Microscopic Characterization and Biosurfactant Potential of Rock-Inhabiting Yeasts

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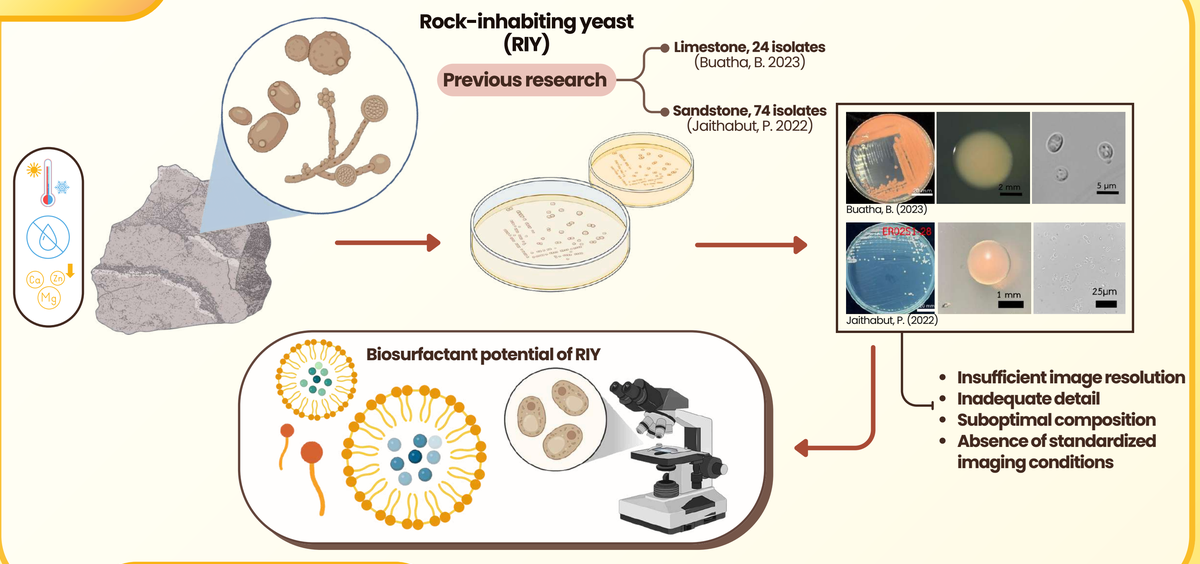
ABSTRACT

Rock-inhabiting yeasts (RIY) thrive in extreme environments characterized by drought, temperature fluctuations, and limited nutrients. This study investigated the microscopic characteristics and biosurfactant production potential of RIY isolated from limestone (24 isolates) and sandstone (50 isolates, selected from an initial 74 isolates). The recovery rates were 100% (24/24) for limestone isolates and 58% (29/50) for sandstone isolates. Microscopic analysis revealed that most RIY colonies shared similar morphological characteristics and were classified as non-pigmented yeasts. Traditional preservation methods were found to be inadequate, particularly for sandstone isolates, due to their low recovery rate. To assess biosurfactant production potential, 20 selected RIY isolates underwent screening using the emulsification index (EI24) test, oil spreading test, and Parafilm M assay. Nine isolates exhibited an EI24 of ≥ 50%, with isolate ER04S1-07 demonstrating the highest biosurfactant production (EI24 = 66.00 ± 2.83%, oil spreading diameter = 2.73 ± 0.25 cm, and a positive Parafilm M test). These findings highlight the potential of RIY as novel sources of biosurfactants for biotechnological applications.

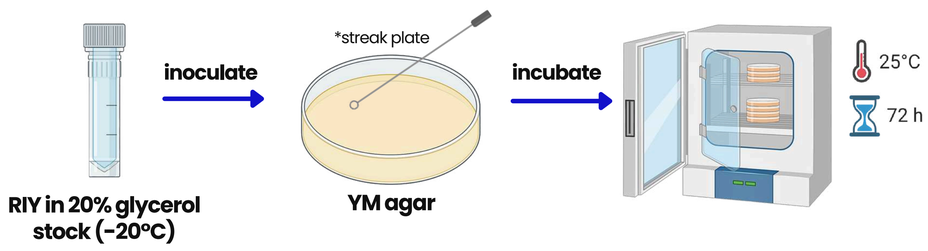


OBJECTIVES

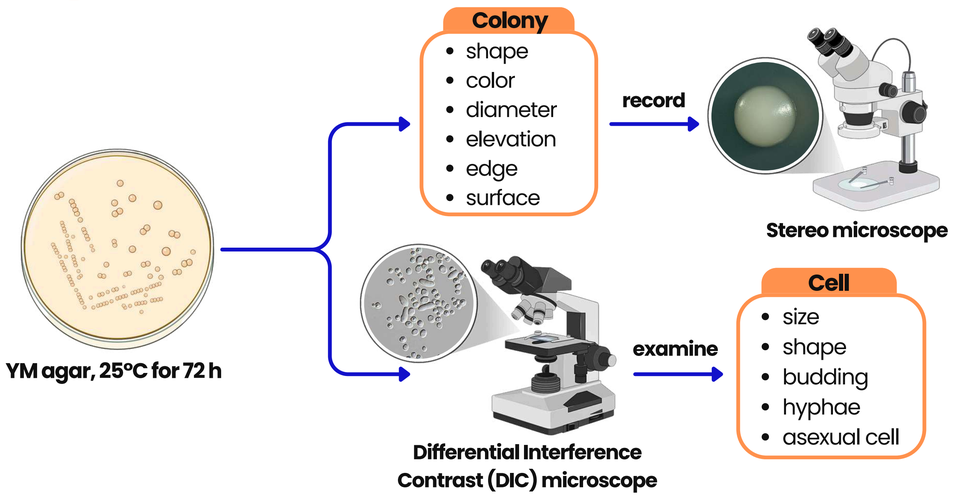
- To investigate the microscopic characteristics of rock-inhabiting yeasts
- To evaluate the biosurfactant production potential of rock-inhabiting yeasts

METHODOLOGY

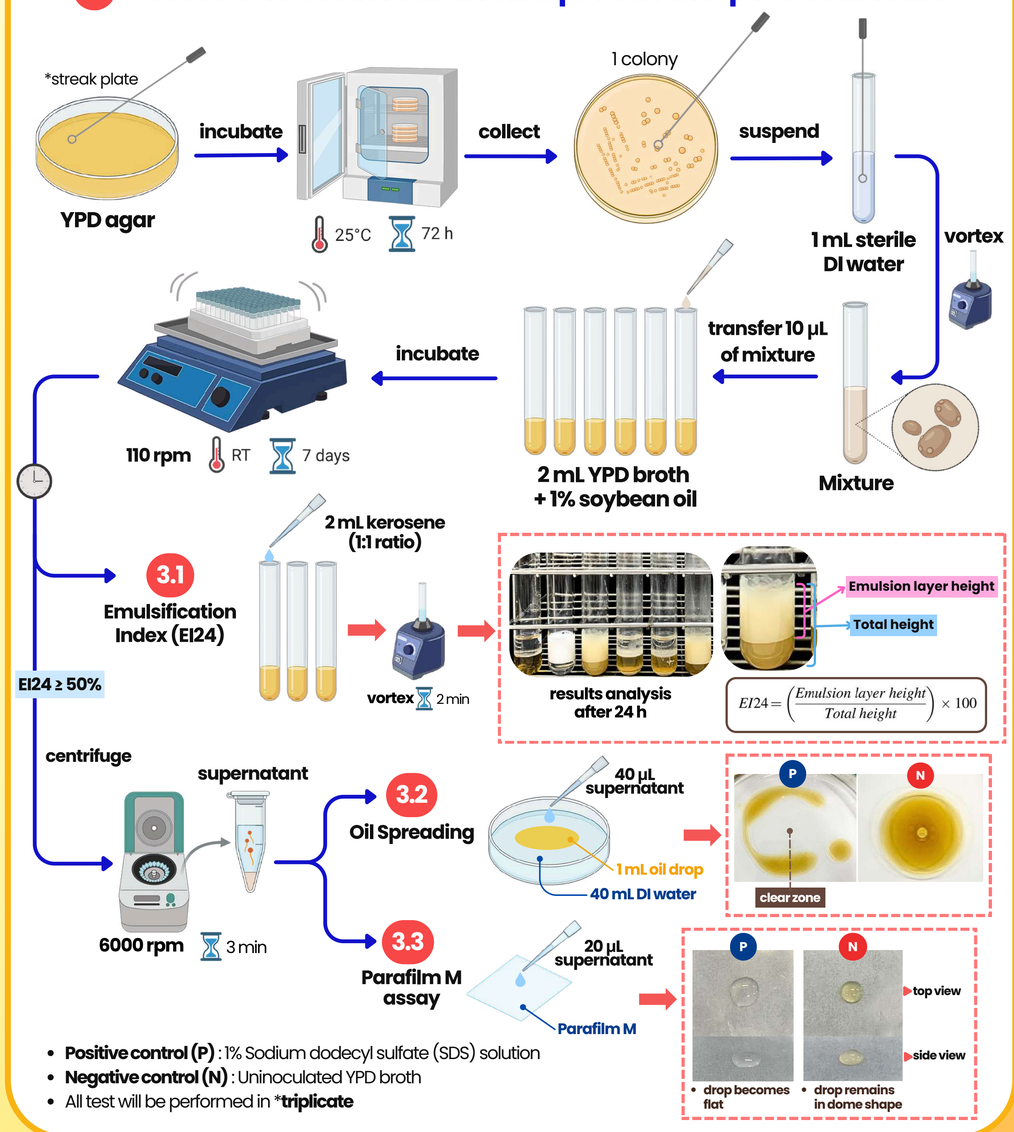
1 Recovery of RIY from glycerol stocks



2 Investigation of the microscopic characteristics of RIY



3 Assessment of biosurfactant production potential of RIY



- **Positive control (P)** : 1% Sodium dodecyl sulfate (SDS) solution
- **Negative control (N)** : Uninoculated YPD broth
- All test will be performed in ***triplicate**

RESULTS

1 Recovery of RIY from glycerol stocks

RIY from limestone : **24**/24 isolates → **100% recovery rate**

RIY from sandstone : **29**/50 isolates → **58% recovery rate**

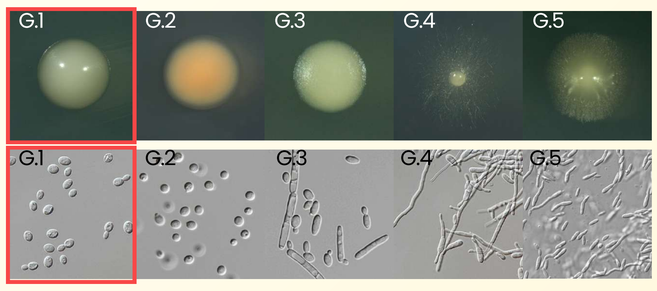
- **Recovery rate of RIY from limestone > sandstone**

2 Investigation of the microscopic characteristics of RIY

Limestone

- Can be divided into **5 groups** based on colony characteristics and **5 groups** based on cell shape
- The most common group is **G.1**

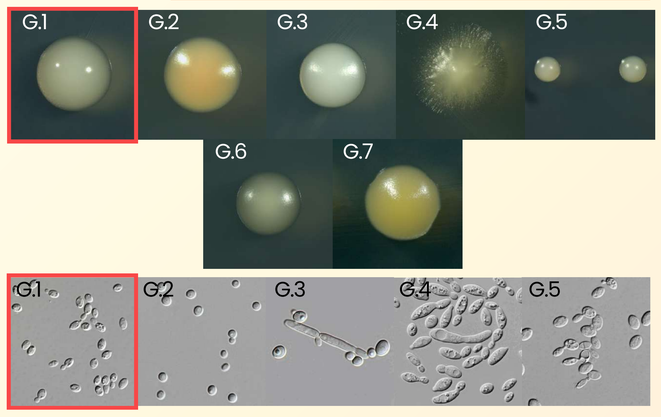
Colony - round, creamy-white, convex, entire edge, and smooth surface
Cell - ellipsoidal unicellular and budding



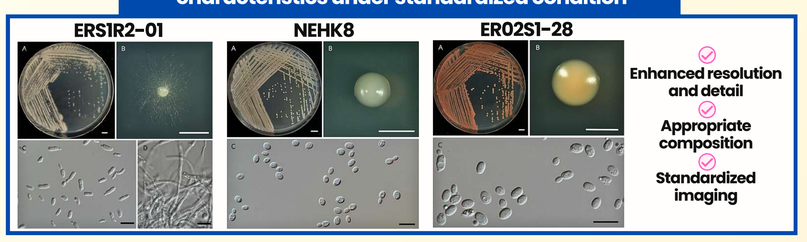
Sandstone

- Can be divided into **7 groups** based on colony characteristics and **5 groups** based on cell shape
- The most common group is **G.1**

Colony - round, creamy-white, convex, entire edge, and smooth surface
Cell - ellipsoidal unicellular and budding



Examples of images after re-examining microscopic characteristics under standardized condition



3 Assessment of biosurfactant production potential of RIY

- 20 selected RIY isolates were evaluated for their biosurfactant production potential by EI24 test, oil spreading test and Parafilm M assay (**Table 1**)

Result

- **9 isolates exhibited an EI24 > 50%**
- **ER04S1-07** showed the highest biosurfactant production

EI24 = **66.00±2.83%**
oil spreading = **2.73±0.25 cm**
Parafilm M = **positive**

Emulsification Index (EI24)

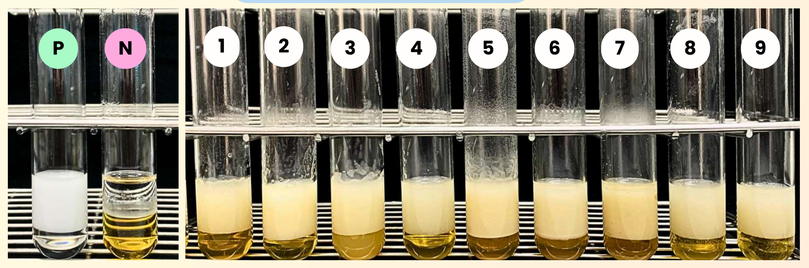


Table 1 : Biosurfactant-producing ability of RIY exhibiting an EI24 > 50% via oil spreading test and Parafilm M assay

Isolate	Methods		
	EI24 (%)	Oil Spreading (cm)	Parafilm M
R02S2-02 (1)	56.70 ± 6.85	0.57 ± 0.06	n
R05S1-01 (2)	65.38 ± 0.00	0.73 ± 0.06	n
R02S2-06 (3)	62.77 ± 1.74	0.37 ± 0.06	n
R01S2-01 (4)	58.00 ± 2.83	1.07 ± 0.21	n
ER03S1-34 (5)	54.00 ± 8.49	2.10 ± 0.36	n
ER04S1-07 (6)	66.00 ± 2.83	2.73 ± 025	+
R01S2-03 (7)	59.62 ± 2.72	2.23 ± 0.25	n
ER03S1-28 (8)	62.00 ± 2.83	1.73 ± 0.21	n
ER02S1-06 (9)	60.00 ± 0.00	0.90 ± 0.10	n
1% SDS (Positive control) (P)	68.00 ± 4.00	11.67 ± 0.58	+
Uninoculated YPD (Negative control) (N)	0	0	n

n - negative

CONCLUSION

- **RIY isolated from limestone exhibited a 100% recovery rate, whereas those from sandstone had a lower recovery rate of 58%.**
- **Most RIY colonies and cells from both rock types shared similar morphological characteristics and were classified as non-pigmented yeasts.**
- **From the emulsification index method, nine isolates were identified as potential produce biosurfactant producers (EI24 > 50%).**
- **Isolate ER04S1-07 exhibited the highest biosurfactant production, with an EI24 of 66.00±2.83%, 2.73±0.25 cm, and positive result in the EI24, oil spreading, and Parafilm M tests, respectively.**
- **The EI24 test was found to be a suitable method for the initial screening biosurfactant-producing yeasts.**

ACKNOWLEDGEMENTS

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