Comparative Glucose and Xylose Coutilization Efficiencies of Soil-Isolated Yeast Strains Identify Cutaneotrichosporon dermatis as a Potential Producer of Lipid

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ABSTRACT: Glucose and xylose are the major hydrolysates of lignocellulose, and therefore, it is of great implication to identify the microbes involved in simultaneous utilization of glucose and xylose. In this study, the strain ZZ-46 isolated from the soil of Nanyang, China, could simultaneously assimilate glucose and xylose efficiently to produce lipid. Upon cultivation with a 2:1 glucose/xylose mixture as the carbon source for 144 h, the cell biomass, lipid concentration, lipid content, and lipid yield of ZZ-46 reached 19.85 ± 0.39 g/L, 9.53 ± 0.60 g/L, 48.05 ± 3.51%, and 0.142 ± 0.003 g/g sugar, respectively. Moreover, C16 and C18 fatty acids were the main constituents of lipid produced by ZZ-46. In addition, ZZ-46 was identified as Cutaneotrichosporon dermatis by the morphology features and phylogenetic analyses. The strain ZZ-46 would have good perspective in practical application for converting lignocellulose into microbial lipid.

INTRODUCTION

Overconsumption of fossil fuels has caused many serious problems including energy crisis, greenhouse effects, toxic gas emission, and pollution.1-3 In order to solve these problems, it is necessary to find alternatives to fossil fuels.4 Biodiesel, as an alternative to fossil fuels, has a lot of environmental advantages over other alternatives.5,6 At present, biodiesel is mainly produced from vegetable oils, which obviously cannot meet the needs of large-scale biodiesel production.7,8 Compared with vegetable oils, the production cycle of microbial oil is shorter, and its production is independent of climate/weather.9,10 In recent years, many microorganisms have been engineered as lipid cell factories.11,12 Oleaginous yeasts are among the most promising microorganisms because of their fast growth and efficient accumulation of lipids (up to 70% of dry cell weight).13,14

For microbial lipid synthesis, lignocellulose used as feedstock has the advantages of abundant availability, low cost, and so on.15,16 For complete utilization of lignocellulose, glucose and xylose (2:1), the hydrolysis products of lignocellulose should both be utilized.17,18 As nearly all oleaginous yeasts can utilize glucose, the ability of xylose utilization has aroused researchers’ interest. For example, Ledesma-Amaro et al. introduced the xylose reductase encoding gene (XYL1) and xylitol dehydrogenase encoding gene (XYL2) from the xylose-fermenting yeast Scheffersomyces stipitis into Yarrowia lipolytica to make it utilize xylose.19 Chu et al. investigated the effect of the xylose isomerase pathway on xylose assimilation and lipid production in the filamentous fungus Mucor circinelloides.20

Although some oleaginous yeasts can utilize xylose, use of xylose is repressed in the presence of glucose, which is referred to as catabolite repression.21-23 The catabolite repression makes the fermentation process too complex and difficult to control and often reduces the productivity and yields.24 Thus, simultaneous assimilation of xylose and glucose is a highly advantageous ability for oleaginous yeasts to completely utilize the hydrolysis product of lignocellulose.25 Thus, identifying oleaginous yeasts that could simultaneously utilize xylose and glucose has been receiving growing attention.26,27 So far, some oleaginous yeasts such as Trichosporon cutaneum (present name, Cutaneotrichosporon cutaneum) and Cystobasidium trimitense have been reported by different research groups to be able to assimilate glucose and xylose simultaneously.

In this study, 10 oleaginous yeast strains isolated from the soil in Nanyang, China (Note S1), were used to explore their abilities of simultaneous utilization of glucose and xylose. The strain ZZ-46 that utilizes mixed sugars to produce lipid most efficiently was selected to analyze the lipid component. Furthermore, ZZ-46 was identified as Cutaneotrichosporon dermatis (now stored in Nanyang Center of Industrial Culture Collection (NICC), Henan province, China, and its serial...
number is NICC30027) by the morphology features and phylogenetic analyses.

■ RESULTS AND DISCUSSION

Biomasses, Lipid Concentrations, and Lipid Contents of 10 Oleaginous Yeast Strains. Ten yeast strains isolated from Nanyang soil were cultured in shake flasks for 144 h, with glucose or xylose as the carbon source. For every strain, the cell biomass and lipid concentration were detected, and the lipid content was calculated. To detect differences of biomass and lipid concentration among different strains, one-way ANOVA was applied (Figure 1). When the carbon source was glucose, the biomass of strain ZZ-29 was the highest, reaching 19.23 ± 0.55 g/L, and the lipid concentration of strain ZZ-31 was the highest, reaching 8.66 ± 0.50 g/L. According to the statistical results, the strains with high biomass included ZZ-29, ZZ-31, ZZ-46, ZZ-03, ZZ-07, and ZZ-10 (Figure 1A), and the strains with high lipid concentration included ZZ-31, ZZ-46, ZZ-29, ZZ-10, ZZ-07, and ZZ-03 (Figure 1B). With a comprehensive consideration of biomass and lipid concentration, the six strains (ZZ-29, ZZ-31, ZZ-46, ZZ-03, ZZ-07, and ZZ-10) were considered efficient in producing lipid using glucose as the carbon source.

When the carbon source was xylose, the biomass and lipid concentration of strain ZZ-16 were both the highest, reaching 16.64 ± 0.54 and 8.81 ± 0.56 g/L, respectively. The strains with high biomass included ZZ-16, ZZ-29, ZZ-03, ZZ-46, ZZ-07, ZZ-10, and ZZ-31 (Figure 1D), and the strains with high
lipid concentration included ZZ-16, ZZ-10, ZZ-31, ZZ-46, and ZZ-29 (Figure 1E). Thus, the five strains (ZZ-16, ZZ-29, ZZ-46, ZZ-31, and ZZ-10) could produce lipid efficiently using xylose as the carbon source.

Taken together, the strains that could both utilize glucose and xylose as the carbon source to produce lipid efficiently were ZZ-31, ZZ-29, ZZ-46, and ZZ-10. Considering the lower lipid content of ZZ-29 (Figure 1C,F), ZZ-31, ZZ-46, and ZZ-10 were selected to determine their ability to utilize glucose and xylose simultaneously.

**Lipid Production of Strains ZZ-31, ZZ-46, and ZZ-10 Using Mixed Sugars.** To determine their ability of lipid synthesis using mixed sugars of glucose and xylose, the strains ZZ-46, ZZ-31, and ZZ-10 were cultured in shake flasks for 144 h. The ratios of glucose and xylose were 1:1, 2:1, and 1:2, respectively. As shown in Figure 2, the biomass of the three strains was all above 17 g/L regardless of the glucose/xylose ratios, indicating that they could grow well using sugar mixtures as the carbon source. In terms of lipid concentration, the strain ZZ-46 was higher than ZZ-31 and ZZ-10 in all conditions. In addition, when the ratio of glucose/xylose was 2:1, the biomass and lipid concentration were the highest, reaching 19.85 ± 0.39 and 9.53 ± 0.60 g/L, respectively. Taken together, the strain ZZ-46 performed better than other strains in lipid production using sugar mixtures of glucose and xylose, so it was selected for further study.

**Growth Curves and Time Course of Sugar Consumption of ZZ-46.** To elucidate the substrate assimilation profile by the strain ZZ-46, growth curves and time course of sugar consumption were determined (Figure 3). The growth of the microorganisms could be roughly divided into two stages: the log phase and stationary phase. When glucose was the sole carbon source, both the growth rate and biomass were higher than those achieved with xylose. However, no classic pattern of diauxic growth behavior was observed when mixed sugars were used as the carbon source, indicating that utilization of xylose by ZZ-46 did not need glucose depletion induction. In addition, the ratios of mixed sugar had no significant effect on the growth rates.

The rates of sugar consumption and final lipid yields for glucose and xylose cultures versus the mixtures were compared (Table 1). When glucose or xylose was used as the sole carbon source, the rates of sugar consumption were about 0.483 and 0.481 g/L/h, respectively. When the carbon source was mixed sugars, the total sugar consumption rates were nearly identical (about 0.48 g/L/h) regardless of the ratios of glucose and xylose, and the consumption rates of glucose and xylose were nearly proportional to their initial concentrations. Moreover, when glucose was the carbon source, the biomass, lipid concentration, and lipid yield were 16.66 ± 0.40 g/L, 7.65 ± 0.45 g/L, and 0.109 ± 0.006 g/g, respectively. When xylose was used, the biomass, lipid concentration, and lipid yield were 13.57 ± 0.58 g/L, 7.05 ± 0.52 g/L, and 0.101 ± 0.007 g/g, respectively. When the carbon source was mixed sugars, the biomass, lipid concentration, and lipid yield were 18.13–20.06 g/L, 9.55–9.95 g/L, and 0.136–0.142 g/g, respectively.

Moreover, the utilization of xylose by the strain ZZ-46 was not suppressed by the glucose existed at the beginning. In addition, the total sugar consumption rates were nearly constant (about 0.48 g/L) regardless of the ratios of mixed sugars. Taken together, glucose and xylose were assimilated simultaneously rather than sequentially. Similar sugar consumption behaviors for glucose and xylose have been reported in other microorganisms, such as *Sulfolobus acidocaldarius* and *T. cutaneum*. Although the cause of this growth behavior is not clear at the moment, we speculated that there were at least two reasons accounting for this phenomenon in ZZ-46: (i) the transport systems of glucose and xylose might function at similar efficiencies; and (ii) there was no carbon catabolite repression (CCR) system in ZZ-46. Further studies need to be carried out to determine the mechanism of simultaneous metabolism of mixed sugars in ZZ-46.

In recent years, coutilization of glucose and xylose has been intensively investigated. The previous studies on lipid production during growth on a mixture of glucose and xylose are listed in Table 2. The capability of lipid production using mixed sugars varies greatly with different strains and was also affected by culture conditions. Hu et al. cultured *T. cutaneum*...
AS 2.571 in a 3 L bioreactor with glucose and xylose (G/X = 2:1) as the carbon source. The strain accumulated lipids up to 59% of dry cell weight, and lipid yield reached 0.17 g/g sugar. When *C. curvatus* ATCC 20509 was cultivated with mixed sugars (G/X = 4:2:5), the strain preferentially utilized glucose. The final lipid content was 39.2 ± 0.7%, and the lipid yield was 0.164 g/g sugar. In another study, three *C. iriomotense* strains were cultivated with mixed sugars (G/X = 1:1). All the three strains utilized glucose and xylose simultaneously. The lipid contents were >20%, and the lipid yields were 0.046–0.064 g/g sugar. The strains *L. starkeyi* AS 2.1560 and *L. starkeyi* DSM 70296 both preferred glucose than

Table 1. Results of Lipid Production by ZZ-46 Cultivated on Different Glucose/Xylose Ratios

| number | G/X (g/L) | culture time (h) | cell mass (g/L) | lipid concentration (g/L) | lipid content (%) | lipid yields (g/g sugar) | sugar consumption rate (g/L/h) |
|--------|-----------|------------------|----------------|---------------------------|------------------|--------------------------|-------------------------------|
| 1      | 70:0      | 144              | 16.66 ± 0.40   | 7.65 ± 0.45               | 45.88 ± 1.61     | 0.109 ± 0.006             | G: 0.483                      |
| 2      | 0:70      | 144              | 13.57 ± 0.58   | 7.05 ± 0.52               | 51.92 ± 1.62     | 0.101 ± 0.007             | X: 0.481                      |
| 3      | 47:23     | 144              | 20.06 ± 0.25   | 9.95 ± 0.23               | 49.61 ± 1.72     | 0.142 ± 0.003             | G: 0.324, X: 0.158            |
| 4      | 35:35     | 144              | 18.13 ± 0.68   | 9.55 ± 0.19               | 52.74 ± 1.87     | 0.136 ± 0.003             | G: 0.242, X: 0.241            |
| 5      | 23:47     | 144              | 18.37 ± 0.35   | 9.73 ± 0.20               | 52.96 ± 1.85     | 0.139 ± 0.003             | G: 0.159, X: 0.325            |

Figure 3. Growth curves and sugar consumption profiles of ZZ-46 cultivated in shake flasks with a total sugar concentration of 70 g/L in different mass ratios. (A) Glucose. (B) Xylose. (C) Glucose/xylose (2:1). (D) Glucose/xylose (1:1). (E) Glucose/xylose (1:2).
xylose. *L. starkeyi* AS 2.1560 accumulated lipids up to 54%, and lipid yield reached 0.18 g/g sugar when cultivated with mixed sugars (G/X = 2:1). DSM 70296 accumulated lipids up to 29.5%, and lipid yield reached 0.134 g/g sugar.\(^9,30\) In this study, the strain ZZ-46 could accumulate lipids up to 48.05 ± 3.51% of dry cell weight and lipid yield reached 0.136 g/g sugar.

**Table 2. Conversion of Sugar Mixtures to Lipids by Different Strains**

| strain                  | mode          | glucose/xylose | lipid content (%) | lipid yield (g/g sugar) | substrate utilization pattern | references |
|-------------------------|---------------|----------------|-------------------|-------------------------|-------------------------------|------------|
| *T. cutaneum* AS 2.571  | batch 3 L bioreactor | 2:1            | 59                | 0.17                    | simultaneous                  | 24         |
| Cryptococcus curvata ATCC 20509 | flask         | 45:25          | 39.2 ± 0.7        | 0.164                   | sequential                    | 12         |
| Cystobasidium tritomense | flask         | 1:1            | >20%              | 0.046–0.064             | simultaneous                  | 26         |
| *Lipomyces starkeyi* AS 2.1560 | flask         | 2:1            | 54                | 0.18                    | sequential                    | 9          |
| *L. starkeyi* DSM 70296 | fed-batch 2 L bioreactor | 70:30          | 29.5              | 0.134                   | sequential                    | 10         |
| *C. dermatis* ZZ-46     | flask         | 2:1            | 48.05 ± 3.51      | 0.136                   | simultaneous                  | this study |

**Table 3. Fatty Acid Profile of Lipid Produced by ZZ-46 Cultivated on Different Glucose/Xylose Ratios**

| ratio (G/X) | octoic acid (C10:0) | myristic acid (C14:0) | palmitic acid (C16:0) | palmitoleic acid (C16:1) | stearic acid (C18:0) | oleic acid (C18:1) | linoleic acid (C18:2) | arachidonic acid (C20:0) | unknown IUFA | \(\text{IUFA}^a\) |
|-------------|---------------------|-----------------------|-----------------------|--------------------------|---------------------|-------------------|----------------------|--------------------------|----------------|----------------|--------|
| 1:0         | 0.18                | 0.52                  | 20.07                 | 1.36                     | 9.38                | 43.01             | 22.21                | 3.22                     | 0.05           | 88.79        |
| 0:1         | 0.17                | 0.52                  | 20.12                 | 1.37                     | 9.40                | 43.21             | 21.86                | 3.18                     | 0.17           | 88.30        |
| 2:1         | 0.04                | 0.48                  | 19.09                 | 1.05                     | 10.86              | 45.57             | 20.06                | 2.52                     | 0.33           | 86.66        |
| 1:1         | 0.01                | 0.50                  | 18.93                 | 0.93                     | 12.04              | 46.80             | 18.15                | 2.35                     | 0.29           | 84.03        |
| 1:2         | 0.08                | 0.53                  | 18.85                 | 0.91                     | 12.15              | 47.49             | 17.83                | 2.09                     | 0.07           | 84.06        |

\(^a\)IUFA: index unsaturated fatty acid.

**Figure 4.** (A, B) Morphologies of (A) colonies and (B) cells of the strain ZZ-46.

**Figure 5.** Neighbor-joining phylogenetic tree based on 26S rDNA D1/D2 domain sequences showing the relationship of strain ZZ-46 to the closely related strains. *Haglerozyma chiarellii* FCP540806 (EU030272) was used as an outgroup. GenBank accession numbers were given in parentheses. Bootstrap values (based on 1000 replications, only values above 70%) were shown at branch points. Bar, 0.01 substitutions per nucleotide position.

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sugar when the initial sugar concentration was 70 g/L and G/X was 2:1.

Fatty Acid Composition of the Lipid Produced by ZZ-46. The fatty acid profile was analyzed by GC after converting the TGA containing fatty acids to methylesters (Table 3). In all samples, the major fatty acids were C16 and C18, which accounted for more than 90% of the total fatty acid content. The proportions of oleic acid (C18:1) were the highest (more than 40%), and oleic acid, palmitic acid (C16:0), and linoleic acid (C18:2) were the three predominant components regardless of the glucose/xylose ratios, which was similar to lipid produced by most oleaginous yeasts.34,35 Overall, the composition of fatty acids produced by ZZ-46 was similar to that of vegetable oils, which indicated that it could provide appropriate feedstock for biodiesel synthesis. Moreover, the proportion of unsaturated fatty acid was the highest, reaching 88.79% when the carbon source was glucose, and decreased with the decrease in the glucose/xylose ratios.

Identification of ZZ-46. In order to clarify the taxonomy of ZZ-46, morphology observations and phylogenetic analyses were carried out. The yeast cells of ZZ-46 were cultured on the YPD plate at 30 °C for 72 h before morphology observations. As shown in Figure 4A, the colony of the strain ZZ-46 was white and creamy, with featureless bulges on the surface and irregular edges. Moreover, a large number of hyphae and ellipsoid or rod-shaped conidiospores could be seen under a light microscope (Figure 4B). In addition, the strain ZZ-46 could utilize cellobiose or arabinose (α-arabinose and l-arabinose) as the sole carbon source (Figure S1). Phylogenetic trees were constructed using the 26S (D1/D2) sequences and the rDNA ITS1 sequences (Figure 5 and Figure S2). As shown in the phylogenetic trees, the strain ZZ-46 was in the same branching group with C. dermatis. Together, these analyses suggested that the strain ZZ-46 belongs to C. dermatis.

In recent years, several oleaginous yeasts, such as Y. lipolytica, L. starkeyi, and R. toruloides, have been widely studied because they have many advantages over oil crops: (i) their growth is faster and their lipid content is at least 15 to 20 times higher than that of oil crops; and (ii) single cell oils produced using lignocellulosic feedstocks have the potential to impact food markets depending upon land usage.14,32,33

■ CONCLUSIONS

As shown in Figure 6, in the present study, 10 oleaginous yeast strains isolated from the soil were used to study their ability of lipid synthesis using glucose and/or xylose as the carbon source. Among them, the strain ZZ-46 identified as C. dermatis could efficiently assimilate glucose and xylose simultaneously to accumulate a considerable amount of lipid, regardless of the ratios of glucose/xylose. Our results provided a yeast strain that had the native ability to coconsume glucose and xylose for biochemical conversion of lignocellulosic materials into lipid. More importantly, the fatty acid composition of lipid produced by ZZ-46 was similar to that of vegetable oils, which means that it could provide appropriate feedstock for the synthesis of biodiesel.

■ MATERIALS AND METHODS

Strains, Media, and Culture. In total, 48 soil samples were collected from two districts and four counties in Nanyang. The two districts were Wancheng (33.00378N, 112.53955E) and Wolong (32.98615N, 112.53479E). The four counties included Xixia (33.29772N, 111.48187E), Tongbai (32.37917N, 113.42886E), Tanghe (32.69453N, 112.83609E), and Fangcheng (33.25453N, 113.01269E).

To separate oleaginous yeast strains, pure cultures were inoculated into 50 mL of nitrogen-limited medium (NLM) for lipid synthesis (NLM: glucose (or xylose or mixed sugars), 70 g/L; yeast extract, 0.75 g/L; NH4Cl, 0.10 g/L; KH2PO4, 1.0 g/L; MgSO4·7H2O, 0.5 g/L; MgSO4, 1.0 g/L; FeSO4·7H2O, 0.1 g/L; citric acid, 0.5 g/L; pH 4.5–5.5) at 30 °C and 200 rpm for 72 h. The resultant cultures were purified to single colonies by the streak plate method on isolation medium (IM) agar (glucose, 50 g/L; yeast extract, 0.5 g/L; Na2HPO4, 0.5 g/L; urea, 1.0 g/L; (NH4)2SO4, 1.0 g/L; KH2PO4, 2.5 g/L; Na2HPO4·5 g/L; MgSO4, 1 g/L; FeSO4·6H2O, 0.1 g/L; and rose bengal (Shanghai Zhihua ChemTech, Shanghai, China), 0.03 g/L; pH 4.5–5.5) at 30 °C and 200 rpm for 72 h.

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Ten oleaginous yeast strains isolated from the 48 soil samples were used in this study. The YPD medium for seed culture contained glucose (20 g/L), yeast extract (10 g/L), and peptone (10 g/L). When necessary, agar (20 g/L) was added to the medium. Seed inoculums from the preculturing step were introduced to the shake flasks containing nitrogen-limited media after washing with sterile distilled water, resulting in a starting cell biomass of about 0.4 g/L. The NLM medium was used for lipid synthesis. The media containing cellobiose (or α-arabinose or l-arabinose) (20 g/L), yeast extract (10 g/L), and peptone (10 g/L) were used for identification of ZZ-46. The cellobiose and arabinose were purchased from the company Macklin (Shanghai, China). All oleaginous yeast strains were grown at 30 °C.
Fermentation in Shake Flasks. Yeast cells were cultured in 500 mL unbaffled conical flasks containing 50 mL of media at 30 °C with shaking at 200 rpm for 144 h. Plastic sheets with ventilated membranes were used as the caps of flasks. Cell cultures were collected at the 24 h interval to detect the concentrations of glucose and/or xylose. All experiments were performed at least in triplicate.

Analytical Methods. The biomass was expressed as dry cell weight (DCW). Cell suspension samples were taken and centrifuged at 12,000 rpm for 10 min followed by being washed with distilled water. Finally, the samples were dried in an oven at 105 °C to a constant weight.

Glucose concentration was quantified using an SBA-40D biosensor analyzer (Institute of Biology of Shandong Province Academy of Sciences, Shandong, China). The total reducing sugars were determined by the dinitrosalicylic acid (DNS) method (a sugar reduction assay). Xylose concentration was obtained by subtracting glucose from the total reducing sugars. The total lipid was extracted from yeast cells using methanol and chloroform. The lipid content was expressed as gram lipid per gram dry cell mass. The fatty acid compositional profiles of lipid samples were determined by gas chromatography (GC7980, Techcomp, Shanghai, China) after transmethylation.

The results were expressed as mean values ± standard deviation (SD). Comparisons among experimental sets were conducted using the software named Statistical Product and Service Solutions (SPSS). The significance of differences among different samples was analyzed using post-hoc statistical test in analysis of variance (ANOVA). A P value (P < 0.05) was considered statistically significant.

DNA Extraction and PCR Amplification. The PCR was performed in a 50 μL reaction volume containing 10 μL of 5X PCR buffer, 50 μM dNTPs, 0.2 μM each primer, 0.5 U Q5 high-fidelity DNA polymerase (New England Biolabs, NEB), and 100 ng of genomic DNA as the template. PCR reactions were conducted under the following reaction conditions: initial denaturing step was 30 s at 98 °C followed by 25 cycles of denaturing at 98 °C for 10 s, annealing at 62 °C for 30 s, and extension at 72 °C for 60 s, and final extension was done at 72 °C for 5 min.

Phylogenetic Analyses. The purified PCR products were sequenced by Beijing Genomics Institute (BGI, Beijing, China). DNA quality determination was carried out by agarose gel electrophoresis, and DNA concentration was determined using a NanoDrop 2000. Then, the DNA fragments were measured by Sanger sequencing technology with an ABI 3730. The reference sequences were downloaded from GenBank. Phylogenetic trees were generated with MEGA 5.0 (https://www.megasoftware.net/older_versions).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c02089.

Note S1, summary of strain isolation; Figure S1, growth status of ZZ-46; and Figure S2, neighbor-joining phylogenetic tree (PDF)

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Author Contributions

S.G. and L.W. conceived and designed the study. L.W., D.W., Z.Z., C.W., B.L., and R.L. performed the experiments. L.W., S.G., and R.L. performed all data analysis. L.W., D.W., and S.G. wrote and revised the manuscript. All authors read and approved the final manuscript.

Notes

The authors declare no competing financial interest.

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