplets containing *A. rhizogenes*.

After inoculation, hairy roots appear within one week to four weeks. They may appear directly at the site of inoculation or a callus may be formed initially and then hairy roots appear subsequently from it. Treatment with acetosyringone and ultrasonication followed by exposure to *A. rhizogenes* results in enhanced transformation frequency in recalcitrant plants (Vinod Kumar *et al.*, 2005). Different factors such as carbon source and its concentration, the ionic concentration and pH of the medium, light, temperature, phytohormones and inoculum, influence the growth and secondary metabolism for hairy root cultures (Morgan *et al.*, 2000).

interconnected tissues unevenly distributed throughout the vessel. Bioreactor technology involving large scale operations can reduce labour cost and time factor which translates into economic advantage in commercial process.

## REFERENCES

- Azlan, G.J., Marziah, M., Radzali, M, Johari, R., 2002. Expression of functional mammalian P 450 2 E 1 in hairy root cultures. *Biotechnol. Bioeng.* 77 : 462-466.
- Carron T.R., Robbins M.P., and Morris P., 1994. Genetic modification of condensed tannin biosynthesis in *Lotus corniculatus*. 1. Hererologous antisense digydroflavonol reductase down-regulates tannin accumulation in hairy root cultures. *Theor Appl Genet* 87, 1006-1015.
- Cho H.J., and Wildholm J.M., 2002. Improved shoot regeneration protocol for hairy roots of the legume *Astragalus sinicus*. *Plant Cell Tiss Org Cult* 69,259-269.
- Christey M.C., and Sinclair B.K., 1992. Regeneration of transgenic kale (*Brassica oleracea* var. *acephala*), rape (*B. napus*) and turnip (*B. campestris* var. *rapifera*) plants via *Agrobacterium rhizogenes* mediated transformation. *Plant Sci* 87,161-169.
- Giri, A., Ravindra, S.T., Dhingra, V., Narasu, M.L., 2001. Influence of different strains of *Agrobacterium rhizogenes* on induction of hairy roots and artemisinin production of *Artemisia annua*. *Curr. Sci.* 81: 378-382.
- Hughes E.H., Hong S.B., Shanks J.V., San K.Y., and Gibson S.I., 2002. Characterization of an inducible promoter system in 126 *Journal of Integrative Plant Biology* Vol. 48 No. 2 2006. *Catharanthus roseus* hairy roots. *Biotechnol Prog* 18, 1183-1186.
- Hooykass, P. J.J., Klapwijk, P.M., Nuti, P.M., Shilperoot, R.A. and Rorsch, A. 1977. Transfer of the *Agrobacterium tumifaciens* Ti plasmid to avirulent *Agrobacteria* and *Rhizobium* ex planta. *J. Gen. Microbiol*, 98 : 477-484.
- Kittipongpatana N., Hock R.S., and Porter J.R., 1998. Production of solasodine by hairy root, callus and cell suspension cultures of *Solanum aviculare* Forst. *Plant Cell Tiss Org Cult* 52, 133-143.
- Krolicka, A, Staniszewska, I, Bielawski, K, Malinski, E., Szafranek, J, Lojkowska, E., 2001. Establishment of hairy root cultures of *Ammi majus*. *Plant Sci.*160: 259-264.
- Menzel G., Harloff H.J., and Jung C., 2003. Expression of bacterial poly (3-hydroxybutyrate) synthesis genes in hairy roots of sugar beet (*Beta vulgaris* L.). *Appl Microbiol Biotechnol* 60,571-576.
- Morgan J.A., Barney C.S, Penn A.H., and Shanks J.V., 2000. Effects of buffered media upon growth and alkaloid production of *Catharanthus roseus* hairy roots. *Appl Microbiol Biotechnol* 53, 262-65.
- Sevon N., Oksman C., and Kirsi M., 2002. *Agrobacterium rhizogenes* mediated transformation: Root cultures as a source of alkaloids. *Planta Med* 68, 859-868.

Shio T.L., Ellis M.H., Dolferus R., Dennis E.S., and Doran P.M., 2003. Overexpression of alcohol dehydrogenase or pyruvate decarboxylase improves growth of hairy roots of reduced oxygen concentrations. *Biotechnol Bioeng.* 77,455-461.

Vinod Kumar, Ashwani Sharma, Bellur Chayapathy Narasimha Prasad, Harishchandra Bhaskar Gururaj and Gokare Aswathanarayana Ravishankar, 2006. *Agrobacterium rhizogenes* mediated genetic transformation resulting in hairy root formation is enhanced by ultrasonication and acetosyringone treatment. *Plant Biotec.* 9 (4)

Wongsamuth, R. and Doran, P.M. 1997. Hairy root as an experimental system for production of antibodies. In : Doran, P.M. ed. *Hairy roots, Culture and applications*. Amsterdam, Harwood:89-97.

Zhi-Bu Hu and Min Du 2006. Hairy root and its application in plant genetic engineering. *Journal of Integrative Plant Biology* 48 (2). 121-127.