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In Vitro Production of Genetically Transformed Carrot Hairy Roots

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Agrobacterium rhizogenes (recently revised as Rhizobium rhizogenes) is a soil-borne gram-negative bacterium that induces hairy roots of monocot and dicot plants. Its plasmid, containing transfer DNA encoding Root Locus (rol) genes (rolA, rolB, and rolC), is responsible for the stable introduction of genetic material of host cells. This can trigger the production of highly branched hairy roots which can be maintained in culture media. It was shown that auxin signaling, but not ethylene signaling, is required for hairy root production. These Hairy roots modified hormonal balance and allows profuse growth on culture media and have been used for a variety of purposes over the last 30 years, ranging from recombinant protein production and metabolic engineering to analyses of rhizosphere physiology and biochemistry. The objective of this study was to establish an optimized system for hairy root production from carrot. Bacterial strain prepared on LB broth medium and overnight incubation on shaker at 150rpm at 28°C. Then, bacterial strain cultured on LB, YMA and modified media and incubated 36 hours at 28°C in darkness. Then, growing colonies mix in these media for inoculation. Basal parts of carrot discs inoculated by bacterial

colonies and plates incubated in darkness at 25°C for 48-72 hours followed by inverted onto the MS medium amended with different concentrations of cefotaxime antibiotics (500mgL⁻¹, 300mgL⁻¹ and 150mgL⁻¹) and incubated at 25°C. Two types of roots started forming on carrot discs after 8-10 days. The first type was delicate, aerial hairy roots without any growth after cutting from discs and rotted after one week. The second type was slightly thicker transformed roots with numerous lateral roots and negative geotropic of growth. Roots were initiated from both sides of discs and occurred from 8-10 days to 3-4 weeks. Carrot is one of the most amenable species for transformed hairy roots production. Although these hairy roots have been produced before but an optimized method for carrot transformed hairy roots production have not been described before. These roots were better adapted to growth in culture media than normal roots with a longer survival time without any subculture. Also, the bacteria have a higher ability to produce hairy roots on the basal sides corresponding to apical sides since on a higher endogenous auxin level.

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