HPTLC as a tool to rapidly assess the elicitor responsiveness of hairy roots cultured in the Liquid Lab™ reactor

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Abstract

Hairy roots obtained via *Agrobacterium rhizogenes*-mediated transformation offer a sustainable plant tissue-based system for bioproduction of valued specialized metabolites. Recent progress in the scale-up of hairy roots has made this system an attractive tool for industrial processes.

Hairy roots reflect the metabolic phenotype of the host plant, and are genetically stable, providing advantages when compared to cell suspensions. Moreover, the biosynthetic potential of the roots can be expanded by the use of elicitors, which lead to the induction of novel bioactive molecules that could be secreted into the culture medium. In order to develop a low-cost-bioreactor for scale up of hairy root biomass and production of specialized metabolites, we tested the simple-to-operate Liquid Lab™ (Southern Sun Biosystems, Inc.) rocker reactor. Hairy roots of *Hyoscyamus muticus* were cultured in the reactor and elicited with copper sulfate every two weeks. In order to determine the responsiveness of the hairy roots in production and secretion of specialized metabolites, the medium was collected and analyzed for phenolics and sesquiterpenes by HPTLC. A particular type of inducible phenolic chemical was only elicited in the 2-week old cultures. However, sesquiterpenes were elicited in 2-, 4-, and 6-week old cultures with the highest production observed in 2-week old cultures. In order to confirm these observations, we performed GC-MS analyses with the same extracts. Our data showed correlations between the HPTLC and GC profiles suggesting that HPTLC could be used to rapidly determine production and secretion of metabolites in a scalable bioreactor system.

Culture of hairy roots in the Liquid Lab™ bioreactor

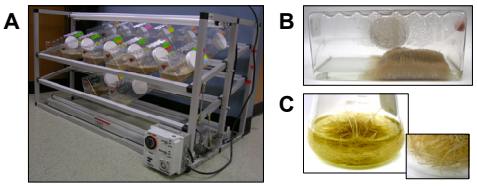


Figure 1. **(A)** Rocker system and Liquid Lab™ reactors (Southern Sun Biosystems, Inc.) used to grow the hairy roots. **(B)** Hairy roots of *H. muticus* cultured in the Liquid Lab™ reactor. **(C)** Effect of copper sulfate elicitation on hairy roots. Note the dark areas in the root tips.

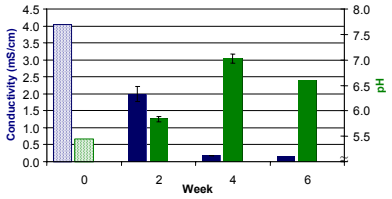
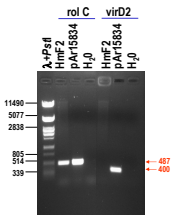


Figure 2. Growth of *H. muticus* hairy roots line F2 in the Liquid Lab™ reactor with media exchange every 2 weeks, during a 6-week period. The conductivity of the medium was drastically reduced every two weeks even after 6-weeks of culture, suggesting that roots were still growing at this stage. To prevent the growth of contaminants, B5 medium containing 0.02% PPM™ (Plant Preservative Mixture, Plant Cell Technology, Inc.) was used every time the medium was exchanged.

Figure 3. PCR to detect the presence of the *rol C* gene in transgenic *H. muticus* hairy roots, line F2 (HmF2). This gene is transferred during the transformation process from the plasmid pAr15834 in *A. rhizogenes* (used as PCR positive control) to the plant cell; this is shown as a 487 bp fragment. The gene *virD2* resides in pAr15834, but outside of the transferred region, and was used to discard the presence of bacterial contamination in the DNA preparations 400 bp fragment). Phage lambda digested with *Pst*I (λ+*Pst*I) was used as molecular weight marker.



Elicitor-induced sesquiterpenes in solanaceae species

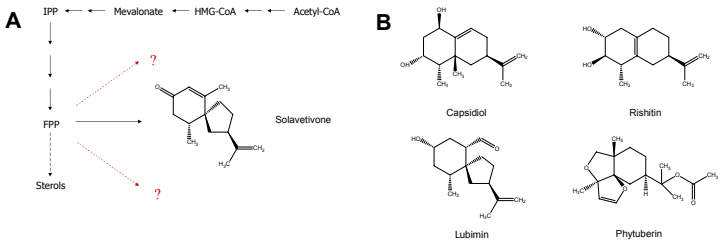


Figure 4. **(A)** Biosynthetic pathway of the sesquiterpene solavetivone produced and secreted in hairy roots of the solanaceae species *H. muticus* upon copper sulfate elicitation. Other sesquiterpenes are also induced in these roots (not shown). **(B)** Chemical structures of inducible sesquiterpenes identified in various solanaceae species.

HPTLC analyses of the medium of hairy roots cultured in the Liquid Lab™ reactor

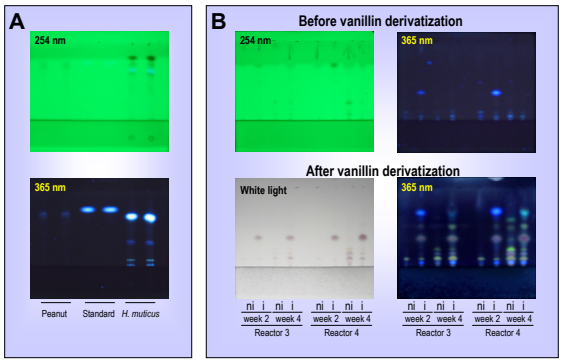
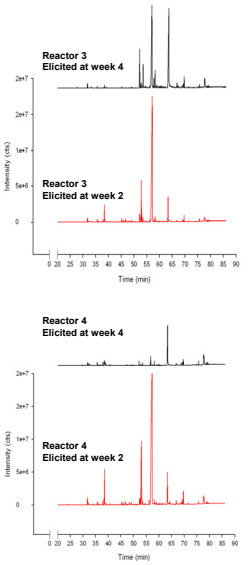


Figure 5. HPTLC of media extracts from hairy root cultures.

Panel A shows fluorescent chemicals secreted to the growth media after 24 h elicitation of hairy roots with 600 µM $CuSO_4$. **B**: ethyl acetate extracts from peanut hairy roots cultured for 12 days in Erlenmeyer flasks. **Reactor 4**: chloroform extracts from *H. muticus* hairy root cultured for 2 weeks in the Liquid Lab™ system. **Umb**: umbelliferone was run as control (standard).

Panel B shows the fluorescence of chemicals elicited after induction with 600 µM $CuSO_4$ (i) compared to non-induced *H. muticus* hairy roots (ni), on the week 2 or 4 of culture. Results before (upper plates) or after (below plates) derivatization with vanillin reagent are shown, as well as visualization under white and UV (365 and 254 nm) light, as indicated.

GC-MS analyses of the medium of hairy roots cultured in the Liquid Lab™ reactor



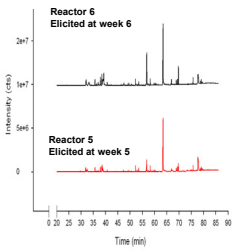


Figure 6. GC chromatograms of chloroform extracts from 24 h copper sulfate elicited media of *H. muticus* hairy root cultured in the Liquid Lab™ reactor.

Reactors 3 and 4: Grown for 4 weeks and elicited at weeks 2 and 4.

Reactor 5: Grown for 4 weeks and elicited at week 4 only.

Reactor 6: Grown for 6 weeks and elicited at week 6 only.

GC-MS analyses of the medium of hairy roots cultured in flasks

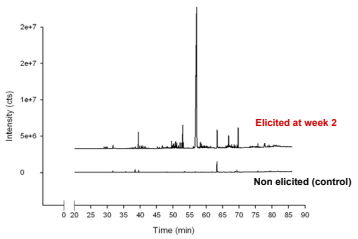


Figure 7. GC chromatogram of chloroform extracts from 24 h copper sulfate elicited media of *H. muticus* hairy root cultured in 250 ml Erlenmeyer flasks.

Conclusions

- The Liquid Lab™ reactor can be used to grow hairy roots and effectively produce and secrete inducible chemicals.
- HPTLC and GC profiles of independent reactors (3 and 4) are comparable at week 2. Differences are observed when elicitation was done in older cultures.
- HPTLC analyses can be used to rapidly assess the elicitation responsiveness of hairy root cultures.

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