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Biotransformation of Uridine Monophosphate (UMP) and Glucose to Uridine Diphosphate-Glucose (UDPG) by *Candida saitoana* KCTC7249 Cells

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ABSTRACT

The present study investigates the biotransformation of glucose with uridine monophosphate (UMP) to obtain sugar nucleotide, UDP-glucose (UDPG), by the dried cells of *Candida saitoana* KCTC7249. The biotransformation was optimized by varying the concentrations of substrates and phosphate ion. UDPG (24 m*M*) was biotransformed from 200 m*M* glucose and 37.5 m*M* UMP by dried cells of *C. saitoana*. The glucose yields about 64% UDP-glucose, based on UMP concentration. The addition of glucose-1-phosphate to the reaction mixture accelerated the formation of UDPG from a concentration of UMP. The structure of UDP-glucose obtained was determined with ^{13}C NMR and FAB mass spectra. These results indicate that the yeast-dried cells could be used for the production of nucleotide sugars for donor molecules of complex carbohydrate synthesis.

Index Entries: Uridine diphosphate-glucose; biotransformation; *C. saitoana*.

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INTRODUCTION

In biosynthesis of complex carbohydrates, a variety of sugar-nucleotides are required, which are active forms of sugar and are needed as substrates of glycosyltransferases. Since uridine 5'diphosphate-glucose (UDPG) was first identified from yeast by Leloir and co-workers (*1*), it has been known that activated nucleotide sugars are used for the transformation of monosaccharides and transglycosylation to give complex polysaccharides (*2*). Many other sugar-nucleotides containing different sugar and nucleoside moieties have been found in nature. For example, another activated nucleotide sugar, ADP-glucose, is important as activated monomer in the synthesis of plant starch (*3*) and in developing pea embryos (*4*).

While sugar nucleotides in vitro can be prepared by chemical (*5*) and enzymatic synthesis (*6*), as well as by extraction from microbial cells, the sugar-nucleotide synthesis in numerous cellular metabolic pathways in vivo is catalyzed by the action of sugar nucleotide pyrophosphorylases. For example, UDP-glucose is synthesized from α-D-glucose 1-phosphate (glucose-1-phosphate) and UTP by the action of UDP-glucose pyrophosphorylase (UDPGase). This enzyme catalyzes the transfer of nucleotidyl groups from a nucleoside triphosphate to a sugar phosphate, with the formation of inorganic pyrophosphate (PPi). UDP-glucose was first synthesized enzymatically from UTP and glucose-1-phosphate, with an enzyme preparation of yeast (*7*).

For the preparation of UDPG in yeast, UDPG was extracted from commercial Baker's yeast autolyzed with toluene (*8*). Another improvement was made by a fermentative method for preparation of UDPG, using ground cells of Baker's yeast and acetone-dried cells of brewer's yeast as enzyme sources (*9*). This method was also applied to a potent producer of UDPG, *Torulopsis candida* IFO 0768 (*10*).

In this article, we report a fermentative method for the preparation of UDP-glucose, using dried cells of *C. saitoana* as an enzyme source. The final yield of UDP-glucose was about 23%, based on UMP concentration. The structure of the UDPG obtained was analyzed with ^{13}C-NMR spectra and FAB-mass spectroscopy.

MATERIALS AND METHODS

Chemicals and Strains

Sodium uridine-mono-phosphate (UMP) was obtained from Miwon (Seoul, Korea). Authentic samples of UDPG, UTP, UDP, and monosaccharides were purchased from Sigma (St. Louis, MO). *C. saitoana* KCTC 7249 was obtained from Korean Culture Type Collection (KCTC), Korea Research Institute of Bioscience and Biotechnology, KIST, Taejon, Korea.

Microorganism Culture and Preparation of Dried Cells

C. saitoana KCTC7249 was grown at 28°C in a medium containing 5% glucose, 0.5% peptone, 0.2% yeast extract, 0.2% KH_2PO_4, 0.2% $(NH_4)_2HPO_4$, and 0.1% $MgSO_47H_2O$ (pH of the medium was adjusted to 6.8). The cultivation was carried out for 24 h in a jar fermentor containing 10 L of the medium after addition of innoculum (500 mL) of culture. *C. saitoana* cells were harvested by centrifugation at 7000*g* for 10 min and washed with water. The cells were freeze-dried and stored at −20°C before their use.

Bioconversion (Reaction System) of Monosaccharide

Glucose (400 m*M*) and UMP (75 m*M*) in 2 mL of reaction solution containing magnesium sulfate (20 m*M*) were incubated at 28°C with dried cells (100 mg/mL) by shaking in a test tube. After incubating the reaction mixture, the solution was put in boiling water for 3 min, and followed by centrifugation at 12,000*g* for 5 min to remove cell debris. The supernatant was analyzed by Beckman HPLC (Fullerton, CA) equipped with programmable UV detector module and ODS C-18 (5 μm) reverse-phase column (4.6 × 300 mm). The injection volume for their analysis was 50 μL. Peaks in HPLC chromatograms, obtained from the absorbance at 254 nm, were identified by comparing their retention times with those of authentic samples. Mobile phase was 25 m*M* potassium phosphate (pH 4.0) containing 5 m*M* tetrabutyl ammonium chloride (TBA-Cl) and 60% acetonitrile gradient (*11*). UDPG was purified on a preparative scale with Dowex-1 anion exchange column (2 × 3 cm), which was equilibrated with 10 m*M* HCI and eluted by NaCl step gradient. The reactions were also analyzed by PEI celluose thin-layer chromatography. Elunts were 1*N* acetic acid for prerun, and the mixed solution with 1-*M* LiCl, 1 N acetic acid and water (3:1:6, v/v) for the developments.

NMR and Mass Spectra

^{1}H-spectra were recorded from Varian 300 and 500 MHz, and ^{13}C-NMR spectra were from 75 MHz NMR. The samples exchanged with D_2O two times were used for acquisition. MeOD was added as internal standard for ^{13}C NMR spectra. Fast atom bombardment (FAB) mass spectra were obtained from Kratos CONCEPT-1S spectrometry, adjusted to 7 Kev and 0.3 mA as its energy and current. Glycerol was used as a matrix, and argon was the fast atom.

RESULTS AND DISCUSSION

In a preliminary experiment, yeast *C. saitoana* was grown on a medium containing glucose as carbon source. This was found to be an effective

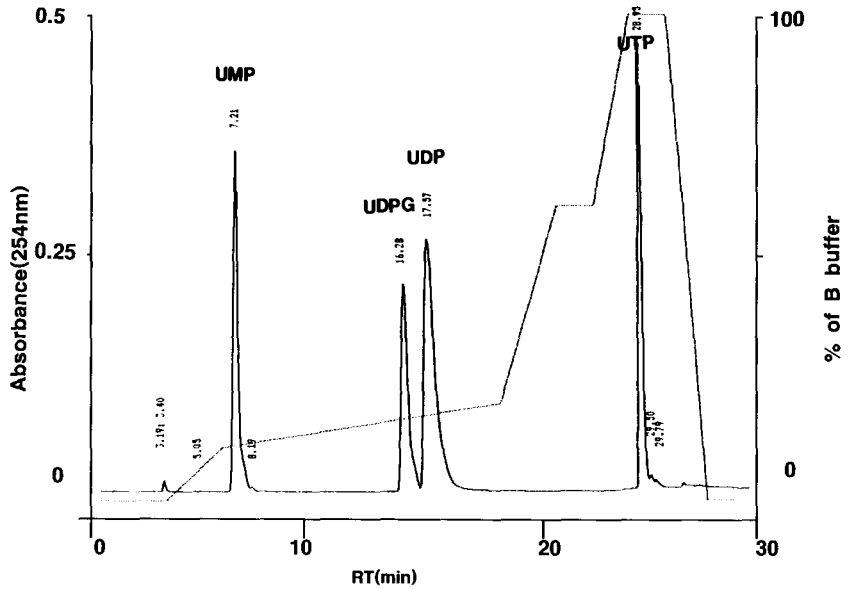

Fig. 1. HPLC chromatogram of Standard (50 μmol of UMP, 50 μmol of UDPG, 150 μmol of UDP, 50 μmol of UTP): 50 μL of standard mixture was applied to an ODS C-18 (5 μm) reverse-phase column (4.6 × 300 mm) with buffer and acetonitrile. Peaks were identified from their retention times and absorbance at 254 nm (——). ----, % of B buffer.

enzyme source for fermentative production of UDPG from UMP and glucose. UDPG was produced in relatively high yields from UMP and glucose, with the dried cells of the yeast grown on a glucose medium.

In cells during the biosynthesis of UDPG, a number of biochemical reactions occur:

1. Glucose-1-phosphate is formed via glucose-6-phosphate from glucose;
2. At the same time, UTP is also synthesized (phosphorylated) from UMP through UDP;
3. Finally, UDPG is synthesized by condensation of glucose-1-phosphate with UTP in the presence of UDPGase.

The results of UDPG production were calculated from HPLC chromatogram (Fig. 1) and plotted to obtain yields.

During the production of UDPG, UTP was initially formed, then UDP-glucose and UDP were synthesized as UTP was consumed during its prolonged incubation (Fig. 2A and 2B). However, the UDP-glucose produced was gradually decreased, and UDP and uridine increased during prolonged incubations (data not shown). UDP-glucose, however, was not significantly degraded within 15 h. The rate of UDPG formation seems to be slowed by increasing the concentration of glucose (Fig. 2A and 2B).

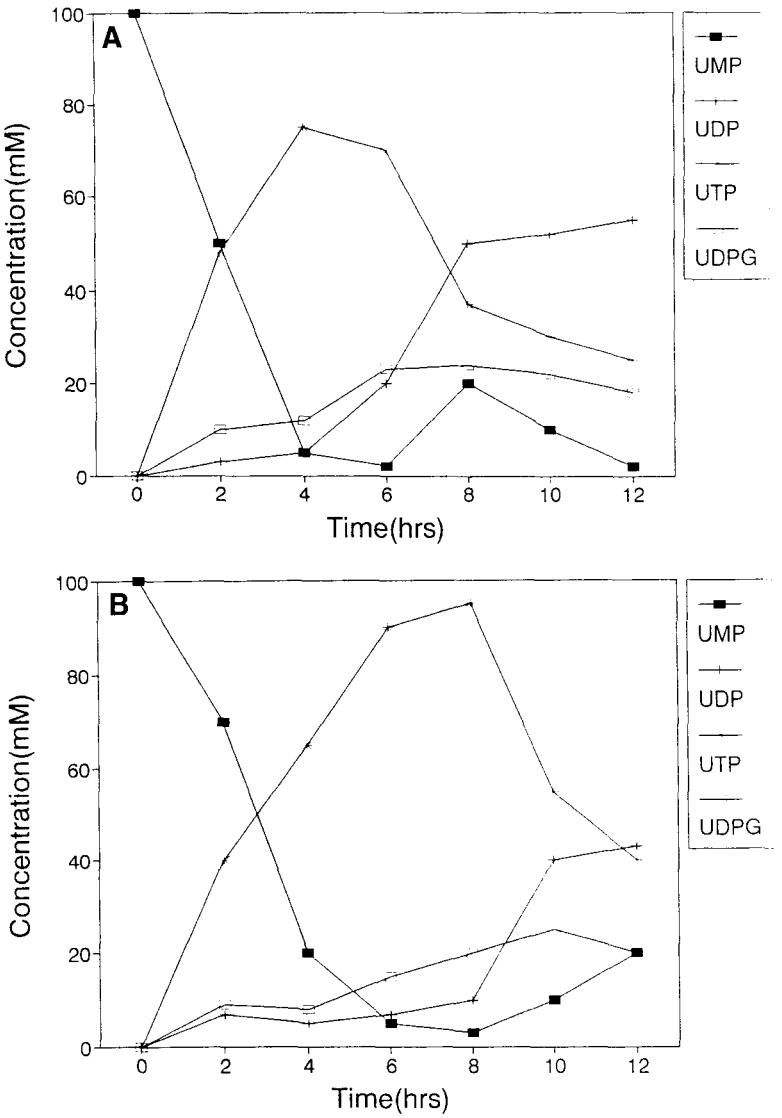

Fig. 2. Time-course of bioconversion for UDPG formation with glucose substrate at (**A**) 400 m*M* and (**B**) 600 m*M* concentrations. The product concentrations were estimated by measuring the peak areas in HPLC chromatograms. (■) UMP; (+) UDP; (*) UTP; (□) UDPG.

The amount of UDPG formed in 6 h reached 24 m*M*/mL (55–76% yield from UMP) in reaction containing 400 m*M* glucose. However, the same amount of UDPG formed was produced after 10 h in the reaction containing 600 m*M* glucose. The results also indicate that addition of 400 m*M* glucose is sufficient for a maximal formation of UDPG in the reaction. The yield of UDP-glucose was higher than that of *T. candida* (20 μmol/mL), indicating that a *C. saitoana* system is superior to the *T. candida* system described by Kawai et al. (*10*).

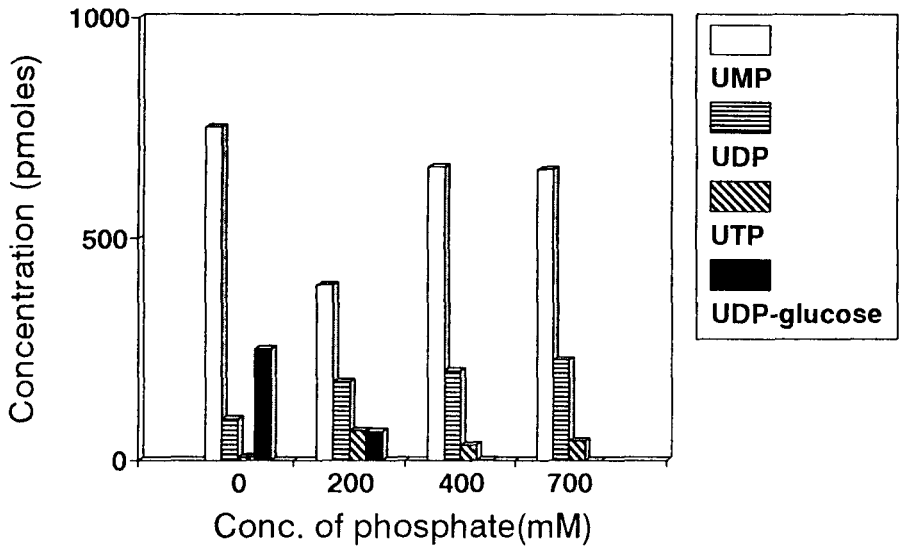

Fig. 3. The effect of phosphate ion in bioconversion for UDPG. The formation of (□) UMP, (▤) UDP, and (▨) UTP, as well as (■) UDPG during the bioconversions by *C. saitoana*, were carried out under various phosphate concentrations for 10 h and monitored on HPLC.

The effect of phosphate ions in the reactions with *C. saitoana* was also examined. The addition of phosphate ions (0, 200, 400, and 700 m*M*) gave a significant effect on UDPG formation (Fig. 3). Concentrations above 200 m*M* seemed to inhibit its formation. Such effect might result from the high ionic strength of phosphate ion in reaction of UMP phosphorylation; alternatively, phosphate ion may inhibit the formation of UDPG through product inhibition (PPi and Pi). Furthermore, the addition of glucose-1-phosphate to the reaction mixture accelerated the formation of UDPG from a concentration of UMP (data not shown). These results are similar to those observed for *T. candida* (*10*).

Finally, to confirm the molecular structure of the UDP-sugar that was formed, the product was purified by Dowex-1 anion exchange column chromatography, and the NMR spectra (Fig. 4) and mass spectra (Fig. 5) were obtained. Based on these spectral data, the product was assumed to be UDP-glucose.

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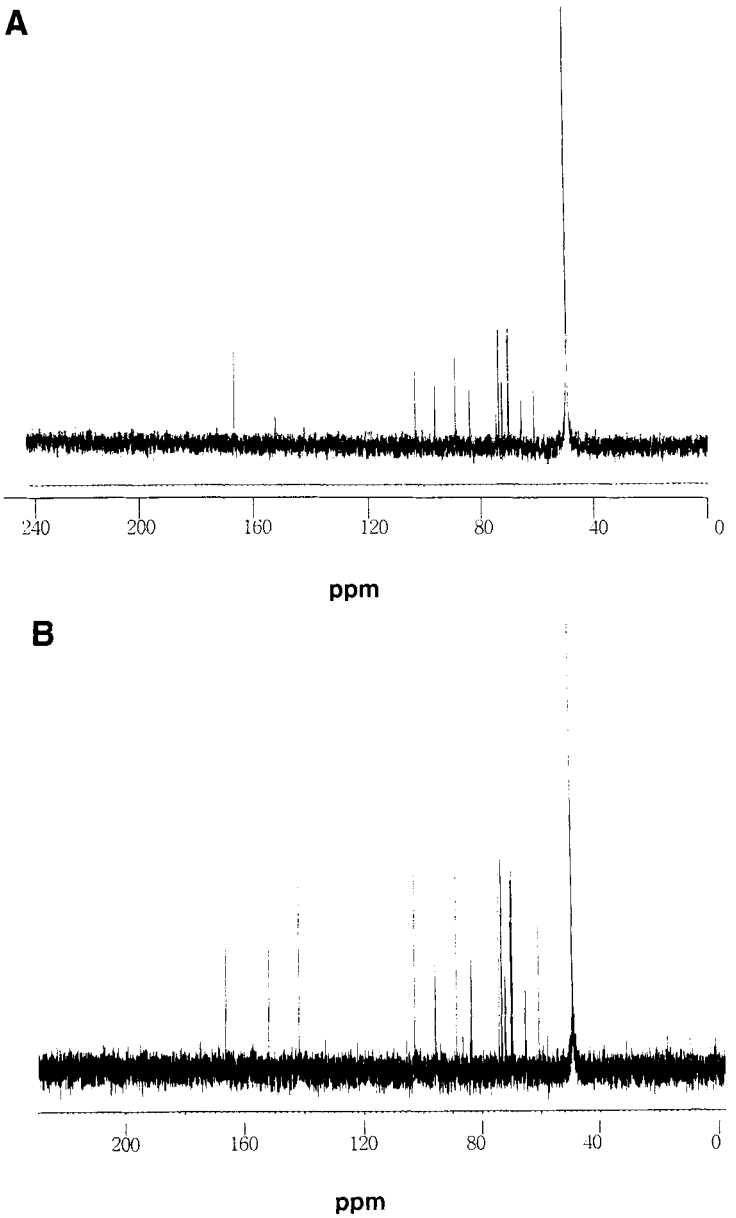

Fig. 4. ^{13}C NMR spectra of products from glucose and UMP. UDP-sugar, purified with Dowex-1 column, was lyophilized two times in D_2O, filtered over a small magnet through glass fiber. Methanol was added for reference (**A**) standard of UDP-glucose (3 mmol/mL) purchased from Sigma (**B**) UDP-glucose formed from the work.

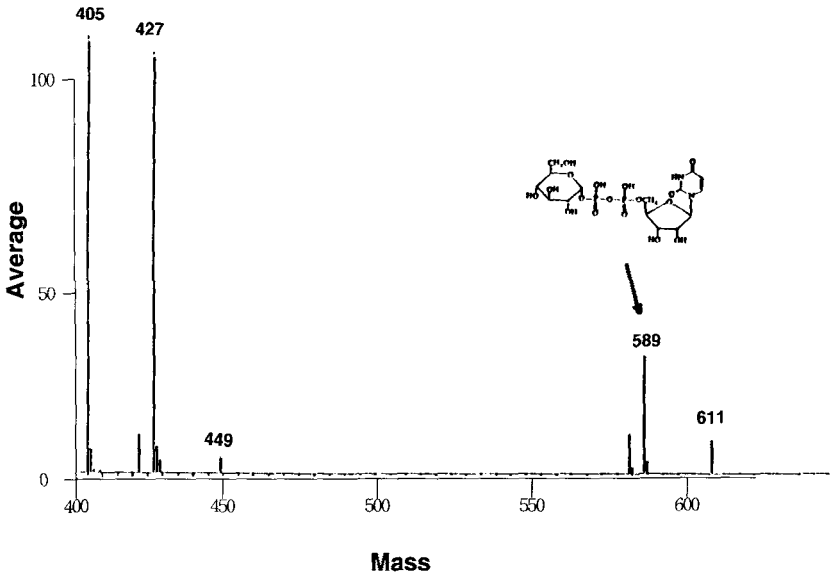

Fig. 5. Mass spectrometric analysis of product. The reaction product from UMP and glucose was identified as UDP-glucose by FAB mass spectrometry.

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