Genome and Growth Responses of Selected Rock-Inhabiting Fungi to Environmental Stress

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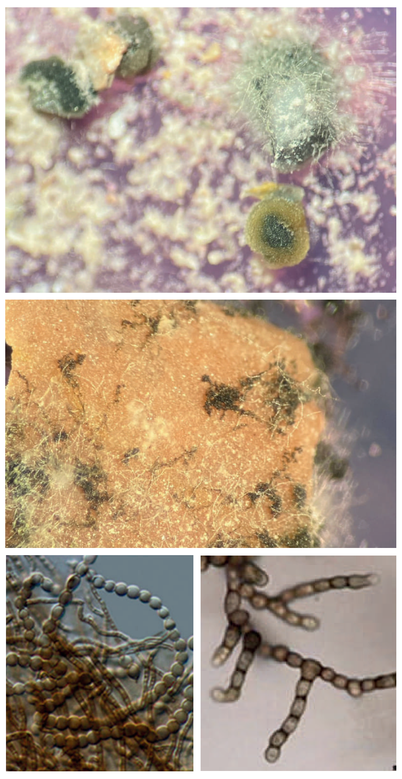
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Introduction

Rock-inhabiting fungi (RIF) are a specialized group of fungi that colonize the surfaces of rocks or inhabit fissures and internal rock substrates [1]. Their remarkable ability to survive in extreme environments is attributed to various adaptive traits, particularly their slow-growing, compact colonies that are typically dark brown to black due to melanization [1,2]. Research on RIF has contributed to a better understanding of microbial life in extreme environments and the evolutionary strategies that enable survival under environmental stress. In Thailand, research on RIF diversity and ecological roles remains relatively limited. In recent years, RIF have attracted increasing scientific interest due to their ecological significance and potential biotechnological applications [3,4]. However, the mechanisms underlying these adaptive traits remain poorly understood.



Objectives

1. To investigate the whole genome of selected rock-inhabiting fungi
2. To study the response of selected rock-inhabiting fungi to drought stress

Methodology

1. Rock-inhabiting fungal strains in this study



SDBR-CMU2-01



SDBR-CMU6-72

The Sustainable Development of Biological Resources Laboratory, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

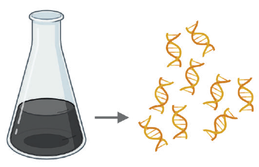
2. Investigate the whole genome



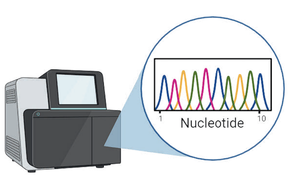
Shake culture
in potato dextrose broth
120 rpm, Room temp. for 7 days



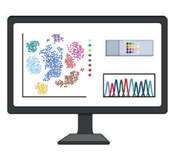
Harvest mycelium
by Cetrifuge



DNA was extracted by
Thermolysis protocol (Zhang et al. 2010)
and CTAB method.



DNA sequence



Bioinformatic analysis



Whole genome

3. Drought tolerance assays



Shake culture
in 2% Malt extract broth
adding 0%, 5%, 10%, 20%
and 40% PEG for 14 days



Removal PEG
by 60 C° DI water



Drying mycelium
overnight



Weigh the dried
mycelium



Spectophotometric
measurement



Sonicate
15 min



Autoclave
121 ˚C, 15 min



1 mL
of 1M KOH

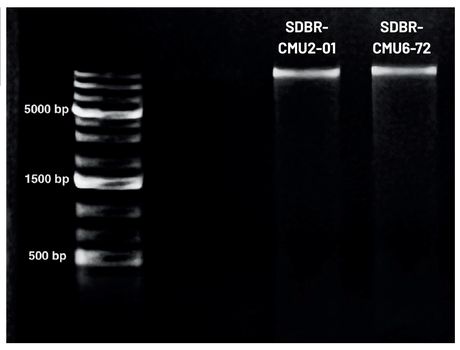


10 mg
of dried mycelium

Results

1. Genomic DNA quality

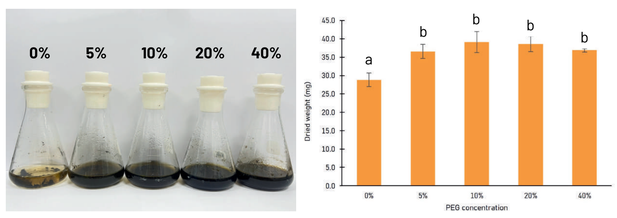
Strain	ng/µL	A260/A280	A260/A230
SDBR-CMU2-01	970.8	2.13	2.22
SDBR-CMU6-72	707.6	2.17	2.52



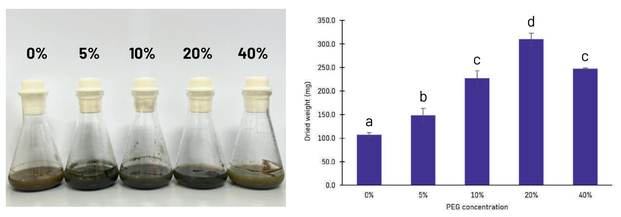
2. Effects of drought stress on growth and melanin production

Effects of drought stress on growth

Dried weight of SDBR-CMU2-01 under drought stress



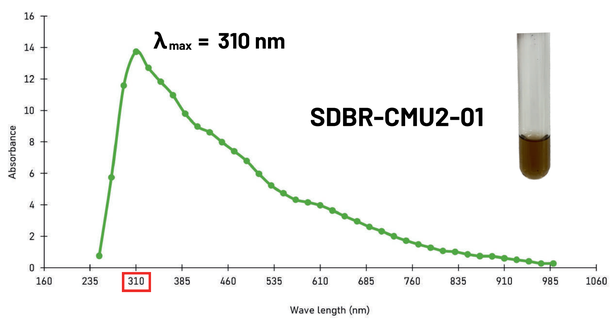
Dried weight of SDBR-CMU6-72 under drought stress

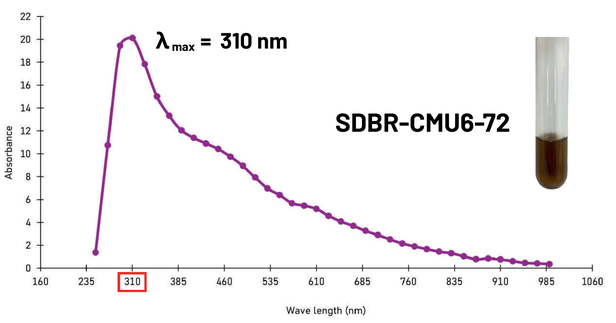


Note: Different letters (a, b, c,) indicate significant differences between treatments

Melanin characterization

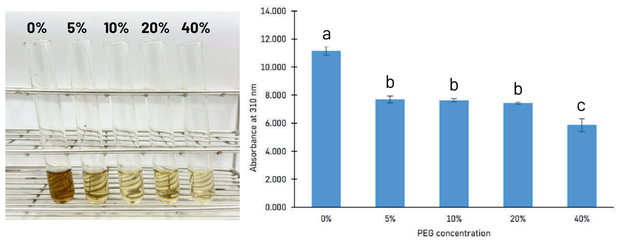
UV-Vis absorption curve of crude melanin extraction (250 - 990 nm)



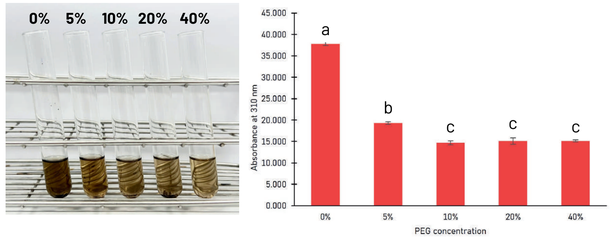


Effects of drought stress on melanin production.

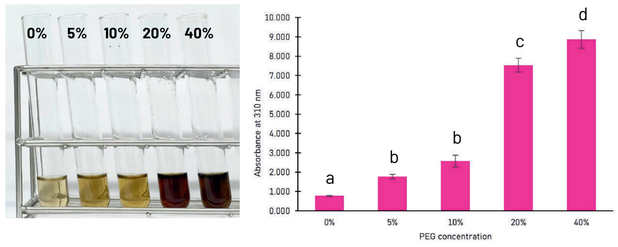
Melanin in dried mycelium of SDBR-CMU2-01



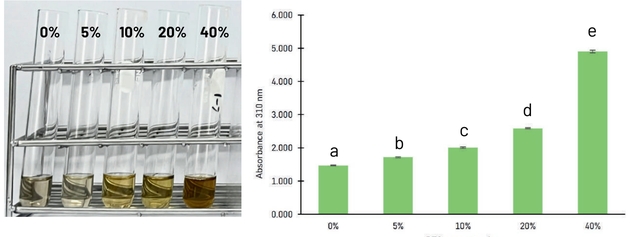
Melanin in dried mycelium of SDBR-CMU6-72



Melanin in culture filtrates of SDBR-CMU2-01



Melanin in culture filtrates of SDBR-CMU6-72



Note: Different letters (a, b, c, d, e) indicate significant differences between treatments

Conclusions

1. Genomic DNA concentration is 700-900 ng/µL that suitable for sequencing.
2. RIF strains SDBR-CMU2-01 and SDBR-CMU6-72 exhibited robust growth under drought stress conditions in liquid medium containing 5% to 40% PEG-6000.
3. Increasing PEG-6000 concentrations correlated with enhanced melanin secretion.
4. Melanin production represents an important adaptive trait for survival under drought stress conditions.

References

[1] Coleine, C., et al. (2021). Beyond the extremes: Rocks as ultimate refuge for fungi in drylands. Mycologia, 113(1), 108-133.

[2] Liu, B., et al. (2022). Rock-inhabiting fungi: terminology, diversity, evolution and adaptation mechanisms. Mycology, 13(1), 1-31.

[3] Fu, R., et al. (2023). Genome and transcriptome reveal lithophilic adaptation of *Cladophialophora brunneola*, a new rock-inhabiting fungus. Mycology, 14(4), 326-343.

[4] Paiva, D. S., et al. (2024). First Genome Sequence of the Microcolonial Black Fungus *Saxispiralis lemnorum* MUM 23.14: Insights into the Unique Genomic Traits of the Aeminiaceae Family. Microorganisms, 12(1), 104.

Acknowledgement

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