Evaluation of Biosurfactant Production Potential of Rock-Inhabiting Yeasts

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Abstract

The demand for microbial biosurfactants has continuously increased due to their safety and environmental friendliness. This study aimed to evaluate the biosurfactant production potential of rock inhabiting yeasts. In a previous study, 29 yeast isolates obtained from limestone and sandstone in northern Thailand were screened based on their emulsification index at 24 h (EI24), and 13 isolates exhibiting EI24 values greater than 50% were selected. The present study further evaluated these 13 selected isolates using several biosurfactant-related assays, including determination of EI24, oil spreading assay, Parafilm M assay, drop-collapse test, CTAB–methylene blue agar plate method, and growth assessment at different temperatures. Eight yeast isolates exhibited EI24 values greater than 60% using kerosene, with Kwoniella heveanensis R05S1-01 showing the highest value (67.42 ± 1.34%). The EI24 values obtained using diesel oil were lower than those obtained using kerosene, with the highest value observed in isolate S1R2-01, a putative novel species (40.47 ± 1.07%). This isolate also exhibited the largest oil spreading diameter (0.74 ± 0.01 cm). Positive results in the drop-collapse test and Parafilm M assay were observed in certain isolates, and blue halos on CTAB–methylene blue agar were detected in several isolates. In addition, only two isolates (R01S1-01 and ER03S1-28) were able to grow well at 40 °C. These findings indicate that rock-inhabiting yeasts represent a promising microbial resource for biosurfactant production and suitable candidates for further investigation of biosurfactant stability and future applications.

Introduction

Problem Chemical surfactants → Environmental pollutions



Alternatives Need eco-friendly surfactants → **Biosurfactants** → **Rock-inhabiting yeasts (RIY):** promising potential biosurfactant producer



Previous studies

Isolation of RIY Jaitabut (2023) and Buatha (2024) → **1st screening for biosurfactant** Kathongthung (2025) → **13 isolates: EI24 >50%**

Research Screening of 13 RIY for biosurfactant activity

- EI24
- Oil spreading
- CTAB-MB
- Parafilm M
- Drop collapse
- Growth at different temperatures

→ Identification of promising biosurfactants-producing isolates



Objective

To evaluate the biosurfactant production potential of rock-inhabiting yeasts

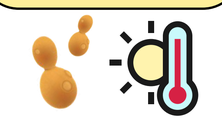
Methodology

13 isolates (EI24 > 50%)

Biosurfactant assays

1. Emulsification Index at 24 h (EI24)
2. Oil spreading assay
3. Parafilm M assay
4. Drop collapse assay
5. CTAB-MB Agar Plate Method (BAPM)

Growth at different temperatures

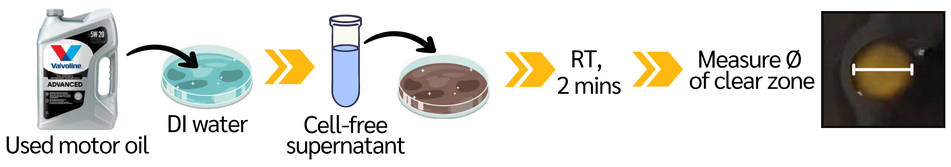


Biosurfactant assays

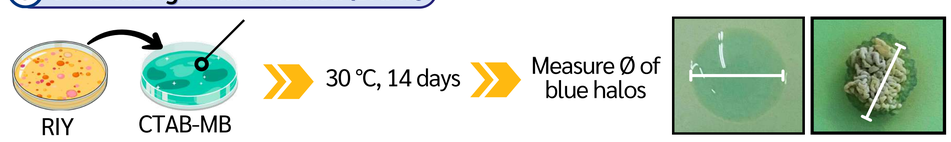
1 Emulsification Index at 24 h (EI24)



2 Oil Spreading Assay



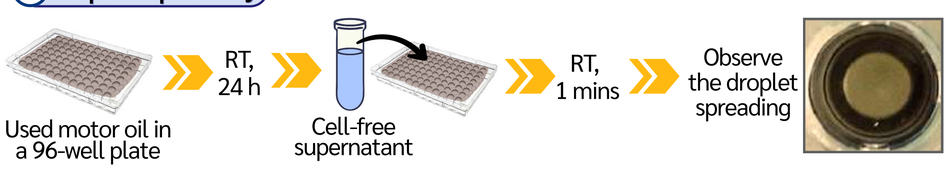
3 CTAB-MB Agar Plate Method (BAPM)



4 Parafilm M Assay



5 Drop Collapse Assay



Growth at different temperatures



Results

Biosurfactant assays

1 EI24 (kerosene and diesel)

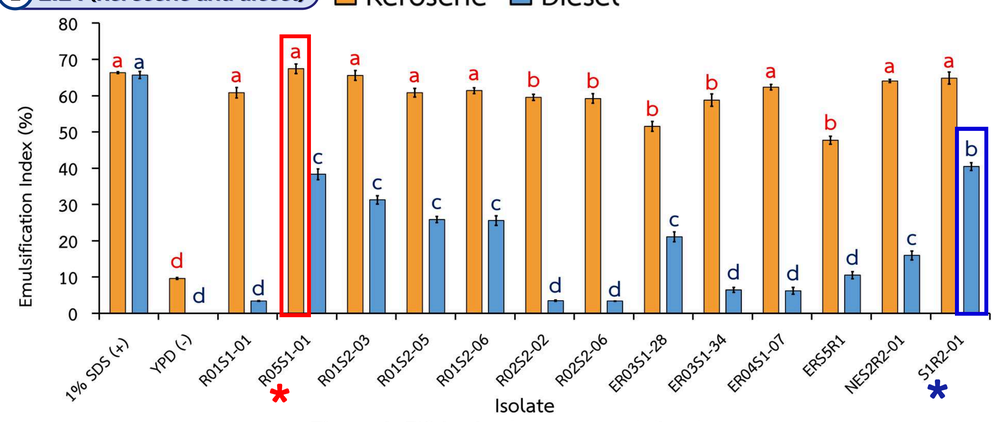
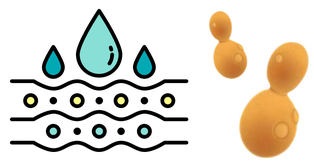


Figure 1. EI24 using kerosene and diesel

Most isolates exhibited emulsification activity, with R05S1-01 showing the highest EI24 with kerosene, while S1R2-01 showed the highest EI24 with diesel oil.



2 Oil Spreading Assay

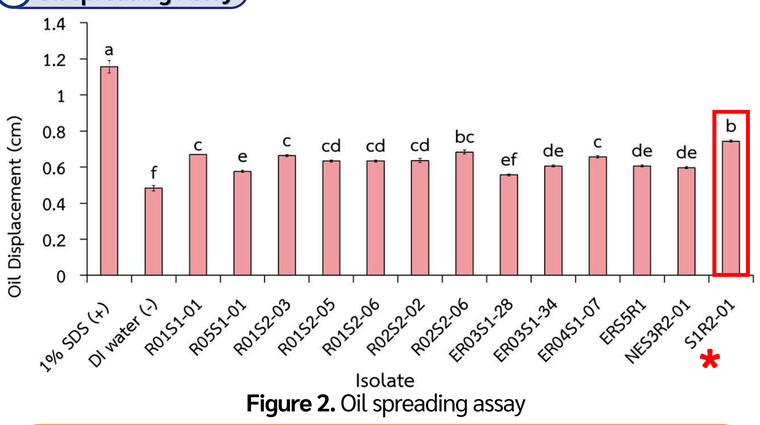


Figure 2. Oil spreading assay

Isolate S1R2-01 produced the largest oil displacement, indicating strong biosurfactant activity.

3 CTAB-MB Agar Plate Method (BAPM)

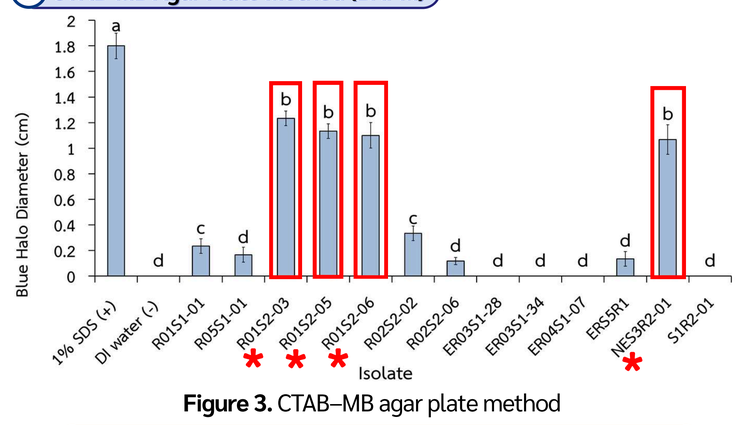


Figure 3. CTAB–MB agar plate method

Several isolates formed blue halos, suggesting potential production of anionic biosurfactants.

4 Parafilm M Assay

Isolate	Parafilm M
1% SDS (+) control	3
YPD (-) control	0
R01S1-01	0
R05S1-01	0
R01S2-03	0
R01S2-05	0
R01S2-06	0
R02S2-02	0
R02S2-06	0
ER03S1-28	0
ER03S1-34	0
ER04S1-07	0
ERS5R1	1
NES3R2-01	0
S1R2-01 ★	2

Figure 4. Parafilm M assay

Only a few isolates showed positive droplet spreading, indicating detectable biosurfactant activity.

5 Drop Collapse Assay

Isolate	Drop Collapse
1% SDS (+) control	3
YPD (-) control	0
R01S1-01	1
R05S1-01	0
R01S2-03	1
R01S2-05	0
R01S2-06	0
R02S2-02	1
R02S2-06	1
ER03S1-28	1
ER03S1-34	1
ER04S1-07	1
ERS5R1	0
NES3R2-01	1
S1R2-01 ★	2

Figure 5. Drop collapse assay

Positive collapse reactions were observed in several isolates, indicating surface tension reduction by biosurfactants.

6 Growth at different temperatures

Growth at different temperatures (°C)	25 °C		30 °C		37 °C		40 °C		45 °C	
	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h
K. marxianus	3	4	3	4	3	4	3	4	3	4
S. passalidarum	3	4	3	4	2	3	2	3	0	0
ER03S1-28★	3	4	3	4	3	4	3	4	0	0
R01S1-01★	3	4	3	4	3	4	3	4	0	0
R05S1-01	3	4	2	3	1	1	0	0	0	0
ERS5R1	2	4	2	3	0	0	0	0	0	0
S1R2-01	2	4	3	4	0	0	0	0	0	0
NES3R2-01	2	3	2	4	0	0	0	0	0	0
R01S2-03	3	4	2	3	0	0	0	0	0	0
R01S2-05	3	4	2	3	0	0	0	0	0	0
R01S2-06	3	4	2	3	0	0	0	0	0	0
R02S2-02	2	3	2	3	0	0	0	0	0	0
R02S2-06	2	3	3	3	0	0	0	0	0	0
ER03S1-34	3	4	3	4	0	0	0	0	0	0
ER04S1-07	3	4	3	4	0	0	0	0	0	0

Figure 6. Growth at different temperatures

Most isolates grew well at 25–30 °C, while only ER03S1-28 and R01S1-01 tolerated 40 °C.

7 Weighted score ranking

Isolate	EI24 (Kerosene)	EI24 (Diesel)	Oil spreading	Parafilm M	Drop collapse	CTAB-MB	Max Temp	Weighted score	Rank
S1R2-01	0.86	1.00	1.00	1.00	1.00	0.00	0.00	0.82	1
R01S2-03	0.91	0.75	0.56	0.00	0.50	0.50	0.00	0.56	2
R05S1-01	1.00	0.94	0.11	0.00	0.00	0.00	0.70	0.53	3
R01S1-01	0.68	0.00	0.61	0.00	0.50	0.00	1.00	0.41	4
NES3R2-01	0.83	0.34	0.22	0.00	0.50	1.00	0.00	0.40	5
R01S2-06	0.70	0.60	0.39	0.00	0.00	0.50	0.00	0.39	6
R01S2-05	0.68	0.61	0.39	0.00	0.00	0.50	0.00	0.39	7
ER04S1-07	0.75	0.08	0.56	0.00	0.50	0.00	0.00	0.33	8
ER03S1-28	0.22	0.48	0.00	0.00	0.50	0.00	1.00	0.31	9
R02S2-06	0.60	0.00	0.67	0.00	0.50	0.00	0.00	0.30	10
R02S2-02	0.61	0.00	0.44	0.00	0.50	0.00	0.00	0.26	11
ER03S1-34	0.58	0.08	0.28	0.00	0.50	0.00	0.00	0.24	12
ERS5R1	0.00	0.19	0.28	0.50	0.00	0.00	0.00	0.15	13

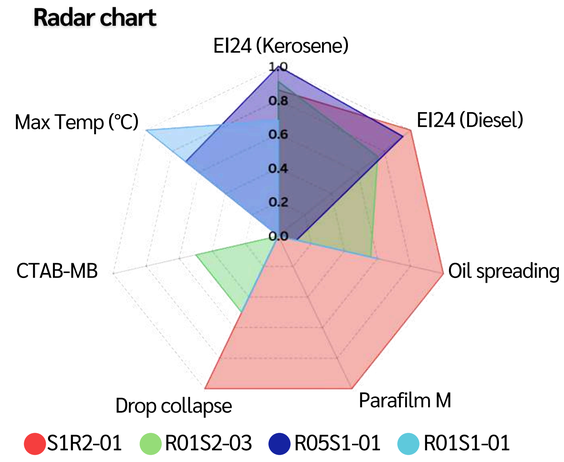
Figure 7. Weighted score ranking **High value** **Low value**

Four isolates (S1R2-01, R01S2-03, R05S1-01 and R01S1-01) showed the highest overall biosurfactant potential.

Weighted score

Metric	Weight
EI24 (Kerosene)	0.20
EI24 (Diesel)	0.25
Oil spreading	0.20
Parafilm M	0.10
Drop collapse	0.10
CTAB-MB	0.05
Max Temp (°C)	0.10

Radar chart



Conclusion

- Rock-inhabiting yeasts are promising **biosurfactant-producing microorganisms.**
- Four isolates (**S1R2-01, R01S2-03, R05S1-01 and R01S1-01**) showed the **best overall performance.**
- These isolates are **potential candidates for further biosurfactant characterization and application studies.**

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