Co-production of microbial oil and exopolysaccharide by the oleaginous yeast *Sporidiobolus pararoseus* grown in fed-batch culture

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1 Thin layer chromatography

The preparation of samples was carried out according to the reports of Han et al.[1]. When analyzing the fat soluble nutrients in *S. pararoseus* oil by TLC, extracts were spotted on a silica gel plate (60GF254 plate; Amresco, Ohio, USA) with benzinum:ethyl acetate:acetone (1:1:1, v/v) solvent as developing solvent. The standard sample was used to compare spots with extracts.

2 The separation of the fat soluble nutrients in *S. pararoseus* oil by High-performance chromatography (HPLC)

The major components were quantified by a high-performance liquid chromatopgraph (HPLC; Hitachi L-2000, Japan) equipped with a photodiode array detector and using C_{18} column (25 mm×4.6 mm; 4.6 μm particle size; Agilent, USA). Isocratic elution analysis was carried out with acetonitrile:tetrahydrofuran=60:40 described in our laboratory previous study[2].

3 The component identification by mass spectrometry (HPLC-MS)

The identifications of oils and carotenoids were analyzed by a mass spectrometry (MS) equipped with a Waters ACQUITY PDA detector and BEH C_{18} column (2.1 mm×100 mm and filler diameter is 1.7 μm; Waters, USA). The detail of operation was carried out according to the description of Han et al.[3].

Table S1 The composition of exopolysaccharide produced by different yeasts

Yeast strain	Molecular weight (kDa)	Monosaccharide composition	References
Sporobolomyces salmonicolor AL$_1$	>1000	54.1% of glucose, 42.6% of mannose, and 3.3% of fucose	[4, 5]
Cryptococcus laurentii AL$_{100}$	4.2	61.1% of arabinose, 15.0% of mannose, 12.0% of glucose, 5.9% of galactose, and 2.8% of rhamnose	[6]
Cryptococcus flavus A51	1010	55.1% of mannose, 26.1% of glucose, 9.60% of xylose, and 1.90% of galactose	[7]
Rhodotorula acheniorum MC	Component 1: 310 Component 2: 249	Component 1: 92.8% of mannose Component 2: 90.6% of mannose	[8]
Rhodotorula glutinis KCTC 7989	100-380	85% of neutral sugars (mannose:fucose:glucose:galactose=67:2:1:1) and 15% of uronic acid	[9]
Pichia (Hansenula) holstii NRRL Y-2448	5000-39000	mannose:phosphorus:potassium=5:1:1	[10, 11]
Sporidiobolus pararoseus JD-2	1300	galactose:glucose:mannose=16:8:1	This study

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                    ┌─────────────────────────────┐
                    │  Sporidiobolus pararoseus JD-2  │
                    └─────────────────────────────┘
                                   │
                                   ▼
                         ┌──────────────────┐
                         │   Fermentation   │
                         └──────────────────┘
                                   │
                                   ▼
                         ┌──────────────────┐
                         │  Centritugation  │
                         └──────────────────┘
                         │                  │
                 ▼                                  ▼
          ┌─────────────┐                    ┌─────────────┐
          │ Supernatant │                    │   Biomass   │
          └─────────────┘                    └─────────────┘
                 │                                  │
                 ▼                                  ▼
    ┌──────────────────────────┐     ┌──────────────────────────┐
    │ Precipitation with ethanol │     │   Break the yeast with HCl │
    └──────────────────────────┘     └──────────────────────────┘
                 │                                  │
                 ▼                                  ▼
        ┌──────────────┐              ┌──────────────────────────┐
        │    Drying    │              │ Extraction with ethyl acetate │
        └──────────────┘              └──────────────────────────┘
                 │                                  │
                 ▼                                  ▼
       ┌───────────────────┐             ┌───────────────────┐
       │ Exopolysaccharide │             │     Yeast oil     │
       └───────────────────┘             └───────────────────┘
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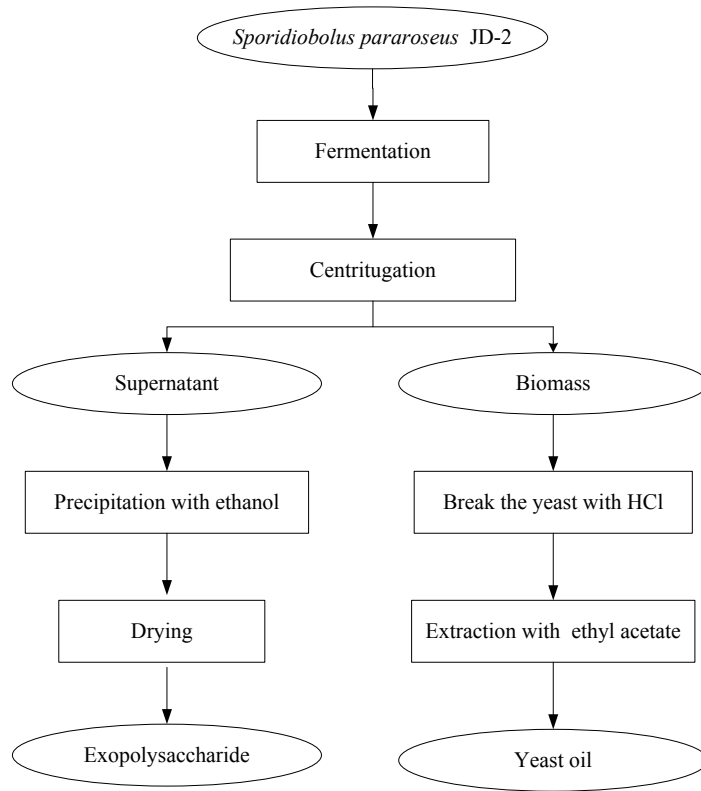
Figure **S1.** The scheme for co-production of exopolysaccharide and oil by *S. pararoseus* JD-2.
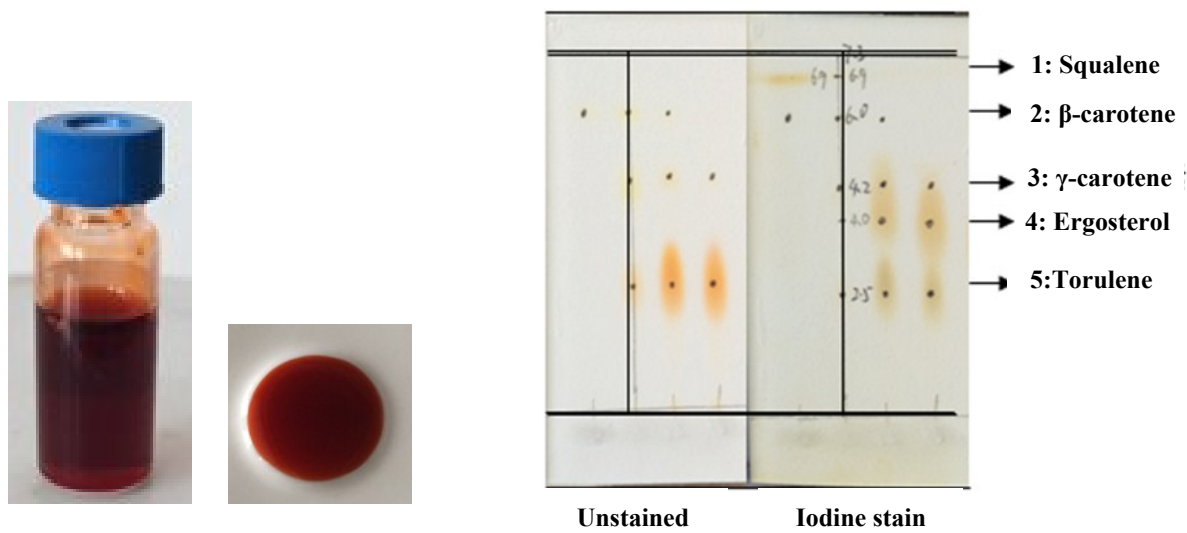
Figure S2. The sample and its thin-layer chromatography of oil produced by *S. pararoseus* JD-2.
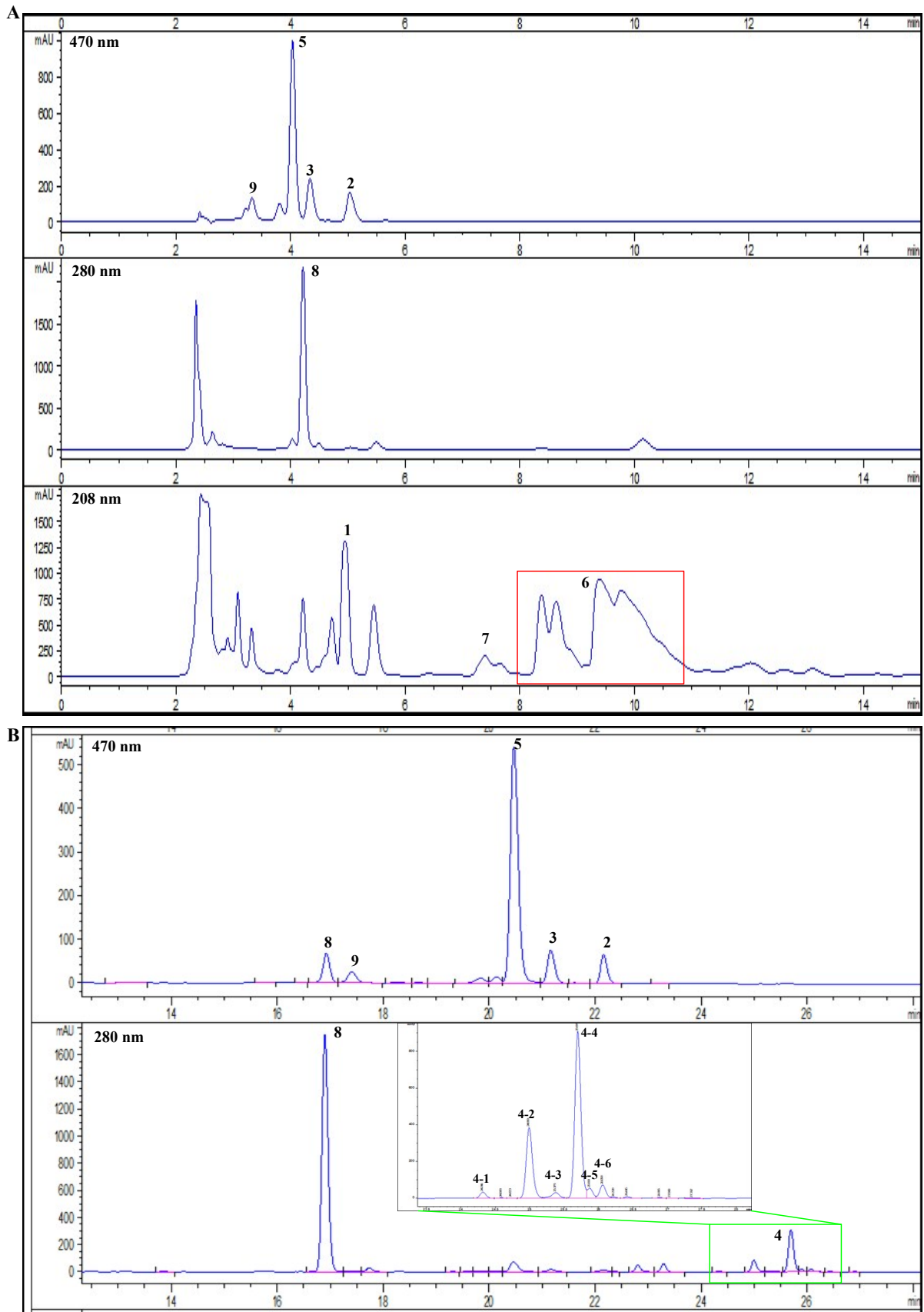
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Figure S3. The main compositions of oil produced by *S. pararoseus* JD-2 separated by isocratic elution (A)

and by gradient elution (B). Chromatographic peaks: peak 1 - Squalene; peak 2 - β-carotene; peak 3 - γ-carotene; peak 4-1~4-6 - Ergosterol ester; peak 5 - Torulene; peak 6 - Triglyceride; peak 7 - Free fatty acid; peak 8 - Ergosterol; peak 9 - Torularhodin. The red frame represents the same composition, and the springgreen frame represents data amplification.
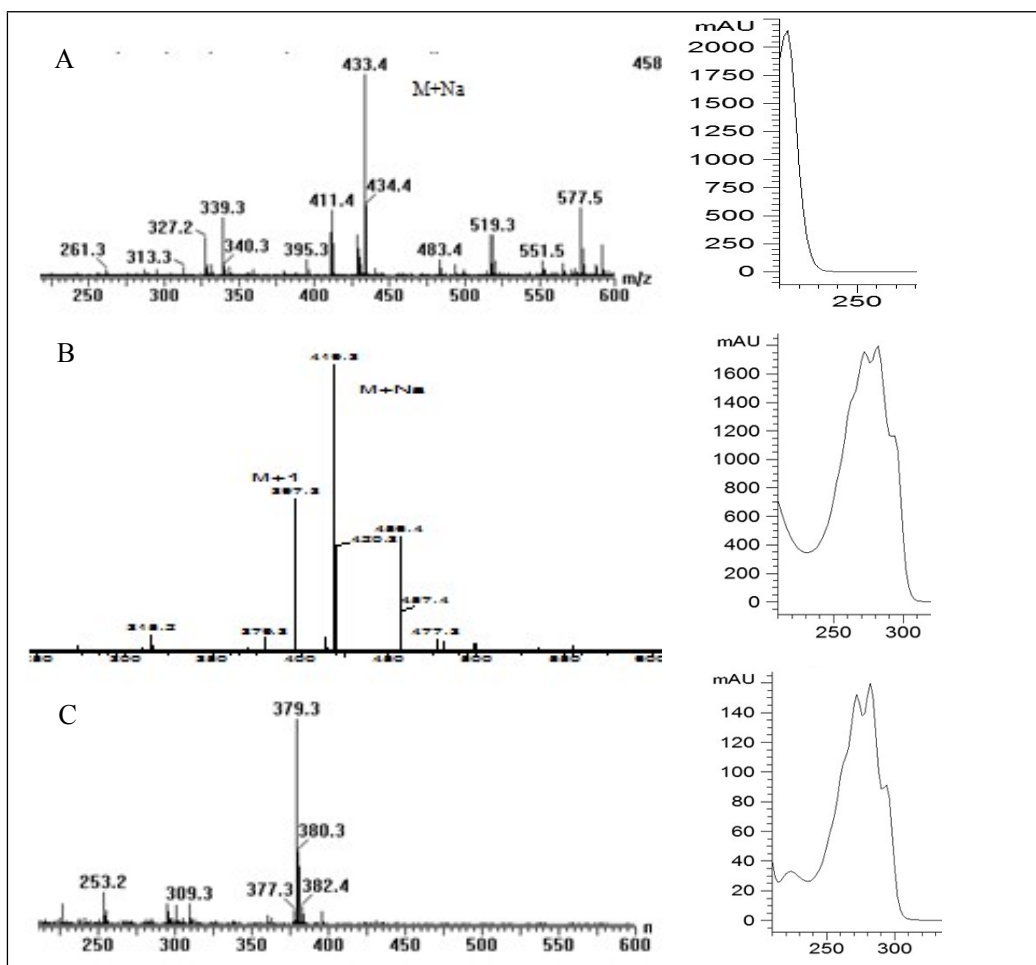
Figure **S4**. HPLC-MS and UV spectrum of squalene (A), ergosterol (B) and ergosterol esters (C).
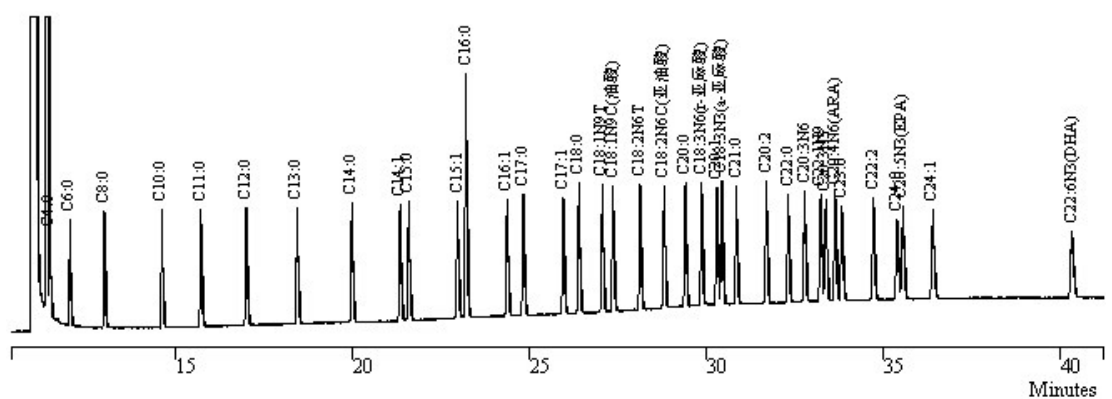
Crude oil
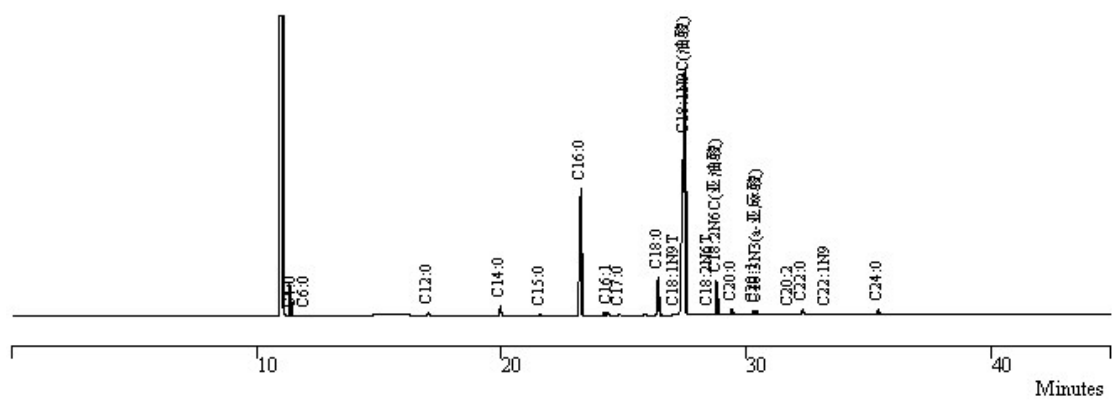
Standards

Figure **S5**. The main compositions of fat soluble nutrients in *S. pararoseus* oil separated by HPLC.

Supplementary References

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