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Enzymes of The Intermediary Carbohydrate Metabolism of
Polyangium cellulosum

By

Renu Sarao.

A Thesis
submitted to the Faculty of Graduate Studies
through the Department of
Biology in Partial Fulfillment of
the requirements for the Degree
of Master of Science at
The University of Windsor.

Windsor, Ontario, Canada
1985

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ABSTRACT

Crude extracts of vegetative cells of the cellulolytic myxobacter *Polyangium cellulosum* contained significant levels of the enzymes of the tricarboxylic acid cycle and the glyoxylate cycle. Key enzymes of glycolysis and the pentose phosphate shunt were also detected. Specific activities of hexokinase and fructose–1,6–diphosphate aldolase exhibited a tenfold increase when the cells were grown in complex medium containing glucose.

Cytochromes of a, b and c type were demonstrated.

By the use of a dispersly growing strain of *P. cellulosum*, its generation time was determined to be 22–24 hours on SP.

This study suggests that the organism probably uses glycolysis and citric acid cycle for complete oxidation of glucose. The exact role of the glyoxylate cycle and pentose phosphate shunt cannot be deduced from this study.

This is the first report on the study of intermediary carbohydrate metabolism in any member of the family Polyangiaceae.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

ADP – adenosine 5'–diphosphate
APAD – 3–acetyl pyridine adenine dinucleotide
ATP – adenosine 5'–triphosphate
BSA – bovine serum albumin
CoA – coenzyme A
CoA–SH – reduced coenzyme A
DCIP – 2,6–dichlorophenolindophenol
NAD – nicotinamide adenine dinucleotide
NADH – reduced nicotinamide adenine dinucleotide
NADP – nicotinamide adenine dinucleotide phosphate
TCA – Tricarboxylic acid
Tris–HCL – Tris (hydroxymethyl) amino methane hydrochloride
TPP – thiamine pyrophosphate

INTRODUCTION

Polyangium cellulosum is a cellulolytic bacterium belonging to the family Polyangiaceae (order Myxobacterales) (McCurdy, 1974). The vegetative cells are cylindrical rods with blunt rounded ends. As in other myxobacteria, depletion of nutrients leads to aggregation of vegetative cells which then convert to myxospores. During this process of morphogenesis, the slender rods shorten and fatten and become physiologically dormant myxospores. The myxospores resemble the vegetative cells, are enclosed in sessile sporangia, and are neither refractile nor phase dense (McCurdy, 1974). The swarming colonies etch or erode the agar and may be pigmented yellow, red or orange. Dispersed growth can be achieved in liquid medium by frequent transferring (Reichenbach and Dworkin, 1980).

An understanding of the nutritional requirements of an organism provide insight into its ecological role as well as metabolism. There also appears to be a relationship between the nutritional milieu of a microbe and its morphological development (Dworkin, 1962). The fruiting body formation by *Mysococcus* (Oetker, 1953) and *Chondromyces* (Kuhlwein, 1950) has been shown to depend upon the nature of the medium.

Myxobacteria can be divided into two physiological groups: the bacteriolytic group and the cellulolytic group. Bacteriolytic myxobacteria feed cooperatively on other bacteria or yeast by secreting into the common milieu, hydrolytic enzymes that degrade other cells and their macromolecules, and then share the pooled products of digestion (Dworkin, 1972). The minimal nutritional requirements of the bacteriolytic myxobacters are still poorly

understood. When grown on a chemically defined medium, the few strains studied so far require complex amino acid mixtures and relatively high concentrations of Mg^{2+} or Ca^{2+} for growth (Dworkin, 1962; Hemphill and Zahler, 1968; McCurdy and Khouw, 1969). Growth is much more efficient on complex media containing enzyme hydrolyzed casein (casitone). Loebeck and Klein (1956) and Dworkin (1962) suggested that peptides might be more efficiently utilized than amino acids.

Unlike these, the cellulolytic myxobacteria examined so far have simple nutritional requirements. They can grow in a defined medium containing an inorganic base supplemented with a single carbohydrate (Krzemieniewska and Krzemieniewski, 1937; Coucke, 1969). Cellulose will function as the sole carbon and energy source for *Polyangium cellulosum.* It metabolizes insoluble crystalline cellulose as well as soluble cellulose by producing both cell bound and extracellular cellulase and cellobiase activities (Godsey, 1976). In the laboratory the organism can use various carbohydrates as carbon sources. Growth takes place on xylose, maltose, dextrin, glucose, starch, cellobiose, arabinose and fructose (Krzemieniewska and Krzemieniewski, 1937). Potassium nitrate can be used as the sole source of nitrogen (Coucke, 1969). It can also use ammonia, urea, protein hydrolyzate and a few amino acids as nitrogen sources. Fruiting body formation occurs on media in which the carbohydrates are almost exhausted (Coucke, 1969). The best growth is obtained on Staniers medium containing 0.25% cellobiose (Peterson, 1969). Various concentrations of simpler carbon sources such as glucose and xylose, used in lieu of cellobiose, yield markedly inferior growth. Some isolates are glucose sensitive, and glucose is clearly toxic in concentrations higher than that used for cellobiose (Peterson, 1969).

Detailed knowledge of the nutrition and metabolism of myxobacteria is restricted to *Mysococcus xanthus*. The conclusions derived from these studies probably apply, at least in part, to other bacteriolytic species, but the difference in nutrition of *P. cellulosum* suggests that the same may not apply to this cellulose decomposing organism. The pathway of glucose metabolism in this organism is unknown (Parish, 1979).

Studies on the intermediary metabolism of *M. xanthus* have indicated the absence of hexokinase and pyruvate kinase in both vegetative cells and microcysts. All other glycolytic activities are present (Watson and Dworkin, 1968). The inability of this organism to metabolize sugars, and its nutritional dependence on amino acids as the source of carbon and energy suggest that the principal function of the Embden–Meyerhof enzymes is in gluconeogenesis. This is corroborated by the absence of pyruvate kinase as it catalyzes an essentially irreversible glycolytic reaction and has no role in gluconeogenesis. Amino acids are sources of carbon and energy for *M. xanthus*, as are acetate, glycerol, pyruvate and many TCA cycle intermediates. But no hexose, pentose or polysaccharide has clearly been shown to serve as a carbon or energy source (Bretscher and Kaiser, 1977). All the enzymes of the tricarboxylic acid cycle have been shown to be present, and the two enzymes of the pentose phosphate pathway, 6–phosphogluconate dehydrogenase and glucose 6–phosphate dehydrogenase, were also detected in *M. xanthus* (Watson and Dworkin, 1968).

The levels of the TCA cycle enzymes in the extracts of *M. xanthus* vegetative cells and microcysts were seen to be the same with the exception of isocitrate dehydrogenase. This enzyme is present in microcysts at a level five times that of vegetative cells (Watson and Dworkin, 1968). There is also a

striking difference in the ability of vegetative cells and microcysts to metabolize acetate. The microcysts metabolize acetate at about 40% of the rate of vegetative cells. The presence of the glyoxylate cycle could account for these data if carbon flow were shifted away from oxidative metabolism of the TCA cycle into the glyoxylate cycle (Watson and Dworkin, 1968).

The glyoxylate cycle enzymes are thought to account for the net synthesis of four-carbon dicarboxylic acids from acetate in a wide variety of microorganisms and plants (Calvin and Beevers, 1961 and Kornberg and Elsden, 1961). It is not clear why the glyoxylate cycle should be necessary in *M. santhus* during microcyst formation, since the process is induced in the presence of casitone, which presumably provides precursors for four-carbon dicarboxylic acids. It is possible that the induction of the glyoxylate cycle reflects a shift to endogenous metabolism, perhaps of lipid (Orlowski *et al.*, 1972).

Hanson (1968) found that the activity of isocitrate lyase rose during the early stages of microcyst formation. Bland *et al.* (1971) showed that the vegetative cells contained low levels of both isocitrate lyase and malate synthase. These enzyme activities exhibit a rapid increase a few minutes before morphological changes begin to occur. Orlowski *et al.* (1972) concluded that the activities of the glyoxylate cycle enzymes change in a characteristic manner during myxospore formation and that the changes in activity are related to the development of the myxospore.

Dworkin and Neiderpreum (1964) have shown that *M. santhus* vegetative cells and microcysts have a functional electron transport system similar to that found in most aerobic bacteria. They have also identified the presence of a, b and c type cytochromes.

McCurdy and Khouw (1969) carried out similar studies on another myxobacterium, *Stigmatella brunnea*, which belongs to the family Cystobacteraceae. *S. brunnea* and myxococci are alike nutritionally, as they both require a large number of amino acids as sources of carbon and energy. Their growth is stimulated by the presence of polysaccharides in liquid medium even though neither uses carbohydrates or their hydrolytic products.

Although α-ketoglutarate dehydrogenase activity could not be demonstrated, the results of enzymatic and respiration studies indicate the presence of an operational TCA cycle with a complete terminal electron transport pathway. Cytochromes of a, b and c type were also detected spectrophotometrically (McCurdy and Khouw, 1969). It has been suggested that the absence of hexokinase in *M. xanthus* might explain why it fails to utilize carbohydrates (Dworkin, 1966). The same reasoning, however, does not hold for *S. brunnea.* Both hexokinase and aldolase activities have been demonstrated in this organism indicating the presence of the glycolytic pathway. No explanation has been offered for the failure of *S. brunnea* to use carbohydrates. Enzymes of the glyoxylate cycle, namely isocitrate lyase and malate synthase, were not detected in the vegetative cells of *S. brunnea* (McCurdy and Khouw, 1969).

Considerable data have been accumulated regarding the nutrition, metabolism and morphogenesis of bacteriolytic myxobacteria but very little is known concerning the enzymes and nutrition of the cellulolytic organism *P. cellulosum*. This work is a preliminary study of the intermediary metabolism of *P. cellulosum.*

MATERIALS AND METHODS

Organism and Growth Conditions

Polyangium cellulosum strain M209, obtained from the University of Windsor culture collection, was used for all experiments.

Working cultures were grown for 36–40 hours at 30°C with shaking, in 250ml screw cap flasks containing 70 ml of SP broth (Raffinose 0.1%, sucrose 0.1%, galactose 0.1%, soluble starch 0.5%, casitone 0.25%, $MgSO_4.7H_2O$ 0.05%, and K_2HPO_4 0.025%. McCurdy, 1963) or Staniers medium (KNO_3 0.1%, K_2HPO_4 0.1%, $MgSO_4.7H_2O$ 0.02%, $CaCl_2$ 0.01% and $FeCl_3$ 0.002%. Stanier, 1942) plus 1% cellobiose. The cells were also grown on CkI (KNO_3 0.2%, $MgSO_4.7H_2O$ 0.3%, $CaCl_2$ 0.14%, Fe^{3+} citrate 20 mg. per litre, glucose 1%, autoclaved separately and K_2HPO_4 0.025%, autoclaved separately. Kleinig *et al*, 1971) and Ck6 ($MgSO_4.7H_2O$ 0.15%, Fe^{3+} citrate 0.002%, autoclaved separately: KNO_3 0.2% + K_2HPO_4 0.025%; and: glucose 0.5% + $CaCl_2$ 0.15%. Coucke and Voets, 1967) media. Dispersed cultures were initially established with repeated transfer of cell suspensions after omnimixing (McCurdy, 1963). These were used for nutritional and enzymological studies as well as growth rate determination, as growth could be measured turbidometerically.

Chemicals

All biochemicals were of reagent grade, unless otherwise specified.

Nicotinamide adenine dinucleotide (NAD), reduced NAD (NADH), nicotinamide adenine dinucleotidephosphate (NADP), 3–acetyl pyridine adenine dinucleotide (APAD), coenzyme A (CoA), reduced CoA (CoA–SH), S–acetyl CoA, 2,6–

dichlorophenolindophenol (DCIP), adenosine triphosphate (ATP), thiamine pyrophosphate (TPP), phenazine methosulfate, bovine serum albumin, phenyl hydrazine hydrochloride, cis–oxaloacetic acid,α–ketoglutaric acid, L–cysteine, DL–isocitric lactone, glutathione, 6–phosphogluconic acid, D–fructose 1,6–diphosphate, glycine, L–malate, sodium glyoxylate and D–glucose 6–phosphate were obtained from Sigma Chemical Co. (St. Louis, Mo. U.S.A.).

Sodium fumarate and sodium arsenate were obtained from BDH Chemicals Canada Ltd. (Toronto, Canada). Potassium cyanide was obtained from Fisher Scientific Co. (Don Mills, Ontario) and succinic acid was obtained from Mann Research Laboratory Inc. (New York, New York).

Preparation of Cell–free Extract

After growth for 36–40 hours, cells were harvested by centrifugation, washed twice with 0.05 M Tris–HCl buffer, pH 7.5, and suspended in 5 ml of the same buffer. The cells were then disrupted by ultrasonic treatment with a Bronwill Biosonik (Bronwill Scientific, Rochester, New York) set at full power for 2 minutes, in 20 second pulses. The cell extract was then centrifuged at 20,000 x g for 20 minutes to remove the cell debris. The supernatant was either used directly for enzyme assays or was first dialysed against 0.05 M Tris–HCl buffer, pH 7.5 for 4 hours. For assaying aconitase, succinic dehydrogenase, fumarase and malic dehydrogenase, the cell free extract was prepared in 0.05 M phosphate buffer, pH 7.4.

All operations were carried out at 0–4°C.

Enzyme Assays

All spectrophotometric assays were carried out at 25°C, using 3.0 ml quartz cuvettes (1 cm light path) in a Beckman Spectrophotometer, model DU, equipped with a Gilford automatic absorbance meter. In spectrophotometric

methods based upon oxidation or reduction of pyridine nucleotides, a molar extinction coefficient of 6.22×10^6 at 340 nm was used. One unit of activity is defined as the amount of enzyme mediating the conversion of 1 umole of substrate per minute at $25°C$ unless otherwise specified. Enzyme activities were recorded as specific activities. Final concentrations of protein in reaction mixtures were between 350-700 ug unless otherwise specified. Specific activities were calculated accordingly. The results represent an average of at least three measurements.

The Citrate condensing enzyme (citrate-oxaloacetate lyase, CoA acetylating, EC. 4.1.3.7) was assayed by the method of Srere and Kosicki (1961) by measuring the disappearance of acetyl-CoA at 233 nm. The reaction mixture contained Tris-HCl buffer, pH 8.0, 200 umoles; oxaloacetate, 1.2 umoles and acetyl CoA, 0.44 umoles in a total volume of 3.0 ml. The reaction was initiated by adding 0.1 ml of cell free extract.

Aconitase (citrate isocitrate hydrolyase, EC. 4.2.1.3) was assayed by following the increase in absorbance at 240 nm, of cis-aconitic acid and other α,β-unsaturated carboxylic acids (Anfinsen, 1955). The reaction mixture contained D,L-isocitrate, 30 umoles and phosphate buffer, pH 7.4, 150 umoles in a final volume of 3.0 ml. The reaction was started by adding 0.1 ml of the dialysed cell free extract.

Isocitrate dehydrogenase (L_s-isocitrate:NADP oxidoreductase EC. 1.1.1.42) was assayed spectrophotometerically by measuring the reduction of NADP at 340 nm as described by Daron et $al.$ (1966). The reaction mixture contained: Tris-HCl buffer, pH 7.5, 60 umoles; $MnCl_2$, 6 umoles; NADP, 1.5 umoles; D,L-isocitrate, 1.5 umoles and 0.15 ml of the cell free extract in a total volume of 3.0 ml.

α-ketoglutarate dehydrogenase (EC. 1.2.4.2) was assayed by the method of Amarasingham and Davis (1965), by measuring the reduction of 3-acetyl NAD (APAD) spectrophotometerically at 365 nm. The reaction mixture contained in 3.0 ml: Tris-HCl buffer, pH 8.5, 500 umoles; L-cysteine, 7.8 umoles; α-ketoglutarate , 25 umoles; CoA-SH, 0.26 umoles; APAD, 6 umoles; $MgCl_2$, 0.1 umoles TPP, 0.1 umoles and cell free extract 0.1 ml. All components were preincubated for a period of 3 minutes, then the reaction was initiated by adding APAD and the initial increase in absorbance was used to calculate the specific activity.

Succinic dehydrogenase (Succinate:oxidoreductase, EC. 1.3.99.1) was assayed spectrophotometrically as described by Ohne *et al.* (1973). The reaction mixture contained potassium phosphate buffer, pH 7.4, 30 umoles; KCN, 3 umoles; succinate, 30 umoles; BSA, 21 nmoles; DCIP, 5 umoles; phenazine methosulfate, 5 umoles. The succinate dependent reduction of dichlorophenolindophenol was followed at 600 nm. A molar extinction coefficient of 16.1×10^3 M^{-1} cm^{-1} for DPIP was used to calculate the specific activity.

Fumarase (L-malate hydro-lyase, EC. 4.2.1.2) was assayed by the method of Hill and Bradshaw (1969) in a reaction mixture containing phosphate buffer, pH 7.3, 150 umoles; L-malate, 150 umoles and 0.1 ml cell free extract in a total volume of 3.0 ml. The increase in absorbance was measured at 250 nm.

The presence of malate dehydrogenase (L-malate:NAD oxidoreductase, EC. 1.1.1.37) was detected by the method of Ochoa (1955) by measuring the rate of NADH oxidation at 340 nm in the presence of oxaloacetate. The reaction mixture contained phosphate buffer, pH 7.4, 75 umoles; oxaloacetate, 1.2 umoles; NADH, 0.3 umoles and 0.1 ml cell extract to be assayed in a total volume of 3.0 ml.

Fructose diphosphate aldolase (fructose–1,6–diphosphate D–glyceraldehyde–3–phosphte lyase, EC. 4.1.2.12) was assayed as described by Christian (1953). The reaction mixture contained NAD, 0.9 umoles; fructose–1,6–diphosphate, 50 umoles; glycine, 80 umoles, sodium arsenate, 52 umoles and 0.1 ml cell free extract in a total volume of 3.0 ml.

Hexokinase was detected by the reduction of NADP, using the method of McCurdy and Khouw (1969). The reaction mixture contained ATP, 17 umoles; NADP, 3.0 umoles; $MgCl_2$, 10 umoles; glucose, 40 umoles; tris–acetate buffer, pH 7.4, 150 umoles and 0.1 ml cell extract in a total volume of 3.0 ml. Controls containing the complete reaction mixture except NADP or ATP were also run.

Pyruvate kinase (EC. 2.4.1.40) was determined by the coupled assay of Bucher and Pfleiderer (1955) as modified by Watson and Dworkin (1968). The reaction mixture contained Tris (pH 7.5), 150 umoles; $MgCl_2$, 10 umoles; KCl, 100 umoles; ADP, 1 umole; phosphoenolpyruvate, 5 umoles; NADH 0.45 umoles; lactate dehydrogenase and cell–free extract (1–2 mg protein) in a final volume of 3.0 ml. The decrease in absorbance was measured at 340 nm.

Malate synthetase (L–malate glyoxalate lyase (CoA acetylating) EC. 4.1.3.2) was assayed by the method of Dixon and Kornberg (1959), by measuring the absorption at 232 nm due to breakage of the thio ester bond of acetyl coenzyme A in the presence of glyoxylate. The reaction mixture contained, in 3.0 ml, Tris buffer, pH 7.1, 108 umoles; $MgCl_2$, 12 umoles; acetyl CoA, 0.15 umoles; sodium glyoxylate, 6 umoles and 0.1 ml of cell free extract.

Isocitrate lyase (L$_s$–isocitrate glyoxylate–lyase EC. 4.1.3.1) was assayed by the method of Dixon and Kornberg (1959), by measuring the absorbance at 324 nm due to the formation of glyoxylic acid phenyl hydrazone from glyoxylate. The assay system contained, in 3.0 ml, Tris buffer, pH 7.5, 100 umoles, phenyl

hydrazine HCl, 20 umoles, cysteine HCl, 0.1 umoles; MgCl₂, 20 umoles and isocitrate, 2 umoles. An extinction coefficient of 1.7×10^4 for glyoxalate phenylhydrazone is used to calculate the specific activity.

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:NADP oxidoreductase EC. 1.1.1.49) activity was measured by the method of DeMoss (1955). The reaction mixture contained, in 3.0 ml, Tris buffer, pH 7.8, 150 umoles, NADP or NAD, 0.54 umoles; MgCl₂, 10 umoles; glucose-6-phosphate, 10 umoles and 0.1 ml cell extract.

6-phophogluconate dehydrogenase (6-phospho-D-gluconate: NADP oxidoreductase (decarboxylating) EC. 1.1.1.44) was also assayed by the method of DeMoss (1955). The reaction mixture contained glycylglycine buffer, pH 7.5, 600 umoles; NADP, 0.54 umoles; MgCl₂, 10 umoles; 6-phosphogluconate, 5 umoles and 0.1 ml of cell extract.

Determination of Doubling Time

Screw cap flasks with side arms, containing 100 ml of SP broth, were inoculated with 1.0 ml aliquots of a dispersly growing isolate of *Polyangium cellulosum*. Turbidometric readings were taken every 4 hours, using a Klett-Summerson colorimeter.

Cytochrome Absorption Maxima

Cytochrome spectra were measured at room temperature by the method of Weston and Knowles, 1973. The cells were disrupted by sonication. Oxidized minus reduced spectra were obtained by reducing one cuvette, containing cell suspension, with Na₂S₂O₄ and oxidizing the other cuvette by vigorous aeration.
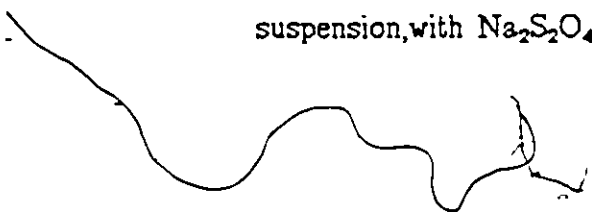
RESULTS

Nutrition and Vegetative Growth

Polyangium cellulosum grew quite well on both a complex medium (SP) and a chemically–defined medium (Staniers + cellobiose). This is in concurrence with earlier reports that the organism can grow on a simple salts medium containing a nitrogen source and a carbon source (Couke, 1969; Krzemieniewska and Krzemieniewski, 1937; Imschenetsky, 1959). The colonies had a bright orange pigment and seemed to etch the agar.

In liquid shake cultures, the organism grew non–dispersly either as clumps or adhering to the sides of the flask. Repeated transfers into fresh medium resulted in a dispersly growing strain. This strain was used for all subsequent studies.

Most efficient growth was observed in SP broth and Staniers medium + cellobiose. When cellobiose was substituted with glucose, it was seen that concentrations higher than 0.1% inhibited the growth of *P. cellulosum*. This was corroborated by poor growth of the organism on Ck1 and Ck6 media, both of which contain higher concentrations of glucose.

Vitamin B_{12} was not essential for growth. The organism grew equally well in media with and without the supplement.

The doubling time of *P. cellulosum* in SP was about 22 to 24 hours.

Enzymatic Activities.

Key enzymes of the glycolytic pathway, namely hexokinase and fructose–1,6–diphosphate aldolase, were found to be present in the cell–free extract of the vegetative cells (Table 1). The specific activities of these enzymes were seen

Table 1: Glycolytic activities in crude extracts of *P. cellulosum* vegetative cells.

ENZYME	SPECIFIC ACTIVITY* (umoles/mg. prtn./min.)		
	SP	SP₁ + Glucose	Staniers medium + cellobiose
Hexokinase	0.0006	0.0054	0.0016
Fructose–1,6–diphosphate aldolase	0.0026	0.0267	0.0033
Pyruvate kinase	0.0024	ND	ND

ND: not determined

* – One unit of enzyme activity is defined as that amount of enzyme which converts 1 umole of substrate per minute. Specific activity is defined as the number of enzyme units per mg. protein.

to increase almost tenfold when the cells were grown in SP + glucose as compared to SP alone or Staniers + cellobiose. Pyruvate kinase was also detected.

Glucose–6–phosphate dehydrogenase and 6–phosphogluconate dehydrogenase activities were present in the crude extracts of vegetative cells. The enzymes were active with both NAD and NADP, however, higher activities were obtained when NADP was used. Both enzymes showed higher specific activity when the cells were grown in Staniers medium + cellobiose, rather than in SP (Table 2).

As shown in table 3, the vegetative cells of *P. cellulosum* were seen to possess all the enzymes of the TCA cycle. The two enzymes of the glyoxylate cycle, isocitrate lyase and malate synthase, were also detected (Table 4).

Crude sonicated fractions of the vegetative cells exhibited absorption maxima at 601, 560, 525, 440 and 422 nm, as determined spectrophotometrically by difference spectra in the presence of $Na_2S_2O_4$ (Table 5).

All tables present typical results of at least three measurements.

Table 2 : Pentose phosphate activities in crude extracts of vegetative
cells of *P. cellulosum*

ENZYME	SPECIFIC ACTIVITY* (umoles/mg. protein/minute)	
	SP	Staniers medium + cellobiose
Glucose–6–phosphate dehydrogenase	0.177	0.471
6–phosphogluconate dehydrogenase	0.237	0.373

*– one unit of enzyme activity is defined as that amount of enzyme which
effects a rate of change of optical density $(\triangle E_{340})$ of 1.0 per minute
during the 15–30 second interval under specified assay conditions.
Specific activity is defined as the number of enzyme units per mg. protein
(DeMoss, 1955).

Table 3 : Specific activities of the TCA cycle enzymes in crude extracts of *P. cellulosum* vegetative cells.

ENZYME	SPECIFIC ACTIVITY[b] (Enzyme units[a]/mg protein)
Citrate synthase	9.46
Aconitase	0.171
Isocitrate dehydrogenase	0.0034
Succinic dehydrogenase	5.12
Fumarase	0.028
Malic dehydrogenase	0.0215
α–Ketoglutarate dehydrogenase	0.012

a : One unit of enzyme activity is defined as the amount of enzyme which converts 1 umole of substrate per minute.

b : Specific activity is defined as the number of enzyme units present per mg. protein.

Table 4 : Glyoxylate shunt in crude extracts of vegetative cells of
P. cellulosum

ENZYME	SPECIFIC ACTIVITY (Enzyme units/mg protein)
Isocitrate lyase	0.179[a]
Malate synthetase	0.206[b]

a : One unit is the amount of enzyme catalyzing the formation of 1.0 umole of glyoxylic acid phenylhydrazone in 1 minute.

b : One unit is the amount of enzyme catalyzing the glyoxylate dependent cleavage of 1.0 umole of acetyl–S–CoA in 1 minute.

Table 5 : Position of maxima in Absorption Spectra of sonicated *Polyangium cellulosum* cells.

Absorption maxima[*] -(nm)	Cytochrome
422	cyt c
440, 601	cyt a
525.	cyt b
560	cyt b_1

* : $Na_2S_2O_4$-reduced minus oxidised difference spectrum.

DISCUSSION

Polyangium cellulosum is the only myxobacter known to decompose cellulose, all others are bacteriolytic. Due to this unique property, it plays an important role in the degradation of cellulosic materials and hence in the carbon cycle. The major respiratory and carbohydrate degradation pathways have been studied in *Myxococcus xanthus* (Watson and Dworkin, 1968) and *Stigmatella brunnea* (McCurdy and Khouw, 1969). This thesis is a preliminary study of these pathways in *Polyangium cellulosum.*

P. cellulosum was seen to grow on a complex medium (SP), with and without glucose, and on a chemically defined medium (Staniers medium + cellobiose) indicating that it is able to utilize carbohydrates as sole source of carbon and energy, as has been previously reported (Coucke, 1969; Peterson, 1969). Better growth was observed on medium containing cellobiose rather than glucose. Godsey (1976) showed the presence of cellulase and cellobiase activities in intact and cell free extracts of this organism. Cellulolytic bacteria, as unrelated as *Ruminococcus flavefaciens* (Ayers, 1959), *Clostridium thermocellum* (Sih *et al.*, 1957) and *Cellvibrio gilvus* (Hulcher *et al.*,1958) apparently possess a cellulase which yields cellobiose as the sole hydrolytic product. Glucose is either not fermented by these bacteria or there is a distinct preference for the disaccharide. It seems possible that this type of activity is widespread among cellulolytic bacteria (Ayers, 1959). Hulcher *et al.* (1958), suggest that enhanced growth on cellobiose may be due to the fact that the cell derives slightly more energy per mole of hexose metabolized from cellobiose, than is obtained from glucose.

Dworkin (1962) showed that *M. santhus* does not metabolize low molecular weight sugars and depends on amino acids or peptides for carbon, nitrogen and energy. Similarly, *Stigmatella brunnea* does not utilize carbohydrates, although growth may be stimulated by starch, glycogen or other polysaccharides for liquid cultivation (McCurdy and Khouw, 1969).

The enzymes of the tricarboxylic acid cycle were assayed and activities of citrate synthase, aconitase, isocitrate dehydrogenase, succinic dehydrogenase, fumarase, malic dehydrogenase and α-ketoglutarate dehydrogenase were detected in *P. cellulosum*.

Watson and Dworkin (1968) demonstrated the presence of a complete Krebs cycle in *M. santhus* vegetative cells and microcysts. Dworkin (1972) suggests that amino acids are deaminated and enter the Krebs cycle, which is linked to the gluconeogenic pathway; carbon thus proceeds upwards for polysaccharide biosynthesis in *M. santhus*. The vegetative cells of *S. brunnea* possess most of the TCA cycle except α-ketoglutarate dehydrogenase (McCurdy and Khouw, 1969). However, the results of the enzyme and respiration studies indicate an operational TCA cycle with a complete terminal electron transport pathway. Taking into account the growth rate of *P. cellulosum*, the levels of TCA cycle enzymes are seen to be comparable with those of *M. santhus* (Watson *et al.*, 1968) and *B. subtilis* (Ohne, 1973).

This study demonstrated the presence of the key enzymes of glycolysis, namely hexokinase and fructose 1,6-diphosphate aldolase, in the vegetative cells of *P. cellulosum*. The activity of these enzymes was low when the cells were grown in SP or Staniers medium + cellobiose while there was almost a tenfold increase in specific activities of both enzymes when the cells were grown in SP + glucose (Table 1).

Watson and Dworkin (1968) showed that the vegetative cells of *M. xanthus* lack hexokinase and pyruvate kinase though all other glycolytic activities are present. Dworkin (1972) suggests that the absence of hexokinase could be of regulatory advantage to the organism and the glycolytic direction serves solely to metabolize endogenous carbohydrate. Watson *et al.* (1968) suggest that the principal function of the Embden–Meyerhof pathway in this organism is gluconeogenic. This is corroborated by the absence of pyruvate kinase, which is not required for gluconeogenesis, but catalyses an irreversible glycolytic step. Pyruvate kinase activity was detected in *P. cellulosum.*

Stigmatella brunnea, does possess a hexokinase, but does not utilize carbohydrates (McCurdy and Khouw, 1969). Some other factor must therefore be responsible for the inability of this organism to use sugars. However, in *Stigmatella aurantiaca,* addition of glucose to casitone medium results in higher population densities and the organism has been shown to metabolize glucose slightly (Gerth and Reichenbach, 1978; Reichenbach and Dworkin, 1969). The presence of hexokinase and aldolase and the fact that the cells can grow on media containing glucose as sole source of carbon indicates the presence of a functional glycolytic pathway in *P. cellulosum.* This is further supported by the presence of pyruvate kinase.

A number of different organisms are known which metabolize glucose mainly through the Embden–Meyerhof pathway although they also possess enzymes of the hexose monophosphate pathway (Brown *et al.*, 1971). The pentose phosphate shunt can be used for degradation of hexoses to generate pentoses, which are required for nucleic acid synthesis, and reducing power in the form of NADPH. It can also provide energy to the cell as an alternate pathway for the oxidation of glucose.

Glucose–6–phosphate dehydrogenase, which is the point of departure for at least two versions of hexose monophosphate pathway, was detected in the cell-free extracts of *P. cellulosum* vegetative cells. The presence of 6-phosphogluconate dehydrogenase was also demonstrated. The specific activities of both enzymes were higher when the cells were grown in medium containing cellobiose rather than in SP. The activity of the enzymes with NAD in addition to NADP may be due to endogenous production of NADH in the crude extract. Both the enzyme activities have been demonstrated in *M. santhus* vegetative. cells and microcysts (Watson and Dworkin, 1968). *P. cellulosum* may use the pentose phosphate shunt either as a biosynthetic pathway or for glucose oxidation. Further studies need to be done before any conclusions can be drawn.

The glyoxylate cycle, a modified form of the TCA cycle, is present in most plants and microorganisms. Its primary function is to enable them to utilize fatty acids or acetate as sole carbon source, and for the biosynthesis of carbohydrates from fatty acids.

The two enzymes of the glyoxylate cycle, malate synthetase and isocitrate lyase, were detected in *P. cellulosum* vegetative cells. The activities of both enzymes increases during microcyst formation in *M. santhus* (Bland *et al.* 1972). Induction of the glyoxylate cycle may reflect a shift to endogenous metabolism, perhaps of lipids (Orlowski *et al.* 1972). The glyoxylate cycle enzymes were not detected in *S. brunnea* (McCurdy and Khouw, 1969).

In conclusion, this study confirms that *Polyangium cellulosum* can use both sugars and amino acids as sources of carbon or energy. The key enzymes of glycolysis, pentose shunt, TCA cycle and the glyoxylate shunt are present in significant levels in the crude extracts of vegetative cells. Being a strict

aerobe, it probably uses glycolysis in conjunction with the tricarboxylic acid cycle for carbohydrate metabolism. The role of the pentose phosphate shunt, whether catabolic or biosynthetic cannot be determined from the preliminary results obtained in this study.

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