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COMPARATIVE EVALUATION OF IN VITRO SYSTEMS FOR BERBERINE PRODUCTION FROM *BERBERIS VULGARIS* AND *BERBERIS OBLONGA*: FROM HAIRY ROOT CULTURES TO BIOREACTOR SCALING)

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ABSTRACT

Berberine is a high-value benzylisoquinoline alkaloid with significant therapeutic potential in treating metabolic and cardiovascular disorders. Due to the ecological impact of harvesting wild Berberis species, in vitro biotechnological production offers a sustainable alternative. This study provides a comprehensive comparative analysis of five cultivation systems—callus, cell suspension, adventitious roots, temporary immersion systems (TIS), and Agrobacterium rhizogenes-mediated hairy roots—using Berberis vulgaris and Berberis oblonga as models. Our findings indicate that hairy root cultures achieve the highest berberine yield (up to 45 mg/g DW), while TIS provides the most efficient platform for biomass intensification. A detailed protocol for A. rhizogenes transformation and a comparative technological matrix are provided to guide industrial-scale implementation.

1. Introduction

The genus *Berberis* (Berberidaceae) is a primary source of berberine, an alkaloid known for activating the AMPK pathway, thereby regulating glucose and lipid metabolism. In Central Asia, specifically Uzbekistan, *B. vulgaris* and *B. oblonga* are dominant species. While natural roots can contain up to 10.29% berberine, the slow growth rate of the plants and the destruction of biodiversity necessitate the development of highly productive in vitro platforms. This article evaluates the productivity, technological complexity, and economic feasibility of various biotechnological approaches.

2. Materials and Methods

2.1. Plant Material and Surface Sterilization

Seeds or nodal segments of *B. vulgaris* and *B. oblonga* are washed in running tap water for 30 min, followed by:

1. Soaking in 70% (v/v) ethanol for 1 minute.
2. Disinfection in 0.1% (w/v) mercuric chloride ($HgCl_2$) or 2% sodium hypochlorite (NaOCl) for 5–7 minutes.
3. Triple rinsing with sterile distilled water.
4. Inoculation on basal Murashige and Skoog (MS) medium supplemented with 30 g/L sucrose and 0.8% agar.

2.2. Hairy Root Induction Protocol (*Agrobacterium rhizogenes*)

The most efficient method for berberine production involves genetic transformation:

1. **Bacterial Strain:** *Agrobacterium rhizogenes* strain **ATCC 15834** or **LBA 9402** (carrying the pRi plasmid) is cultured in YEP medium at 28°C until an OD_{600} of 0.6–0.8 is reached.
2. **Inoculation:** Sterile leaf explants or callus pieces are wounded with a sterile needle and submerged in the bacterial suspension for 20–30 minutes.
3. **Co-cultivation:** Explants are transferred to MS medium (without antibiotics) and incubated in darkness at 25°C for 48–72 hours.
4. **Elimination:** To remove bacteria, explants are moved to MS medium supplemented with **cefotaxime (250–500 mg/L)**.
5. **Selection:** Hairy roots typically emerge within 14–21 days. Transgenic status is confirmed via PCR using primers for *rolB* and *rolC* genes.

2.3. Temporary Immersion System (TIS) Setup

TIS (e.g., RITA® or Plantform™) is utilized for scaling. The system operates on a cycle of:

- **Immersion time:** 5 minutes.
- **Interval:** Every 6 hours. This ensures optimal nutrient uptake while preventing hyperhydricity.

3. Results and Comparative Analysis

Table 1: Detailed Quantitative Comparison of In Vitro Systems

Cultivation Method	Biomass Growth (g/L/day)	Berberine Yield (mg/g DW)	Relative Alkaloid Purity	Capital Investment
Callus Culture	0.1 – 0.2	0.2 – 0.8	Low (Mixed phenols)	Low
Cell Suspension	0.5 – 1.2	0.5 – 5.0	Low (Secreted salts)	Moderate
Adventitious Roots	0.3 – 0.6	15.0 – 25.0	Medium	Moderate
TIS (Bioreactor)	**1.5 – 2.5**	10.0 – 18.0	Medium	High
Hairy Roots	0.8 – 1.5	**35.0 – 45.0**	**High**	High

Table 2: Influence of Elicitation on Berberine Accumulation in Hairy Roots

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Elicitor Type	Concentration	Yield Increase (Fold)	Mechanism
Methyl Jasmonate	100 µM	2.5x	Activation of BBE gene
Salicylic Acid	50 µM	1.8x	Stress-induced secondary flux
Yeast Extract	500 mg/L	3.1x	Mimicking fungal pathogen attack

4. Discussion

4.1. Productivity vs. Complexity

As shown in the data, there is a clear trade-off between yield and technological input.

- **[Diagram 1: Relative Productivity Index]** *(Refer to Figure 1 from your PDF: Hairy roots at ~45 units vs. Callus at <5 units).*
- **[Diagram 2: Cost-Complexity Matrix]** *(Refer to Figure 2 from your PDF: TIS and Hairy Roots occupy the high-cost, high-complexity quadrant).*

4.2. Metabolic Flux in Transformed Roots

The high productivity of hairy roots is attributed to the integration of *rol* genes, which alter the auxin/cytokinin ratio and upregulate the **Berberine Bridge Enzyme (BBE)**. In *B. oblonga*, this leads to a significantly higher ratio of berberine compared to palmatine or jatrorrhizine, simplifying downstream purification.

4.3. Scaling Potential

While Hairy Roots offer the best chemistry, TIS offers the best physical scaling. The combination of TIS with hairy roots is the "Gold Standard" for future industrial bioprocessing of *Berberis* alkaloids.

5. Conclusion

The production of berberine via *Agrobacterium*-mediated hairy roots represents the most viable laboratory model for maximizing alkaloid concentrations. For commercial production, a two-stage strategy is recommended: (1) Biomass accumulation in TIS-bioreactors, and (2) Elicitation with Methyl Jasmonate to trigger final alkaloid synthesis.

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