ISOLATION OF OLEAGINOUS YEAST *Rhodosporidium toruloides* MUTANTS TOLERANT TO SUGARCANE BAGASSE HYDROLYSATES

Yuki Kitahara[1,2], Xuebing Zhao[1], Masaaki Wachi[2], Wei Du[1], Dehua Liu[1]; [1] Institute of Applied Chemistry, Department of Chemical engineering, Tsinghua University, Beijing 100084, P. R. China, and [2] Department of Bioengineering, Tokyo Institute of Technology, Yokohama 226-8501, Japan; kitahara.y.aa@m.titech.ac.jp

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Introduction. Recently biodiesel has attracted considerable attention in the world as an alternative of the diesel fuel. Vegetable oils such as soybean oil, palm oil, rapeseed oil, and sunflower oil are used as a feedstock of biodiesel, but it may cause the shortage of edible foods and a rise of food prices. Therefore, microbial production of oils by algae, yeasts, fungi and bacteria has been drawing more and more attention. An oleaginous yeast *Rhodosporidium toruloides* accumulates lipids up to 70% of its dry cell weight in a nitrogen-limited culture medium. However, when lignocellulosic hydrolysates such as sugarcane bagasse hydrolysates (SBH) were employed as a carbon source, cell growth of the yeast was severely suppressed. It was probably due to toxic substances such as organic acids, furans, and phenolic compounds produced during the preparation of hydrolysates. In order to solve this problem, we tried to isolate *R. toruloides* mutants which have tolerance to SBH.

Methods. SBH was prepared as follows. Sugarcane bagasse was hydrolyzed by 0.5%(v/v) H_2SO_4 for 60 min at 120°C. After removing a solid fraction, hydrolysate was neutralized to pH 6.0 by $Ca(OH)_2$, and adjusted to 60 g/L of total organic carbon. The prepared hydrolysate was directly used as a SBH liquid medium. SBH was solidified with 2% agar. An oleaginous yeast *R. toruloides* ACCC 20341 was purchased from Agricultural Culture Collection of China. *R. toruloides* cells were mutagenized by Atmospheric Room Temperature Plasma and the mutagenized cells were plated on SBH agar plates. Colonies which grew on the SBH agar plates were picked up.

Results. Three mutants S4P11, S4P13 and S4P18 were isolated, which grew on the SBH agar plates. Growth of the mutants on the normal nutrient medium was almost identical to that of the wild type. They grew well on the SBH liquid medium, where the wild-type strain did not grow at all (Fig. 1). They produced intracellular lipids effectively using SBH as a carbon source. Particularly, the S4P11 strain accumulated intracellular lipids up to 60% of its dry cell weight (Table 1).

Conclusions. In this study, we isolated *R. toruloides* mutants, which grew on SBH medium. They produced intracellular lipids effectively using SBH as a carbon source. This study will contribute for the lipid production by *R. toruloides* using lignocellulosic hydrolysates like sugarcane bagasse hydrolysate.

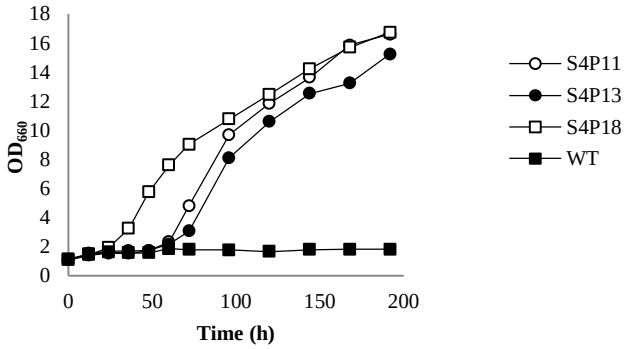

Fig. 1. Growth of *R. toruloides* mutants on SBH liquid medium.

Table.1 Lipid productivity of *R. toruloides mutants* grown on SBH

Strain	Lipid productivity (g/L day^{-1})	Lipid content (%)
S4P11	0.48	63.69
S4P13	0.32	43.19
S4P18	0.45	43.66
Wild type	n.d	n.d

n.d: not detactable

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