Production of Glycolipids by Jelly Mushrooms

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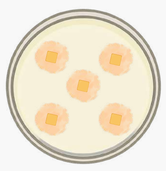
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Abstract

Long-chain glycolipids produced by fungi in the class Dacrymycetes possess surfactant properties capable of inhibiting the growth of Gram-positive bacteria and certain fungi. Due to their consumer safety, they are commonly used as preservatives. This study investigated the optimal conditions for glycolipid production from *Dacryopinax spathularia*. The Da68 and WB2 isolates were initially cultured in yeast-malt-glucose (YMG) medium and potato dextrose broth (PDB) supplemented with 25% coconut water for 3 days, showing no significant difference in growth. Subsequently, cultivation in glucose-yeast extract (GM) medium and corn oil-yeast extract (CY) medium for 10 days revealed that the GM medium yielded the highest fresh weight. Future procedures will involve extracting the glycolipids using NaOH and HCl, followed by analysis via thin layer chromatography (TLC) using an appropriate solvent system against a MEL-A standard.

Methodology

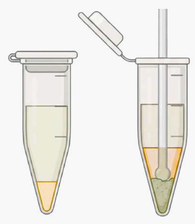
Morphological and Molecular identification



Culture on half PDA,
0.2% malt extract*



- Stereo microscope
- Compound microscope
  - Spore
  - Budding
  - Special structure



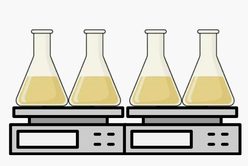
DNA extraction

DNA sequencing

BLAST analysis
(NCBI)

Optimization and Extraction

Inoculum preparation
(*half PDA, 5 days, RT)



(YMG, PDBc)(50 ml)
(3 days, RT, 210 rpm)

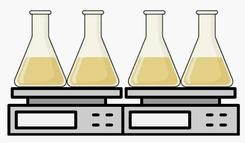


Filtration

Cell dry weight
(60 ºC)

Cell fresh weight

6 ml



(GM, CY)(200 ml)
(10 days, RT, 210 rpm)



Alkalizing to pH 8 with NaOH
(to solubilize glycolipids)



Filtration



Centrifuge at
4,500 rpm 15 min

discard cell



Acidifying to pH 2.2 with HCl
(to induce precipitation)

20 h, 4 ºC

Wash with water
pH 8 (13 ml)

Freeze dry



Thin layer chromatography (TLC) analysis

Objectives

- To isolate and identify fungi belonging to the class Dacrymycetes.
- To investigate the growth and glycolipid production capabilities of the isolated Dacrymycetes fungi.

Result

Morphology investigation



Figure 1 Fungal colonies on *half PDA. A: RT, 19d; B: Basidia; C: Conidia (3d, YMG); D: Yeast cells (3d, YMG).

Molecular characteristics

Description
Dacryopinax spathularia voucher Wu 331 large subunit ribosomal RNA gene, partial sequence

Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Dacryopinax sp…	1600	1739	90%	0.0	99.77%	1335	OL616184.1

Growth in liquid media

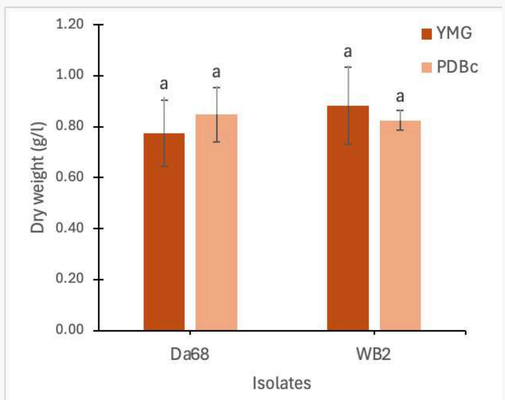


Figure 2 Dry weight in YMG (yeast-malt-glucose broth) and PDBc (potato dextrose broth with 25% coconut water), 3d.

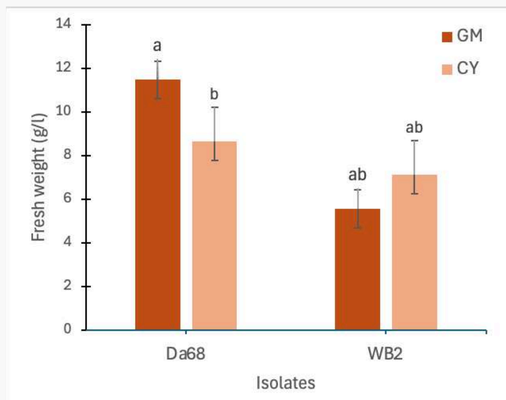


Figure 3 Fresh weight in GM (glucose medium) and CY (corn oil-yeast extract medium), 10d.

Conclusion

- The molecular data confirmed the isolate as *Dacryopinax spathularia.*
- PDBc is an effective alternative medium, showing no significant difference from YMG.
- The glucose-yeast extract medium (GM) is a highly suitable medium for promoting the biomass production of *Dacryopinax spathularia*.
- Qualitative and quantitative comparison of glycolipid production will be conducted in subsequent experiments to confirm the optimal cultivation conditions.

Acknowledgment

- 2812 Laboratory members, Microbiology Division, Department of Biology, Faculty of Science, Chiang Mai University

References

- Stadler, M., Bitzer, J., Köpcke, B., Reinhardt, K., Moldenhauer, J., & GmbH, I. N. S. 2022. Long chain glycolipids useful to avoid perishing or microbial contamination of materials. U.S. Patent 11350628B2 Jun. 7, 2022.
- Bodman, S. M. C. 1938. Morphology and cytology of *Guepinia spathularia*. Mycologia, 30(6), 635–652.