Selection of a Mutant of *Bacillus subtilis* Deficient in Glucose-6-phosphate Dehydrogenase and Phosphoglucoisomerase

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INTRODUCTION

The addition of glucose or other rapidly metabolized carbon compounds into a nutrient sporulation medium suppresses sporulation of *Bacillus subtilis* cells (Freese, Oh, Freese, Diesterhaft & Prasad, 1972). The first step in glucose metabolism in *B. subtilis* (strain 60015) involves phosphorylation to glucose-6-phosphate (G6P), which may either be isomerized to fructose-6-phosphate by phosphoglucoisomerase (PGI) or oxidized to gluconate-6-phosphate by G6P-dehydrogenase (G6PD) (Prasad, Diesterhaft & Freese, 1972).

A mutant lacking only PGI activity (strain 61158) grows slowly on glucose (Freese *et al.* 1972), utilizing the hexose monophosphate shunt; a derivative of this strain that lacked also an enzyme of the shunt ought not to grow on glucose at all. We have isolated such a mutant. All media, such as the minimal salt medium N, tryptose blood agar base (TBAB), and the phosphate-magnesium buffer PM, were as described by Freese *et al.* (1972).

RESULTS AND DISCUSSION

Strain 61158 was grown in N medium containing 0.5% glucose and 0.01% casein hydrolysate to $A_{600}=0.7$ to 0.8. Cells were collected by centrifugation (10000g for 10 min at 4°C) and washed with PM buffer. The washed cells were treated for 90 min at 37°C with 5 mM-ethylmethane-sulphonate. Treated cells were washed twice with PM buffer, resuspended in N medium containing 0.5% gluconate and 0.01% casein hydrolysate, and then shaken on a waterbath shaker (120 strokes/min, 37°C) for 2 h. The cells were diluted in PM and plated on TBAB plates. After overnight incubation at 37°C, the colonies were replicated on N+0.5% glucose agar plates. Isolates unable to grow on glucose were assayed for G6PD, PGI, and gluconate-6-phosphate dehydrogenase (GCN-6-PD) activities, as described earlier (Freese *et al.* 1972; Prasad *et al.* 1972). One isolate (strain 61324) showed a low activity for G6PD (< 20% that of the parent, strain 61158). This mutant grew on plates containing N+0.5% glycerol, malate, citrate, or gluconate but not on glucose or fructose (Table 1). The lack of growth of strain 61324 on fructose was inherited from its parent, strain 61158, and it was unrelated to the G6PD or PGI mutations (C. Prasad and E. Freese, unpublished).

Since G6PD deficiency in strain 61324 appeared to be leaky, the properties of normal and mutant enzymes were examined in crude extract preparations. At 5 mM-G6P and using 50 mM-N-tris(hydroxymethyl)methyl-2-aminoethane-sulphonic acid (TES), the G6PD activity was similar at all pH values for both enzymes. A significant difference in the K_m

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Table 1. Growth, enzymic activities and radiorespirometric pattern of glucose metabolism by different strains of *Bacillus subtilis*

Strain	Enzyme deficiency	Enzyme activity*				Growth on N agar + 0.5% carbon source†					Radiorespirometric pattern of glucose oxidation‡		
		PGI	G6PD	GCN-6-PD	Growth on N agar + 0.5% carbon source†					[¹⁴ C]-glucose labelled at:	Total [¹⁴ C]glucose added (c.p.m.)	¹⁴ CO ₂ formed after 60 min (c.p.m.)	CO ₂ (%)
					Glucose	Fructose	Glycerol	Malate	Gluconate				
60015	None	600	120	80	+	+	+	+	+	C ₁ C ₆	57362 92696	6421 66973	11.2 72.3
61158	PGI	5	120	65	(+)	—	+	+	+	C ₁ C ₆	57362 92696	6326 46	11.0 0.05
61324	PGI and G6PD	8	20	75	—	—	+	+	+	C ₁ C ₆	57362 92696	345 48	0.60 0.05

* Enzyme activities were expressed as nmol NADPH formed/min/mg protein.

† +, Growth comparable to strain 60015; (+), poor growth; —, no growth.

‡ Cells were grown in NSMP (Freese *et al.* 1972) to $A_{600} = 1.0$ and harvested by centrifugation at 4 °C; the cells were washed twice with PM buffer and suspended in NSMP + 0.05% glucose (5 A_{600} units/3 ml). Radiorespirometric measurement of CO₂ was made in triplicate as described by Bulla, Julian, Rhodes & Hesselstine (1970).

(G6P) values for the two enzymes (in 50 mM-K-TES, pH 8.0) was observed. The K_m (G6P) for the mutant enzyme (35 mM) was 175 times higher than that of normal enzyme (0.2 mM).

Table 1 also shows the radiorespirometric pattern of metabolism of [^{14}C]glucose labelled at C_1 and C_6 by strains 60015, 61158 and 61324. Since C_1 and C_6 of glucose are metabolised through the hexose monophosphate shunt and the Embden–Meyerhof pathway, respectively (Mahler & Cordes, 1966), the levels of CO_2 production from C_1 and C_6 in strain 60015 demonstrated that the Embden–Meyerhof pathway is the major route of glucose metabolism in *B. subtilis*. In strain 61324, like strain 61158, the Embden–Meyerhof pathway is non-functional due to the PGI mutation, whereas the hexose monophosphate shunt is operating at a very low efficiency due to the leakiness of G6PD.

G6PD and PGI are widely occurring enzymes. Several possible roles in metabolism have been suggested (Mahler & Cordes, 1966). These include glucose oxidation by the Embden–Meyerhof pathway and the hexose monophosphate shunt, the formation of pentose, and the reduction of nicotinamide adenine dinucleotide phosphate. Mutations leading to G6PD deficiency have also been implicated with haemolytic anaemia in man (Beutler, 1966) and morphological variations in *Neurospora crassa* (Lechner, Fuscaldo & Bazinet, 1971). Comparison of the characteristics of normal cells and mutants lacking these enzymes in *B. subtilis* may clarify the situation.

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