

GROWTH AND SUCROSE PHOSPHORYLASE ACTIVITY IN *PSEUDOMONAS SACCHAROPHILA*.

K. BALASUBRAMANIAM*

Department of Biochemistry, Faculty of Medicine, University of Jaffna, Kokuvil.

AND

SUMEDHA WIJERATNA

Department of Obstetrics and Gynecology, Faculty of Medicine, University of Colombo, Colombo 08.

(Date of receipt : 01 December 1992)

(Date of acceptance : 04 June 1993)

Abstract : *Pseudomonas saccharophila* was grown on sucrose phosphate medium. The age of the inoculum when varied from 10h to 70h, the time taken to reach late log phase decreased to a minimal value and again increased. Late log phase was reached at 20h when the inoculum was 30 h old. The effects of incorporating Tween 20 and Tween 80 into the sucrose phosphate medium on growth of *P. saccharophila* and the activity of sucrose phosphorylase were examined. Tween 80 had no effect while Tween 20 reduced the time taken to reach late log phase from 20 h to 10 h and also increased the specific activity of sucrose phosphorylase. Although sucrose, the carbon source for *P. saccharophila*, increased the activity of sucrose phosphorylase, the presence of Tween 20 further increased sucrose phosphorylase activity. *P. saccharophila* failed to grow in Tween 80 indicating that it does not form a carbon source for *P. saccharophila*

Key words: *Pseudomonas saccharophila*, sucrose phosphorylase, surfactant, Tween 20, Tween 80.

INTRODUCTION

Sucrose phosphorylase (E.C. 2.4.1.7) has been isolated from *Pseudomonas saccharophila*.¹ The enzyme catalyzes the transfer of the glucosyl moiety of sucrose to inorganic phosphate forming glucose-1-phosphate. The equilibrium constant for the reaction is 0.95² and hence the reverse reaction has also been used for synthesis of sucrose.³⁻⁶

We are interested in using sucrose phosphorylase for sucrose synthesis, by altering its kinetic parameters. Such studies require large quantities of enzyme and hence an attempt was made to increase the activity and the amount of sucrose phosphorylase using surfactants. Many bacterial enzymes have been induced by using surfactants. Cellulase, an inducible enzyme, has been induced in *Trichoderma viridae* with Tween 80 and sodium salts of fatty acids.⁷ The effects of incorporating the surfactants polyoxyethylene (20) sorbitan monooleate (Tween 20) and polyoxyethylene (20) sorbitan monolaurate (Tween 80) in the sucrose phosphate growth medium on the activity of sucrose phosphorylase from *P. saccharophila* are presented here.

* Corresponding author.

METHODS AND MATERIALS

Microbial culture and growth conditions : The bacterium *Pseudomonas saccharophila* (NCIB 8570) was obtained from the National Collection of Industrial Bacteria, Aberdeen, Scotland.

The culture medium contained 0.033 M KH_2PO_4 - Na_2HPO_4 buffer (pH 6.5), 0.1% NH_4Cl , 0.05% MgSO_4 , 0.005% ferric ammonium citrate, 0.001% CaCl_2 , 0.5% sucrose and distilled water.⁸ This medium was autoclaved at 15 p.s.i for 10 min. Tween 20 (0.2% v/v) and Tween 80 (0.2% v/v) were incorporated into the liquid culture media by mixing in a Waring blender for 10 min before autoclaving. Bacteria were grown at room temperature at pH 6.5. The inoculum always constituted 10% (v/v) of the culture in its mid log phase. Bacterial growth was monitored by measuring turbidity in a Klett-Summerson colorimeter.

Cell free extract : Bacteria were harvested during the late log phase of growth by centrifugation at 13,000 g at 4°C. Crude extract was prepared by disrupting the cells for 15 sec at 4°C in M.S.E. ultrasonicator set at an amplitude of 15. The sonication was repeated six times and the extract was centrifuged at 17,000g for 20 min. The supernatant was used in sucrose phosphorylase assays.⁹

Sucrose phosphorylase assay : The activity of sucrose phosphorylase was measured by the rate of reduction of NADP in a coupled enzyme system consisting of sucrose phosphorylase, phosphoglucomutase and glucose - 6 - phosphate dehydrogenase.¹⁰ When studying the *in vitro* effect of Tween 20 on sucrose phosphorylase, the control had glucose-1-phosphate instead of sucrose and sucrose phosphorylase. The unit of sucrose phosphorylase activity is defined as the amount of enzyme which catalyzes the formation of 1 μm of glucose-1-phosphate (measured as NADPH) per min per ml at 30°C.

Protein estimation : The amount of protein in the enzyme extracts was estimated by the method of Lowry *et. al.*¹¹ using bovine serum albumin as a standard (Fig. 1). The standard curves for protein were determined in the presence and in the absence of 0.2% (v/v) Tween 80.

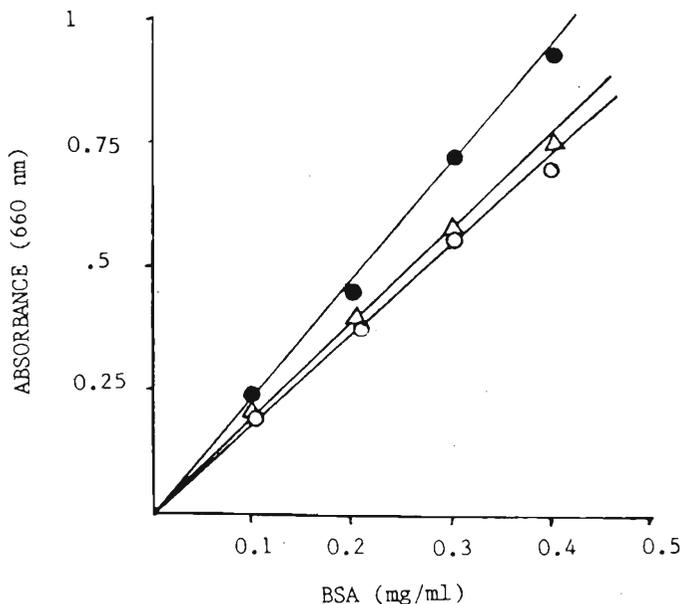


Figure 1: Estimation of protein by Lowry's method using bovine serum albumin as standard (•) in water; (Δ) in 0.2% (v/v) Tween 20; and (o) in 0.2% (v/v) Tween 80.

RESULTS AND DISCUSSION

The growth of *P. saccharophila* was influenced, consistent with other reports for bacterial growth, by the age of the inoculum¹² and by surfactants.¹⁹ Figure 2 shows that, when the inoculum was obtained from early log phase or stationary phase cultures, the time required to reach late log phase was proportionally increased. When 30h inoculum was used, the *P. saccharophila* reached late log phase in 20 h. A 30 h inoculum was used in the subsequent experiments. Cells in the stationary phase undergo considerable changes, significantly a decline in the number of ribosomes which have to be restored before protein synthesis can resume at a high rate¹² in the exponential phase.

The surfactants Tween 20 and Tween 80 appeared to have different effects on growth of *P. saccharophila*. Tween 20 reduced the time required to reach late log phase from 20h to 10h, while Tween 80 increased this time from 20h to 40h (Fig. 3). These results suggest that Tween 20 has a stimulatory effect on the growth of *P. saccharophila* while Tween 80 has an inhibitory effect. Different effects of surfactants have been observed in *E. coli*.¹³ When *E. coli* was grown with the surfactant sodium dodecyl benzene sulphonate (NaDBS) containing nutrient broth, the growth was shown to be partially inhibited by the surfactant without a concomitant drop in the viable cell count.

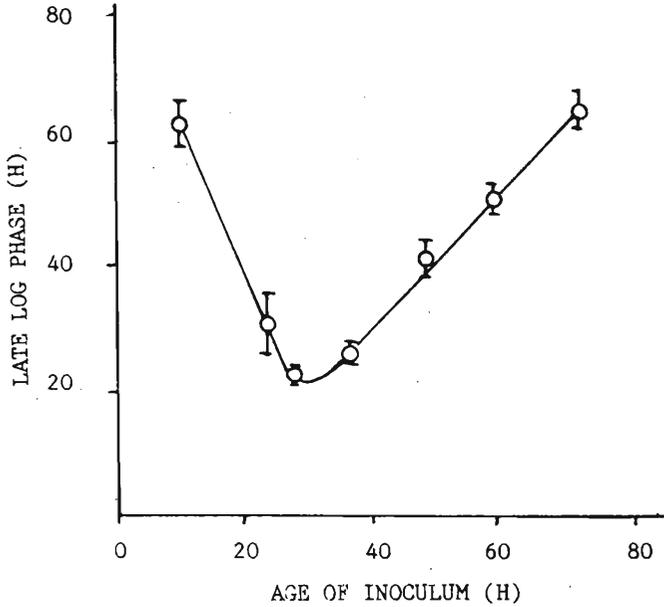


Figure 2: Effect of the age of inoculum on the growth of *Pseudomonas saccharophila*. Values given are the mean of two determinations.

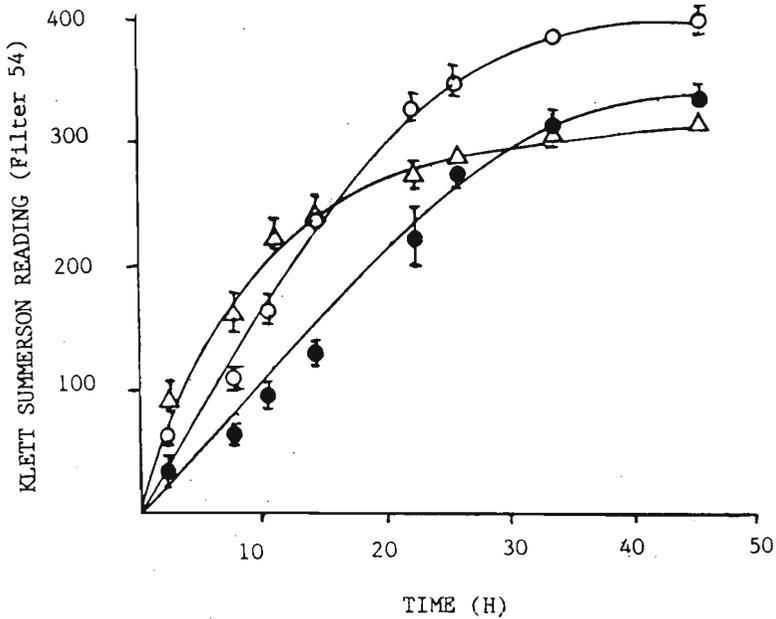


Figure 3: Growth curve of *Pseudomonas saccharophila*. (O) sucrose phosphate medium, (Δ) sucrose phosphate medium containing 0.2% (v/v) Tween 20 and (●) sucrose phosphate medium containing 0.2% (v/v) Tween 80. Values given are the mean of two determinations.

This effect was attributed to a change in osmotic function of bacterial cells which caused the leakage of intracellular components in to the medium.

Sucrose phosphorylase activity: Reese⁷ had studied the induction of cellulase by different surfactants in two strains of *Trichoderma viridae*. The surfactants used in this study were Tween 80, sodium oleate, sodium monopalmitate and sucrose monopalmitate. Unlike our results where sucrose phosphorylase activity was increased by Tween 20 and decreased by Tween 80 (Table 1), all four surfactants increased the synthesis of cellulase to varying degrees. Sodium oleate (0.1%) had the greatest effect on *Trichoderma viridae* strain 9132 and the increase in specific activity was 94 fold. In our studies Tween 20 increased the amount of protein, specific activity and total activity of sucrose phosphorylase by 50% , 57% and 128% respectively (Table 1), whereas *in vitro* it inhibited the activity of sucrose phosphorylase (Fig. 4). These results indicate that the observed increase in specific activity of sucrose phosphorylase in *P. saccharophila* *in vivo* may be due to a net synthesis of sucrose phosphorylase and not due to activation of the enzyme.

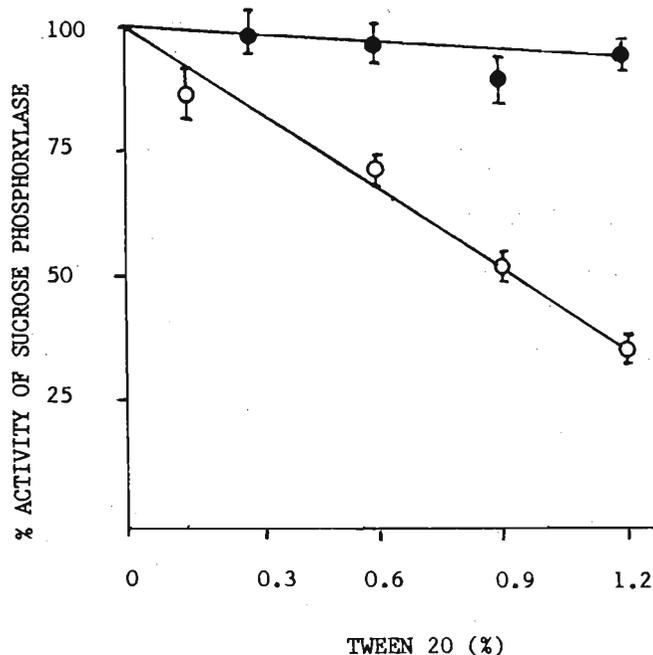


Figure 4: The effect on Tween 20 on sucrose phosphorylase *in vitro*. (●) Control had phosphate buffer containing Tween 20, glucose-1-phosphate, phosphoglucomutase, G-6-P-dehydrogenase and NADP (○) Test had buffer containing Tween 20, sucrose, sucrose phosphorylase, phosphoglucomutase, G-6-P-dehydrogenase and NADP. Values given are the mean of two determinations.

Table 1: The effect of Tween 20 and Tween 80 on sucrose phosphorylase of *P. saccharophila*.

Culture condition	Protein		Specific activity		Total activity	
	mg ml ⁻¹	% change	V(mg pr) ⁻¹	% change	V	% change
Normal	1.21 ± 0.02 (1.0-1.4)	0	1.01 ± 0.24 (0.77-1.35)	0	218 ± 136 (40.5-372)	0
0.2%(v/v) Tween 20	1.83 ± 0.02 (1.5-1.85)	+50	1.58 ± 0.34 (1.17-1.95)	+57	498 ± 275 (105-738)	+128
0.2%(v/v) Tween 80	0.9 ± 0.01 (0.8-1.0)	-25	0.83 ± 0.15 (0.72-0.94)	-25	18.66 ± 0.09 (18.60-18.73)	-95

Sucrose concentration was 0.5% w/v. Values are the mean ± standard deviation of four experiments. The range is given in brackets.

The specific activity of sucrose phosphorylase in *P. saccharophila* increased with increasing sucrose concentration in the medium (Table 2). When there was no sucrose in the medium, there was little or no bacterial growth and there was no detectable activity of the enzyme. The amount of protein and sucrose phosphorylase activity increased with increasing concentrations of sucrose (Table 2). But in the presence of Tween 20, the specific activity of sucrose phosphorylase was more than double that observed in the absence of Tween 20. These results show that sucrose and Tween 20 have a synergistic effect on the synthesis of sucrose phosphorylase by *P. saccharophila*.

Table 2: Effect of sucrose on protein concentration and *P. saccharophila* phosphorylase activity in the presence of Tween 20.

	Protein (mg ml ⁻¹)	Specific activity (U mg protein ⁻¹)	Increase (%)
Sucrose 0.1%	0.80	0.19	0
Sucrose 0.1% + 0.2% v/v Tween 20	0.90	0.24	30
Sucrose 0.2%	0.85	0.23	21
Sucrose 0.2% + 0.2% v/v Tween 20	1.00	0.32	69
Sucrose 1%	1.00	0.27	44
Sucrose 1% + 0.2% v/v Tween 20	1.22	0.40	114

Values are the mean of two determinations.

Acknowledgement

We thank the Natural Resources, Energy and Science Authority for a research grant and H. R. Weerasena and M. S. de Silva for technical assistance.

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