Screening and Identification of Oleaginous Yeasts for Simultaneous Glucose and Xylose Fermentation: Postprint

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Abstract

A yeast strain ZZ-46 capable of simultaneously utilizing glucose and xylose for high-yield lipid fermentation was screened from 10 wild yeast strains isolated from soil in the Nanyang region of Henan Province. Following 120 h of shake-flask fermentation under a glucose:xylose ratio of 2:1, the biomass, lipid yield, lipid content, lipid coefficient, and lipid production rate reached 20.23g/L, 9.89 g/L, 48.91%, 14.64g/100g, and 0.083 g • L-1 • h-1, respectively; the fatty acid composition of lipids produced by this strain using five different ratios of mixed sugars (glucose and xylose) was dominated by C16 and C18 series fatty acids, with oleic acid being the most abundant, followed by linoleic acid, palmitic acid, and stearic acid, and these four fatty acids collectively accounted for over 90% of total fatty acids, which is similar to the fatty acid composition of plant oils, indicating its potential as a biodiesel feedstock. Through morphological characterization and sequence analysis of the 26S rDNA D1/D2 region, strain ZZ-46 was preliminarily identified as Cutaneotrichosporon dermatis, a synonym of Trichosporon dermatis.

Full Text

Preamble

Screening and Identification of Oleaginous Yeast Strains for Simultaneous Utilization of Glucose and Xylose

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Abstract

A wild yeast strain ZZ-46 capable of simultaneously utilizing glucose and xylose for high lipid production was screened from ten oleaginous yeast strains isolated from soil in Nanyang, Henan Province. After 120 h of shake-flask fermentation with a glucose-to-xylose ratio of 2:1, the biomass, lipid yield, lipid content, lipid coefficient, and lipid productivity reached 20.23 g/L, 9.89 g/L, 48.91%, 14.64 g/100g, and 0.083 $g \cdot L^{-1} \cdot h^{-1}$, respectively. Analysis of fatty acid composition from lipids produced using five different glucose-xylose mixtures revealed that C16 and C18 series fatty acids predominated, with oleic acid being the most abundant, followed by linoleic acid, palmitic acid, and stearic acid. These four fatty acids accounted for over 90% of total fatty acids, showing composition similar to plant oils and making the strain suitable as a biodiesel feedstock. Based on morphological characteristics and 26S rDNA D1/D2 domain sequence analysis, strain ZZ-46 was preliminarily identified as *Cutaneotrichosporon dermatis*, a synonym of *Trichosporon dermatis*.

Keywords: Mixed sugar fermentation; Simultaneous utilization; Oleaginous yeast; Screening; Fatty acid composition; Identification

Introduction

Microbial oils represent a novel lipid resource for development following plant and animal oils, offering potential applications not only as edible oil substitutes but also as alternative feedstock for biodiesel production. Compared with conventional plant oil extraction methods, microbial oils offer numerous advantages that provide broad prospects for industrial applications. Lignocellulose, the most abundant renewable resource in China, yields mixed sugars including glucose, xylose, arabinose, and mannose upon complete hydrolysis. Utilizing lignocellulose hydrolysate as substrate for biofuel and bioproduct manufacturing holds significant economic and environmental importance. However, most microorganisms exhibit low utilization efficiency of lignocellulose hydrolysate due to the presence of growth inhibitors such as furfural and because many oleaginous microorganisms exhibit "glucose effect" when glucose and xylose coexist, causing significant lag phases in utilization of other carbon sources and markedly reducing lipid production efficiency. Therefore, screening for strains capable of simultaneously utilizing hexoses and pentoses for lipid production is crucial for microbial oil fermentation.

Previous studies have identified several promising strains. Hu et al. found that *Trichosporon cutaneum* could simultaneously utilize glucose and xylose for lipid production, achieving 59% lipid content and 17 g/100g lipid coefficient at a 2:1 glucose-to-xylose ratio. Kong et al. investigated *Lipomyces starkeyi* using mixed sugars, obtaining 19.0 g/L biomass and 52.6% lipid content after 144 h at a 2:1 ratio. Song et al. screened a high-lipid-yielding yeast strain JM-D that achieved 23.38% lipid content at a 3:1 ratio after 144 h. However, most research has focused on strains from culture collections, with insufficient exploration and utilization of wild oleaginous yeast resources. This study aims to screen high-lipid-producing yeast strains from Nanyang soil that can simultaneously utilize glucose and xylose, and to analyze the fatty acid composition of lipids produced using different mixed sugar ratios, providing a foundation for microbial oil production from lignocellulose hydrolysate.

Materials and Methods

Strains

Ten wild oleaginous yeast strains isolated from Nanyang soil were used: ZZ-03, ZZ-07, ZZ-10, ZZ-16, ZZ-21, ZZ-27, ZZ-29, ZZ-30, ZZ-31, and ZZ-46.

Equipment

- Electric thermostatic incubator (101-2A)
- Digital temperature-controlled shaker (HZQ-B)
- Vertical pressure steam sterilizer (LDZX-50FB)
- Electronic precision balance (FA1004)
- Digital pH meter (PHS-3C)
- Electronic constant-temperature water bath
- UV-Vis spectrophotometer (752N)
- Computerized biological microscope (XSP-13CC)
- Clean bench (SW-CJ-2G)
- Large full-temperature shaker (HZQ-B)
- Gas chromatograph (Agilent Technologies 7890A)
- Liquid chromatograph (Agilent 1100)

Culture Media

1. **Slant preservation medium (YEPD):** Glucose 20.0 g, yeast extract 10.0 g, peptone 10.0 g, agar 15.0 g, distilled water 1000 mL, pH 6.5.
2. **YMA medium:** KH PO 0.1 g, [component name missing] 1.0 g, mannitol 10.0 g, agar 18.0 g, yeast extract 100.0 mL, trace vitamins B +B , [component name missing] 0.2 g, MgSO ·7H O 0.2 g, CaSO ·7H O 0.5 g, NaCl [component name missing] CaCO ·7H O, distilled water 900.0 mL, pH 6.0.

3. **Liquid seed medium:** Glucose 20.0 g, yeast extract 10.0 g, peptone 10.0 g, distilled water 1000 mL, pH 6.5.
4. **Nitrogen-limited fermentation medium:** Glucose 70 g, yeast extract 0.75 g, NH Cl 5.5 mg, ZnSO ·7H O 1.0 g, FeSO ·7H O 0.1 g, KH PO 40 mg, MgCl 3.7 g, MnSO ·4H O 1.0 mg, Na SO 0.1 g, concentrated H SO 0.00184 mg, citric acid 5.2 mg, CaCl 0.76 mg, pH 6.0.

Experimental Procedures

Cultivation Methods

1. **Activation:** Strains were streaked onto YEPD solid slant medium and incubated at 25°C for 2 days.
2. **Seed culture:** Two loops of activated cells were inoculated into liquid seed medium and cultured at 25°C, 140 rpm for 2 days.
3. **Shake-flask fermentation:** Seed culture was inoculated into nitrogen-limited fermentation medium at 10% (v/v) and incubated at 25°C, 140 rpm for 144 h.

Analytical Methods Biomass determination: Cell dry weight was measured using the gravimetric method.

Lipid content determination: During initial screening with single sugars, lipid content was determined by phosphoric acid-vanillin colorimetry. During rescreening with mixed sugars, the acid-heat method was used.

Calculation formulas: - Cell dry weight (g/L) = (total weight of tube + dry cells - empty tube weight) × 1000 / V - Fermentation broth lipid content (g/L) = extracted lipid mass / fermentation broth volume - Cellular lipid content (%) = extracted lipid mass / fermentation biomass - Lipid coefficient (g/100g) = lipid yield / sugar consumption × 100

Glucose and xylose measurement: Concentrations were determined by HPLC using an Eclipse XDB-C18 column (150 mm × 4.6 mm, 5.0 m) with water as mobile phase at 65°C column temperature, 40°C detector temperature, 0.5 mL/min flow rate, and 20 L injection volume.

Fatty acid composition analysis: Samples were methyl-esterified and analyzed by gas chromatography on an FFAP capillary column (25 m × 0.25 mm × 0.25 m). Injection port and detector temperatures were 270°C. Column temperature program: 140°C for 0.5 min, increased at 8°C/min to 165°C (held 2 min), then increased at 2°C/min to 185°C (held 10 min). Split ratio was 20:1 with 1 L injection volume.

Index of Unsaturated Fatty Acids (IUFA) calculation:
IUFA = 1×(monounsaturated fatty acid %) + 2×(diunsaturated fatty acid %) + 3×(triunsaturated fatty acid %) + ···+ n×(n-unsaturated fatty acid %)

Morphological Observation Morphological characteristics were observed according to *The Yeasts: A Taxonomic Study* (5th edition).

Molecular Identification Target strains were identified by 26S rDNA D1/D2 domain sequencing. PCR amplification was performed in 20 L reaction mixture containing 1 L purified PCR product, 8 L BigDye, 1 L primer, and 10 L sterile deionized water. Cycling conditions: 96°C for 1 min, followed by 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min, with final extension at 72°C for 10 min. PCR products were electrophoresed, and target DNA bands were excised and purified using a DNA gel extraction kit (SK1131). After re-electrophoresis verification, sequences were obtained using an automated sequencer. Chromatograms were manually proofread using Chromas software. Corrected sequences were subjected to BLAST search in GenBank, aligned using Clustal X 1.83, and phylogenetic trees were constructed using MEGA 5.0 software with the neighbor-joining method and 1000 bootstrap replicates. Species identification was based on phylogenetic analysis combined with morphological characteristics.

Results

Screening for Efficient Monosaccharide-Utilizing Strains

Ten wild yeast strains isolated from Nanyang soil were cultivated in nitrogen-limited fermentation medium with either glucose or xylose as sole carbon source for 120 h. Their lipid production capabilities are summarized in Table 1 .

Table 1 Lipid production capacity of strains using single sugar fermentation

Strain	Biomass ($g \cdot L^{1}$)	Lipid Yield ($g \cdot L^{1}$)	Lipid Content (%)
ZZ-03			
ZZ-07			
ZZ-10			
...			

Note: Figure translations are in progress. See original paper for figures.