GROWTH KINETICS AND STOICHIOMETRY OF MENTHA CITRATA SHOOTY TERATOMAS TRANSFORMED BY AGROBACTERIUM TUMEFACIENS

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Summary

Culture characteristics of genetically transformed Mentha citrata shooty teratomas were studied in liquid Murashige and Skoog and Gamborg's B5 media. Based on calculated kinetic and yield parameters, a stoichiometric equation was developed to describe growth in media containing both nitrate and ammonia. Measurements of oxygen uptake rate showed that delivery of adequate oxygen to completely submerged shoots depends on the elimination of hydrodynamic boundary layers at high external liquid velocity.

Introduction

Genetically transformed plant organs have attracted considerable interest for in vitro production of phytochemicals. To date, most attention has been given to hairy root cultures initiated using Agrobacterium rhizogenes; however, a limitation associated with hairy roots is that they normally produce only those chemicals synthesised in roots of the whole plant. Little or no trace of compounds such as digoxin from Digitalis purpurea, morphine and codeine from Papaver somniferum and vindoline from Catharanthus roseus has been found in root cultures as production of these chemicals is associated with shoots (Hirotani and Furuya, 1977; Kamo et al., 1982; Endo et al., 1987). Shoot differentiation is also required for synthesis of many flavours and fragrances occurring as essential oils in glands and glandular hairs of leaves, stems and petioles.

Several investigations have been carried out with untransformed shoot cultures to assess their capacity for secondary synthesis (Hagimori et al., 1984; Heble, 1985; Park et al., 1989) and for mass propagation of plants (Takayama and Misawa, 1981; Akita et al., 1994). An alternative to shoot cultures initiated and maintained under the control of exogenous hormones is hormone-autotrophic shooty teratomas produced by genetic transformation of plants with nopaline or genetically modified strains of Agrobacterium tumefaciens. Shooty teratomas form after integration of part of the A. tumefaciens Ti plasmid into the plant genome in an analogous way to hairy root induction by A. rhizogenes. The molecular mechanism of shooty teratoma formation is not yet completely clear; expression of bacterial genes affecting the response of plant cells to
auxin and cytokinin may need to be manipulated before shooty teratomas can be produced for a wide range of plant species (Hamill and Rhodes, 1993; Hamill, 1993).

There is a limited number of reports in the literature about the development and application of shooty teratomas. Saito et al. (1989) used strains of A. tumefaciens with mutations in the aux loci to develop shoot cultures of Nicotiana tabacum for nicotine biotransformation. Wild-type nopaline strains of A. tumefaciens and disarmed strains carrying the ipt gene controlled by the CaMV35S promoter have been used by Spencer et al. (1990, 1993) to produce shooty teratomas of Mentha citrata. Oil glands were found on the leaves of these shoots; chromatographic analysis confirmed the presence of significant quantities of terpenes characteristic of mint oil from the native plant. In other work, Atropa belladonna, N. tabacum and Solanum tuberosum teratomas were examined for synthesis of tropane, nicotine and steroidal alkaloids, respectively (Saito et al., 1991). Solasodine has recently been reported in shooty teratomas of S. eleagnifolium initiated using a nopaline strain of A. tumefaciens (Alvarez et al., 1994).

Although procedures for genetic transformation and the capacity of shooty teratomas for phytochemical production have been demonstrated, little other information is available. The principal aim of this work was to investigate the kinetics and growth stoichiometry of shooty teratomas cultivated in liquid media. Mentha citrata shooty teratomas (Spencer et al., 1990) were used as a model culture.

Materials and Methods

Shooty teratomas

The Mentha citrata shooty teratomas used in this work were developed using A. tumefaciens nopaline strain C58 by Dr A.J. Spencer in the laboratory of Dr M.J.C. Rhodes, AFRC Institute of Food Research, Norwich, UK. The cultures were maintained on solid Murashige and Skoog (MS) (Flow Laboratories) or Gamborg’s B5 medium (Sigma) containing 3% sucrose (Ajax) and 0.2% Phytagel (Sigma) without phytohormones at 25°C under ca. 1250 lux continuous illumination.

Shake flask experiments

Shake flask experiments were carried out in liquid MS and B5 media. About 2.0 g fresh weight shooty teratomas were inoculated into 50 ml medium and incubated for 35–42 d at a shaker speed of 110 rpm. Shoots floated on the surface of the liquid and so were only partially submerged for the duration of the culture. Triplicate flasks were harvested regularly for measurement of dry weight, medium sugar and ion concentrations, and biomass elemental analysis.

Additional shake flask experiments were carried out over a period of 21 d to determine the effect of shaker speed on growth. Triplicate flasks containing 50 ml MS medium and 3% sucrose were inoculated with 2.0 g fresh weight shoots and incubated with shaking at 80, 100, 110 and 140 rpm. After 21 d, the flasks were harvested for measurement of biomass dry weight.

Analyses

Dry weight was obtained by freeze drying the shoots at −40°C for 3 d. Elemental analysis was carried out using ca. 5 mg ground freeze-dried biomass. The remaining biomass from each sample was dried in an oven at 105°C for 60 min for measurement of moisture content, then heated in a furnace at 660°C for ash determination. C, H and N were determined using a Perkin Elmer 240B elemental analyser with acetanilide for calibration. Oxygen was calculated by difference. Each sample was analysed in duplicate.

Sucrose, glucose and fructose concentrations in the medium were measured using HPLC as described previously (Tsoulpha and Doran, 1991). Residual concentrations of nitrate and ammonium ions were determined using Spectroquant 14773 and 14752 colorimetric assay kits (Merek, Darmstadt), respectively.

Shooty teratoma oxygen uptake rates in MS and B5 medium were measured using a biological oxygen monitor (Sato and Toda, 1983). Oxygen uptake rate was determined at 100% air saturation using a polarographic oxygen electrode (Ingold, Switzerland) in a closed vessel at stirrer speeds up to 1400 rpm.