Scrophularia striata CULTURE TECHNIQUE

Bioproduction of phenylethanoid glycosides by plant cell culture of *Scrophularia striata* Boiss.: from shake-flasks to bioreactor

Sedigheh Ahmadi-Sakha, Mohsen Sharifi, Vahid Niknam

Abstract

***Scrophularia striata* cell suspension culture was scaled up from shake flasks to a Winpact FS-01-A05P bioreactor (10 L vessel, 5 L working volume) at pH 4.8, 0.5–1.5 L/min air, 110–170 rpm, 25 °C in darkness. Biomass reached 15.64 g/L DW and acteoside 1404.20 µg/g DW, about threefold the shake-flask level.**



Winpact Model: double-jacketed vessel

Introduction

Motivation: phenylethanoid glycosides (PeG) such as acteoside and echinacoside are plant polyphenols with anti-inflammatory, antioxidant, antibacterial and antitumour activity; their chemical structures are complex and chemical synthesis is costly, so plant cell culture is used as a source of these high-value secondary metabolites.

Gap: transfer from shake flask to bioreactor is the essential step toward commercial production, but scale-up results are not predictable — in *Cistanche deserticola* PeG production fell on transfer to a bioreactor, and in *Podophyllum hexandrum* shake flasks outperformed the bioreactor, so the conditions have to be established for each species.

Objective: establish the optimal bioreactor conditions for cell suspension culture of *S. striata* and compare biomass, cell viability and PeG (acteoside and echinacoside) production against shake flasks.

Materials and Methods

Cell culture and inoculum:

S. striata callus was grown on MS medium with 2 mg/L NAA, 0.5 mg/L BA and 30 g/L sucrose, subcultured every 15–17 days at 26 ± 1 °C. Suspension cultures were initiated from 2 g of white friable callus in 50 mL liquid medium and maintained at 110 rpm, 25 °C in darkness.

Bioreactor cultivation:

A Winpact FS-01-A05P system was used, comprising a 10 L double-jacketed vessel for temperature control, a TruDo dissolved oxygen sensor, a pH probe and an air pump. The vessel with 5 L modified MS medium was autoclaved at 121 °C for 20 min, then inoculated with fresh cells under laminar flow. Agitation 110–170 rpm, air flow held at 0.5 or 1.5 L/min, 25 ± 1 °C in darkness for 25 days; pH was held at 4.8 by automatic addition of 0.5 mol/L NaOH or HCl. 15 mL samples were drawn through the sampling port every 2 days.

Analysis:

Dry and fresh weight for biomass; cell viability by Evan's blue staining; PeG extracted from 0.2 g dried cells with 90% methanol and quantified by HPLC with DAD at 330 nm on a C-18 column (0.04% acetic acid / acetonitrile, 82:18).

Results

Growth in the bioreactor followed the same pattern as in shake flasks but with a longer exponential phase (day 5 to 19 vs day 5 to 15). Maximum biomass was 15.64 g/L DW in the bioreactor and 14.16 g/L DW in shake flasks, but the growth rate was far higher in the bioreactor (68 vs 3.54). Acteoside reached 1404.20 µg/g DW in the bioreactor, roughly threefold the shake-flask value of 459.71 µg/g DW, while echinacoside was 1.36-fold higher in shake flasks (1973.03 vs 1449.39 µg/g DW). Fermentor relevance: the bioreactor allows pH, dissolved oxygen, temperature and agitation to be monitored and controlled, and the authors attribute the higher productivity to reduced cell aggregation and the absence of the shear-induced partial cell lysis seen in shake flasks; air flow rate and aeration regime were found to directly affect cell growth and PeG production.

References

Ahmadi-Sakha S, Sharifi M, Niknam V. Bioproduction of phenylethanoid glycosides by plant cell culture of *Scrophularia striata* Boiss.: from shake-flasks to bioreactor. Plant Cell, Tissue and Organ Culture (PCTOC) (2015). doi:10.1007/s11240-015-0891-3

https://doi.org/10.1007/s11240-015-0891-3