Note

Production of α,α -Trehalose by a Bacterium Isolated from Soil

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Received October 8, 1987

During the course of a study on the production of glycosidases by a bacterium, the 50 b strain,^{1,2)} we found that the bacterium accumulated a disaccharide in the culture filtrate when it was grown on a medium containing glycerol as a carbon source. This paper describes the isolation and identification of the disaccharide.

The bacterium was grown on a medium containing yeast extract 1 g, glycerol 10 g, $(\text{NH}_4)_2\text{SO}_4$ 5 g, K_2HPO_4 1 g, KCl 0.5 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 10 mg, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 5 mg, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 20 mg and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 1 mg in 1000 ml of deionized water, pH 7.0, for 5 days at 28°C with reciprocal shaking. After the culture, the cells were removed by centrifugation. The supernatant (about 500 ml) was concentrated to a minimum volume under reduced pressure at 50°C. The concentrate was applied to a column of Bio-Gel P-2 (3 cm $\times$ 100 cm) and then eluted with deionized water. The disaccharide portions were combined and then passed through columns of Dowex 1 $\times$ 2 (Cl^- , 1 cm $\times$ 5 cm) and Dowex 50 (H^+ , 1.8 cm $\times$ 10 cm) with deionized water successively. The eluate was concentrated to about 1 ml. The concentrate was rechromatographed on Bio-Gel P-2. The eluate was concentrated to dryness under reduced pressure. Thus, about 150 mg of a dried material was obtained. This compound gave a single spot on TLC and only glucose on acid hydrolysis (Table I). Fab-mass spectrometry showed that the molecular weight of this compound was 342 as a disaccharide (data not shown). This compound did not reduce Fehling's reagent suggesting that it is a disaccharide of two D-glucose residues linked through their reducing carbon atoms. As shown in Table I, the R_f values and retention times of this compound were identical with those of authentic α,α -trehalose. The specific rotation of this compound, $[\alpha]_D^{20} = +180^\circ$ (2% in water), was found to be identical with that of authentic α,α -trehalose. $^1\text{H-NMR}$ spectrometry was performed on this compound and the spectral data are shown in Fig. 1. The J values of H-1 and H-1' were found to be 3.6 and 3.7 Hz, respectively, while those of the other protons were 9.4~10.0 Hz. The low J values of H-1 and H-1' indicate that this compound has an α,α -linkage. From the COSY spectrum, the main protons were assigned as shown in Fig.

TABLE I. TLC AND HPLC OF THE ISOLATED COMPOUND AND THE HYDROLYZATE OF THE COMPOUND

Material	R_f value on TLC			Retention time (min)	
	A*	B*	C*	Hitachi 3013N ^a	Hitachi GL-C611 ^b
Glucose	0.38	0.41	0.09	80.0	15.1
Galactose	0.30	0.40	0.07	25.0	16.0
Mannose	0.41	0.47	0.15	13.0	17.4
Hydrolyzate of the isolated compound	0.38	0.40	0.10	80.0	15.1
α,α -Trehalose	0.25	0.35	0.01	—	12.0
Isolated compound	0.25	0.36	0.01	—	12.1

* A, *n*-butanol-ethanol-water (6:2:2, v/v/v); B, *n*-butanol-acetone-water (4:5:1, v/v/v); C, *n*-propanol-ethylacetate-water (2:10:0.5, v/v/v).

^a Eluted with 0.167 M borate buffer (pH 7.5) at a flow rate of 1 ml per min at 65°C.

^b Eluted with deionized water at a flow rate of 1 ml per min at 60°C.

1. These spectral data further confirm that this compound is trehalose. From all the data mentioned above together, it was concluded that this compound is α,α -trehalose.

It is well known that trehalose is widely distributed in the plant and animal kingdoms, from lower to higher species, as a storage carbohydrate. Among microorganisms, this disaccharide has been reported in yeasts^{3,4)} and fungi,^{5,6)} as well as in bacteria.^{7~9)} The pathway for trehalose synthesis from glucose was elucidated in the case of *Saccharomyces cerevisiae*.⁴⁾ However, little is known about the accumulation of the disaccharide in the culture medium of bacteria. In this study, it was found that a glycosidase producing bacterium, the 50 b strain, accumulated the disaccharide in its culture medium when it was grown on a medium containing glycerol as a carbon source. However, the biosynthetic pathway for the disaccharide in this bacterium is unclear. The biosynthetic pathway and the role of the disaccharide in this bacterium are currently under investigation.

Acknowledgment. The authors are grateful to Dr. H. Tamura, Faculty of Agriculture, Kagawa University, for measurement of the mass spectrum.

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FIG. 1. COSY Spectrum of the Isolated Compound.

The spectrum was taken in D₂O with a JEOL-GX400 spectrometer at 400 MHz with tetramethylsilane as an internal standard at room temperature.

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