Phytochemistry, 1974, Vol. 13, pp. 1159–1160. Pergamon Press. Printed in England.

STARCH-TYPE POLYSACCHARIDE AND MANNITOL IN *PLATYMONAS*

HIROSHI SUZUKI

Department of Botany, Faculty of Science, Tokyo Kyoiku University, Otsuka, Tokyo, Japan 112

(*Revised received* 3 *November* 1973)

Key Word Index—*Platymonas* sp.; Prasinophyceae; starch; mannitol.

Abstract—D-Mannitol and a starch-type glucan were isolated from a *Platymonas* species.

CRAIGIE *et al.*[1] have reported the occurrence of D-mannitol and starch as the major photosynthates in *Platymonas* sp. of the Prasinophyceae, a family separated by Manton and Parke[2] from the Chlorophyceae chiefly on morphological grounds. "Prasinophycean starch" has only been presumed to be present on the basis of microscopic observations,[3] so that this work was undertaken to elucidate the nature of the major carbohydrates in a *Platymonas* sp.

Carbohydrate constituents of the EtOH-soluble fraction. Mannitol which was found as a result of survey by PC to be the main component of this fraction was isolated in crystalline form with m.p. and m.m.p. of 165–166° (*ca* 2 mg from 4/5 of the total concentrate). It was further identified by PC in three solvents and by GLC after acetylation. In addition to mannitol, three weak spots of R_{glc} values of 0·35, 0·24 and 0·16, respectively, were detected on PC (by descent in *n*-BuOH-pyridine-water, 6:4:3); the first compound seemed to be a cyclitol, and the latter two were oligosaccharides based on glucose and/or galactose.

Polysaccharides in EtOH–Me_2CO-extracted cells. The carbohydrate composition of the insoluble residue after EtOH and Me_2CO extraction was examined and, since almost all of the water-soluble glycans in the *Platymonas* cells were susceptible to the action of glucoamylase and only glucose was detectable in the enzymic digest, a major fraction seemed to be of the starch-type.

EtOH–Me_2CO-extracted cells (3 g) were boiled in water, sonified and extracted with hot water (× 7). Combined extracts (1 l.) were concentrated and deproteinized with TCA. The starch was precipitated with EtOH, washed and dried (*ca* 0·8 g; anhydroglucose content, 78·2%). A purified starch sample (260 mg; anhydroglucose content, 95·0%) was obtained from the crude preparation (0·75 g) by repeated precipitation as its iodine complex.[4] Fractionation of amylose and amylopectin from the purified starch (202 mg) was carried out by complexing the amylose with thymol and BuOH;[4] yields, amylose, 37 mg, amylopectin,

[1] CRAIGIE, J. S., MACLACHLAN, J., MAJAK, W., ACKMAN, R. G. and TOCHER, C. S. (1966) *Can. J. Botany* **44**, 1247.
[2] MANTON, I. and PARKE, M. (1965) *J. Mar. Biol. Ass. U.K.* **45**, 743.
[3] BONEY, A. D. (1970) *Oceanogr. Mar. Biol. Ann. Rev.* **8**, 251.
[4] LOVE, J., MACKIE, W., MCKINNELL, J. W. and PERCIVAL, E. (1963) *J. Chem. Soc.* 4177.

124 mg. The properties of these fractions were compared with those of commercial preparations of potato amylose and corn amylopectin and the results indicated that the *Platymonas* amylose and amylopectin resemble the corresponding components from starches of Chlorophyceae[4] as well as of higher plants.

Plant material. Platymonas sp. isolated by Chihara[5] was used as inocula. Cells (*ca* 19 g wet wt) harvested from a culture in a modified ESP medium of Tatewaki[6] (T. Ikawa, unpublished) (8 l. in 4 flasks) for 10 days under continuous fluorescent light (4000 lx) and vigorous aeration at 17° were extracted with boiling 80% EtOH, with Me_2CO and finally with Et_2O, and dried *in vacuo* (EtOH–Me_2CO-extracted cells, 3·15 g). Combined EtOH-extracts (*ca* 1·5 l.) were concentrated, extracted repeatedly with Et_2O to remove pigments and further concentrated (EtOH-soluble fraction, 5 ml).

Carbohydrate estimation. Reducing sugar was assayed by the method of Somogyi–Nelson[7] and total carbohydrate by the phenol-H_2SO_4 method,[8] using glucose as the standard. PC and GLC of sugar was carried out by standard procedures.

Periodate oxidation. IO_4^- consumption was followed by the spectrophotometric determination.[9]

β-Amylolysis. The β-amylolysis limit, percentage conversion into maltose, was measured by incubation of the starch fraction (900 μg) with crystalline sweet-potato β-amylase (E.C.3.2.1.2) (0·5 mg) in 5 ml of 0·06 M acetate buffer, pH 5·5, at 30°.

Acknowledgements—The author is indebted to Dr. M. Chihara for supplying the algal strain and to Dr. T. Ikawa for conducting the culture. This work was supported by a grant from the Ministry of Education, Japan.

[5] CHIHARA, M. and HORI, T. (1971) *Proc. 7th Internat. Seaweed Symp., Sapporo*, p. 188.
[6] TATEWAKI, M. (1969) *Sci. Rep. Inst. Algol. Res., Hokkaido Univ.* **6**, 4.
[7] NELSON, N. (1944) *J. Biol. Chem.* **153**, 375.
[8] DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. and SMITH, F. (1952) *Anal. Chem.* **28**, 350.
[9] ASPINALL, G. O. and FERRIER, R. J. (1957) *Chem. & Ind.* 1216.