Occurrence, Structure and Function of Intracellular Polyglucose in the Obligate Chemolithotroph *Thiobacillus neapolitanus*

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Abstract. Nitrogen-limited cells of the obligate chemolithotroph *Thiobacillus neapolitanus* formed an intracellular polymer during growth in the chemostat. This polymer was isolated and characterized as a branched polyglucose composed of units joined by α -1 $\rightarrow$ 4 and α -1 $\rightarrow$ 6 linkages. Polyglucose in *T. neapolitanus* can be considered a storage compound since formation of this compound took place during excess of energy and CO_2 whilst shortage of CO_2 resulted in rapid breakdown of polyglucose. Moreover the breakdown of polyglucose generated metabolically useful energy as could be demonstrated by polyglucose-dependent protein synthesis. Possession of polyglucose did not influence the viability of *T. neapolitanus* during prolonged periods of energy starvation. Activities of key enzymes of the oxidative pentose phosphate cycle, glucose-6-phosphate-dehydrogenase and 6-phospho-gluconate-dehydrogenase, were demonstrated in cell free extracts of *T. neapolitanus* and appeared to increase 5- and 3-fold, respectively, during growth on NO_3^- instead of NH_4^+ as a nitrogen source.

Key words: Polyglucose – Chemolithotroph – *Thiobacillus neapolitanus*

Electron microscopical studies of the obligate chemolithotroph *Thiobacillus neapolitanus* revealed the presence of an intracellular polymer in this organism during nitrogen-limited growth conditions in the chemostat. This paper describes the isolation and characterization of this polymer which appeared to be polyglucose.

Storage compounds such as polyglucose and poly- β -hydroxybutyric acid accumulate in various bacteria when carbon and energy are in excess as for example during nitrogen-limited growth. The function of polyglucose as a storage compound has been extensively investigated in heterotrophic bacteria (for review see Dawes and Senior 1973). During periods of energy- or carbon limitation, or starvation, such storage compounds may generate carbon and energy to sustain the cell metabolism.

For bacteria with a comparable restricted metabolic capacity, such as the obligate photoautotrophic cyanobacterium *Synechococcus* sp. (*Anacystis nidulans*), it has been shown that intracellular polyglucose can serve as a carbon-storage compound (Lehmann and Wöber 1976) whereas in the obligate methylotroph *Methylococcus* strain NCIB 11083

polyglucose can serve as an energy storage compound (Linton and Cripps 1978).

In the light of the inability of obligate chemolithotrophic bacteria including *T. neapolitanus* to use exogenously supplied glucose as an energy source and the very limited capacity to use this compound as a carbon source (Matin and Rittenberg 1971; Smith and Hoare 1977; Matin 1978; Beudeker unpublished) it was of interest to investigate whether *T. neapolitanus* would be able to derive carbon and/or energy from its intracellular polyglucose. The results of these investigations are presented in this paper.

Materials and Methods

Medium and Growth Conditions

Thiobacillus neapolitanus strain X was grown in the chemostat as has been described by Kuenen and Veldkamp (1973).

The basal growth medium for *T. neapolitanus* contained (g/l): NH_4Cl , 0.4; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.8; KH_2PO_4 , 0.5; K_2HPO_4 , 0.5; $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, 10.0. To a litre of medium 2 ml of trace element mixture (Vishniac and Santer 1957) were added.

Ammonium limitation was achieved by lowering the NH_4Cl concentration 10-fold to 0.004% (w/v) and by lowering the thiosulfate concentration to 0.65% (w/v). At this concentration of thiosulfate *T. neapolitanus* was able to oxidize the excess of thiosulfate completely, whilst the nitrogen was still the only growth limiting compound. This made it possible to change over instantaneously from NH_4^+ limitation to thiosulfate limitation.

NO_3^- and urea were also used by *T. neapolitanus* as a nitrogen source when supplied at nitrogen concentrations equivalent to those described for growth on ammonium as a nitrogen source.

CO_2 - and thiosulfate limiting conditions were applied as has been described previously (Beudeker et al. 1980).

CO_2 starvation was achieved by stripping CO_2 from the air with sodiumasbestos and using 1 N NaOH for titration, instead of Na_2CO_3 .

Thiosulfate-starving conditions were created by switching off the medium supply; cysteine (final concentration 0.2 mM) was added to the culture as a sulphur source.

Simultaneous with change-over conditions from NH_4^+ limitation to other limitations or starvation, NH_4Cl (0.04%, w/v) was added to the culture.

Isolation of Polysaccharide

Intracellular polysaccharide was isolated from *T. neapolitanus* by the procedure of Zevenhuizen (1966). The effluent from an ammonium-limited chemostat was collected at 4°C. It appeared necessary to start with about 1,200 mg dry weight to obtain a significant amount of polysaccharide (80 mg). The cells were ruptured by sonification in the presence of 1:1 (w/v) ballotini beads (0.11 mm) and the suspension was centrifuged for 20 min at 40,000 $\times$ g (4°C). The extract was deproteinized by adding icecold trichloroacetic acid (TCA) and the precipitated protein

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was removed by centrifugation for 20 min at $40,000 \times g$ (4°C). To the clear supernatant 1:1 (v/v) ethanol was added after which the polysaccharide precipitated; it was collected by centrifugation for 20 min at $40,000 \times g$ (4°C). The pellet was washed twice with ethanol (100%) and once with diethylether and dried at 100°C .

Analysis of the Isolated Polysaccharide

Isolated polysaccharide (10 mg) was hydrolysed initially for 18 h in 2 ml $24\text{N H}_2\text{SO}_4$ at 4°C . The partly hydrolyzed polysaccharide was hydrolyzed further by diluting the mixture to $1\text{N H}_2\text{SO}_4$ with water and boiling this suspension for 10 h (Sietsma et al. 1969). The acidic solution was neutralized with solid BaCO_3 and the BaSO_4 precipitate was removed by filtration. The neutral solution was subjected to chromatography using cellulose thin layer plates (Merck). References containing $10\text{ }\mu\text{g}$ of glucose, fructose, sucrose or maltose were used. The chromatograms were developed at room temperature in a mixture of equal amounts of solutions A (375 ml n-butanol + 253 ml water) and B (176 ml propionic acid + 224 ml water). Sugars were located by spraying with p-anisidine phosphate. The solution was assayed for glucose using the glucose oxidase system (Boehringer, Mannheim, FRG).

The iodine-polysaccharide complex was prepared by adding $400\text{ }\mu\text{g}$ of polysaccharide to 3 ml of iodine reagent according to Zevenhuizen (1966).

Storage Polysaccharide Content

Total sugar was assayed with the anthrone reagent (Fairbairn 1953) with glucose serving as a standard. The amount of storage polysaccharide in *T. neapolitanus* cells was calculated by subtracting the sugar content of cells without storage polysaccharide.

Infrared Analysis

The infrared spectrum of isolated polysaccharide of *T. neapolitanus* was recorded with a Unicam 1100 infrared spectrophotometer and compared with that of glycogen (Merck, Darmstadt, FRG) using the KBr-disc technique (Barker et al. 1956).

Conditions of Amylolytic

The actions of β -amylase (β -1,4-glucan maltohydrolase EC 3.2.1.2) and α -amylase (α -1,4-glucan 4 glucanohydrolase EC 3.2.1.1) on the isolated polysaccharide of *T. neapolitanus* were tested as has been described by Linton and Cripps (1978).

The action of amyloglucosidase (α -1,4-D-glucan glucosylhydrolase EC 3.2.1.3) on the isolated polysaccharide from *T. neapolitanus* was tested in an identical way as has been described for α -amylase. Reducing sugars were determined by the method of Somogyi (1952) using maltose as a standard.

Electron Microscopy

Pellets were embedded in Epon as has been described previously (Beudeker et al. 1980). To demonstrate carbohydrate compounds the thin sections were stained with aqueous solutions of phosphotungstic acid using golden grids according to Schade (1973).

Demonstration of the Function of Polyglucose in *T. neapolitanus* as an Energy Source

In a typical experiment nitrogen-limited cells (containing 2.4 mg of protein) were incubated at 28°C for 24 h in basal growth medium without thiosulfate. For the detection of polyglucose dependent protein synthesis ^{35}S -cysteine ($100\text{ }\mu\text{Ci}$) was added at a concentration of 0.2 mM to the cell suspensions. Incorporation of ^{35}S -cysteine into several cell fractions concurrent with polyglucose metabolism was followed. Cell fractionation

was carried out according to Cripps (1973). ^{35}S -cysteine was taken as a tracer molecule because this compound can be used as a sulphur source by *T. neapolitanus*. The organism is unable to carry out assimilatory sulfate reduction and S from ^{35}S -cysteine can only be incorporated into protein and into lipid-protein complexes and not into other cell fractions.

Controls were taken with thiosulfate-limited cells which do not contain polyglucose. Incorporation of ^{35}S -cysteine into *T. neapolitanus* was also followed in cells without polyglucose but in the presence of the energy source (thiosulfate). The thiosulfate was exhausted after 1 h of incubation. For comparison a similar experiment has been carried out with addition of $1\text{-}^{14}\text{C}$ -acetate as a tracer molecule instead of cysteine.

Preparation of Cell-Free Extracts

Steady state cultures were harvested by centrifugation for 40 min at $20,000 \times g$ (4°C) and washed in the assay buffer. Cell-free extracts were made by sonification for $5 \times 30\text{ s}$ at 0°C , in the presence of 1:1 (w/v) ballottini beads (0.11 mm diameter). Beads and debris were removed by centrifugation for 20 min at $40,000 \times g$ (4°C).

Enzyme Assays

D-Glucose-6-phosphate: NADP oxidoreductase (EC 1.1.1.49) reaction mixture contained 36 mM tris(hydroxymethyl)methylamine (TRIS)-HCl pH 7.4; 12.5 mM MgCl_2 ; 6 mM glucose-6-phosphate; 1 mM NADP and cell-free extract (0.2 mg protein/ml of reaction mixture). Glucose-6-phosphate-dependent NADP reduction was measured in a Perkin Elmer 124 double beam spectrophotometer at 30°C .

6-Phospho-D-gluconate: NADP oxidoreductase (EC 1.1.1.43) test mixture contained 36 mM TRIS-HCl pH 7.4; 12.5 mM MgCl_2 ; 1.5 mM 6-phosphogluconate; 1 mM NADP and cell-free extract (0.2 mg protein/ml of test mixture). The reaction was followed as described above.

Protein Determination

Protein of cell-free extracts was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Protein of whole cells was determined with a modification of the microbiuret method of Goa (1953) as has been described by Kuenen and Veldkamp (1973).

Contamination by Other Bacteria

The cultures were frequently checked for contaminants by methods described by Kuenen and Veldkamp (1973).

Viability

Viability was measured on appropriate agar media by the method of Postgate (1969). Agar plates were incubated at 28°C and contained the basal growth medium for *T. neapolitanus*.

Chemicals

All radioisotopes were purchased from the Radiochemical Centre Amersham (England). Amyloglucosidase and α -amylase were obtained from Merck (Darmstadt, FRG). β -Amylase came from Sigma Chemicals Co. (St. Louis, Missouri, USA).

Results

Structure of the Intracellular Polyglucose

Polyglucose was isolated from cells of *Thiobacillus neapolitanus*, which had been grown under ammonium limitation at

Fig. 1. The infra-red spectra of polyglucose isolated from *Thiobacillus neapolitanus* (2) compared with commercial glycogen from rabbit liver (1). Note characteristic peaks for glycogen at 765, 850–860 and 930 cm^{-1} wavenumber (equivalent to reciprocal wavelength)

a dilution rate of 0.07 h^{-1} using standard procedures as described in Materials and Methods.

Thin layer chromatograms of the hydrolysed polysaccharide from *T. neapolitanus* showed only one spot with a R_f value of 0.28. This R_f value was identical to that of a standard glucose solution. Quantitative determination of the glucose from the hydrolysed polysaccharide using glucose oxidase confirmed the observation that the intracellular polysaccharide from *T. neapolitanus* consisted for more than 99% of glucose. The infrared spectrum of the polyglucose taken in the region $960-720 \text{ cm}^{-1}$ showed absorbance peaks at 765, 850–860 and at 930 cm^{-1} . Commercial glycogen showed an identical spectrum (Fig. 1). The absorption at $850-860 \text{ cm}^{-1}$ is characteristic for derivatives with α -glucosidic linkages. Polysaccharides containing β -linkages do not show an absorbance peak at this wavelength. These data provide convincing evidence that the *Thiobacillus neapolitanus* polyglucose consists of α -linked glucose units.

To obtain an impression about the branching character of the polyglucose from *T. neapolitanus* the spectrum of the iodine-polyglucose complex was measured. The more branching is present in a polyglucose, the shorter the wavelength at which the absorbance maximum is reached (Zevenhuizen 1966). Addition of the iodine reagent to the polyglucose isolated from *T. neapolitanus* yielded a red-brown solution with an absorbance maximum at 470 nm, characteristic for a highly branched polyglucose (Zevenhuizen 1966; Linton and Crips 1978).

The actions of α - and β -amylase as well as amyloglucosidase on the isolated polyglucose from *T. neapolitanus* were followed to detect whether α -1 $\rightarrow$ 4 and α -1 $\rightarrow$ 6 linkages existed. The percentage of the polysaccharide released as maltose or glucose is shown in Table 1. When α -amylase acts on polyglucose α -1 $\rightarrow$ 4 linkages will be hydrolysed at random, yielding maltose, whilst β -amylase only hydrolyses the outer glucose chains to maltose. Neither amylase attacks α -1 $\rightarrow$ 6 linkages. In contrast, amyloglucosidase will hydrolyse not only the α -1 $\rightarrow$ 4 linkages but also the α -1 $\rightarrow$ 6 bonds.

As shown in Table 1 the amylases did not hydrolyse polyglucose from *T. neapolitanus* completely. However, α -amylase plus amyloglucosidase did, indicating that the poly-

Table 1. The percentage of polyglucose released as maltose by the action of α - and β -amylase (amylolysis limit) and the percentage of polyglucose released as glucose by the combined action of amyloglucosidase and α -amylase on polyglucose isolated from *Thiobacillus neapolitanus*

Enzyme	% Maltose	% Glucose
α -Amylase	71	—
β -Amylase	50	—
Amyloglucosidase + α -amylase	—	99

Fig. 2. Ultra-thin sections of *T. neapolitanus* grown under NH_4^+ limitation in the chemostat ($D = 0.07$; $p\text{O}_2 = 50\%$ air saturation) showing intracellular polyglucose granules ($13,050\times$)

Fig. 3. Ultrathin sections of *T. neapolitanus* grown under NH_4^+ limitation in the chemostat ($D = 0.07$; $p\text{O}_2 = 50\%$ air saturation) stained specifically to demonstrate carbohydrate (see Methods section). Each individual cell contained the polyglucose granules ($34,400\times$)

glucose from *T. neapolitanus* is composed of α -1 $\rightarrow$ 4 and α -1 $\rightarrow$ 6 linked glucose molecules. The amylolysis limit (that is the amount of polyglucose hydrolysed by the β -amylase) for the polyglucose from *T. neapolitanus* was found to be 50% (Table 1). To visualize the deposition of polyglucose within the cells ultrathin sections were prepared for electron microscopy (Fig. 2). Carbohydrate was stained specifically (Fig. 3) showing rosette-like granules of more or less uniform size (25 nm). Each individual cell contained the granules. Poly- β -hydroxybutyrate was never detected in *T. neapolitanus* grown under ammonium limitation in the chemostat ($D = 0.07$; $p\text{O}_2 = 50\%$ air saturation).

Occurrence of the Intracellular Polyglucose

Polyglucose was detected in *T. neapolitanus* during NH_4^+ -, NO_3^- - and urea limitation in the chemostat. The polyglucose

Fig. 4. The content of polyglucose in *T. neapolitanus* as a function of dilution rate under NH_4^+ limitation ($p\text{O}_2 = 50\%$ air saturation)

Fig. 5. The decrease in polyglucose content (corrected for washout) in *T. neapolitanus* during change-over experiments in the chemostat from NH_4^+ limitation ($D = 0.07$; $p\text{O}_2 = 50\%$ air saturation) to: thiosulfate limitation/thiosulfate starvation ($\times$); CO_2 limitation ($\bullet$); CO_2 starvation ($\circ$). Except for the change-over to thiosulfate starvation the dilution rate and the oxygen tension were kept constant during this experiment

content of the culture grown at a fixed dilution rate of 0.07 h^{-1} was independent of the nitrogen source applied and amounted to approximately 8% of the dry weight. The polyglucose content of an ammonium limited culture expressed per mg of protein varied inversely with the applied growth rate (Fig. 4). This phenomenon which has also been observed in other organisms (Herbert 1961; Linton and Cripps 1978) is most likely explained by the relatively high overcapacity of *T. neapolitanus* to oxidize thiosulfate at low dilution rate (Beudeker and Kuenen, unpublished results). During thiosulfate- or CO_2 -limitation in the presence of excess ammonia (7.7 mM) polyglucose was not detected in *T. neapolitanus*. However, when CO_2 -limited chemostat cultures of *T. neapolitanus* were shifted to CO_2 -excess and thiosulfate limitation a transient accumulation of polyglucose was observed which disappeared after prolonged cultivation under thiosulfate limitation.

Function of the Intracellular Polyglucose in the Obligate Chemolithotroph *T. neapolitanus*

The possible function of polyglucose in *T. neapolitanus* as a carbon storage compound was examined by change-over experiments from NH_4^+ -limitation in the chemostat to CO_2 limitation and CO_2 starvation ($D = 0.07$; $p\text{O}_2 = 50\%$ air saturation). Figure 5 shows that polyglucose disappeared from the culture at a rate considerably faster than the dilution

Table 2. Effect of intracellular polyglucose on the incorporation of radioactive cysteine in the obligate chemolithotroph *Thiobacillus neapolitanus*. ^{35}S -cysteine ($330 \text{ nCi}/0.2 \mu\text{mol} \cdot \text{ml}$ cell suspension) had been added to equal amounts (protein) of cells with polyglucose, cells without polyglucose and cells without polyglucose but in the presence of thiosulfate (see Materials and Methods)

	Cells with poly-glucose	Cells without poly-glucose	Cells without polyglucose but with thiosulfate
	nCi		
Total uptake of ^{35}S -cysteine	1,000	55	1,125
Low molecular weight compounds fraction ^a	244	9	292
Lipid fraction ^a	221	30	224
Nucleic acids fraction ^a	0	0	0
Protein fraction ^a	535	16	609

(wash-out) rate. During change-over from NH_4^+ limitation to CO_2 starvation 40% of the polyglucose was degraded within 20 min. This was followed by a reproducible short period of approximately 60 min, during which no further degradation was detected. After this period the breakdown resumed at a much slower rate. Protein content, viability and carbon content of the cells did not change significantly during these experiments (not shown).

The degradation of polyglucose during the change-over from NH_4^+ -limitation to thiosulfate limitation (or starvation) appeared to be slower as compared to the change-over to CO_2 limitation (or starvation) but was still significantly faster than would be expected from mere washout of the culture.

The degradation of the intracellular polyglucose in *T. neapolitanus* after transfer of NH_4^+ -limited cells ($D = 0.07$; $p\text{O}_2 = 50\%$ air saturation) to conditions of energy starvation in batch culture appeared to yield metabolically useful energy. The magnitude of ^{35}S -cysteine incorporation was very similar in cells containing polyglucose and cells supplied with thiosulfate (Table 2). In cells containing polyglucose or cells provided with thiosulfate also the percentage of label incorporated into protein was comparable. These results clearly showed that the breakdown of polyglucose allowed protein synthesis. Cells without polyglucose or thiosulfate hardly incorporated any ^{35}S -cysteine (Table 2) whilst they were able to do so after addition of thiosulfate as an energy source (not shown). The uptake and incorporation of $1\text{-}^{14}\text{C}$ -acetate by *T. neapolitanus* in an identical experiment showed similar results (not shown).

Effect of the Presence of Intracellular Polyglucose on the Viability of *T. neapolitanus*

The effect of intracellularly stored polyglucose on the viability of *T. neapolitanus* incubated in 0.05% (w/v) phosphate buffer at 28°C is shown in Fig. 6. Though polyglucose disappeared from the cells no significant difference in viability could be detected between cells containing polyglucose and cells without polyglucose. Even after 2 months of starvation the viability of both types of cells was still approximately 40%.

Fig. 6. The effect of prolonged starvation in 0.05% phosphate buffer on the viability of cells of *T. neapolitanus* with (x) or without (●) intracellular polyglucose

Table 3. Activities of glucose-6-phosphate dehydrogenase and 6-phospho-gluconate dehydrogenase measured in cell-free extracts of *Thiobacillus neapolitanus* grown in the chemostat ($D = 0.07 \text{ h}^{-1}$; $p\text{O}_2 = 50\%$ air saturation) during NH_4^+ - and NO_3^- limitation

Growth limiting substrate	Glucose-6-phosphate-dehydrogenase	6-Phosphogluconate dehydrogenase
	n · mol/min · mg protein	
NH_4^+	15	24
NO_3^-	77	86

Pathway of Glucose Metabolism

The key enzymes of the Embden-Meyerhof glycolytic pathway and Entner-Doudoroff pathway have been shown to be absent in *T. neapolitanus*, whereas the enzymes for the oxidative pentose phosphate cycle could be detected (Matin and Rittenberg 1971). Therefore, only the key enzymes of the oxidative pentose phosphate cycle were tested for activity. Table 3 shows the activities of both glucose-6-phosphate dehydrogenase and 6-phospho-gluconate dehydrogenase in cell-free extracts of *T. neapolitanus* grown in the chemostat under nitrogen-limitation. During NO_3^- -limited growth these activities were 5 and 3 times higher, respectively, as compared with NH_4^+ -limited growth. The activities measured are high enough to account for the observed breakdown of (poly)glucose through the oxidative pentose-phosphate cycle. Attempts to elucidate the pathway of glucose metabolism in *T. neapolitanus* by radiorespirometry according to Wood et al. (1977) were unsuccessful due to the very limited uptake of exogenous ^{14}C -glucose by *T. neapolitanus*.

Discussion

According to the criteria of Wilkinson (1959) the intracellular polyglucose of *T. neapolitanus* can be considered to be a storage compound:

1. Polyglucose was formed in *T. neapolitanus* during N-limited growth with excess of carbon and energy.
2. Polyglucose functioned as a storage carbon compound by *T. neapolitanus* as can be concluded from the rapid degradation of polyglucose during change-over from NH_4^+ -

limitation to CO_2 starvation or to CO_2 -limitation (Fig. 5). The observed lag period during the rapid degradation of polyglucose in *T. neapolitanus* during CO_2 starving conditions might be needed to induce enzymes for the further degradation of polyglucose.

3. Degradation of polyglucose resulted in production of metabolically useful energy as could be judged from the polyglucose dependent protein synthesis (Table 2).

The importance of polyglucose for the survival of *T. neapolitanus* obviously lies in its role as reserve material during short periods of energy- or carbon starvation. The importance of polyglucose for the maintenance of viability is less obvious since *T. neapolitanus* turned out to be extremely resistant to starvation (Fig. 6). For heterotrophic bacteria such as *Streptococcus mitis* (van Houte and Jansen 1970) and *Escherichia coli* (Dawes and Ribbons 1963) it has been shown that the possession of glycogen resulted in a prolonged viability during energy-starvation periods. It has been shown that the facultatively anoxygenic cyanobacterium *Oscillatoria limnetica* is able to ferment glucose derived from intracellular polyglucose under anaerobic conditions (Oren and Shilo 1979). The possibility that the aerobic *T. neapolitanus* would be capable of anaerobic metabolism of polyglucose for survival under anaerobic conditions is presently under investigation.

Polyglucose in cyanobacteria is metabolized aerobically through the oxidative pentose phosphate cycle as was shown by radiorespirometric experiments combined with enzyme studies (Pelroy et al. 1972). Radiorespirometric studies in *T. neapolitanus* have been unsuccessful hitherto due to the extremely low uptake of ^{14}C -glucose by these cells. All enzyme data, however, indicated that glucose metabolism in *T. neapolitanus* proceeds through the oxidative pentose phosphate cycle (Table 3). The increase in activities of the key enzymes of this cycle during growth on NO_3^- as a nitrogen source was not due to the presence of activators in the cell-free extract as was concluded from experiments using mixed extracts. Whether glucose-6-phosphate dehydrogenase and 6-phosphogluconate-dehydrogenase were increased in activity by modification or by enzyme induction in this organism is still unknown. The increase in activities of both enzymes has also been found in plants during growth on NO_3^- as a nitrogen source and is most likely explained by the demand for NADPH needed for the reduction of NO_2^- to NH_4^+ (Sarkissian and Fowler 1974).

The clear demonstration of the ability of *T. neapolitanus* to derive both energy and carbon from intracellular (poly)glucose is the first report on the importance of polyglucose as reserve material in an obligately chemolithotroph. This is another example of the interesting parallels between obligately photoautotrophs and the obligately chemolithotrophs (see reviews Smith and Hoare 1977; Doolittle 1979). Both groups of organisms are not able to grow on glucose which is due to the absence of specific carriers for glucose in obligately photoautotrophic cyanobacteria (Beauclerk and Smith 1978).

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References

- Barker SA, Bourne EJ, Whiffen DH (1956) Use of infrared analysis in the determination of carbohydrate structure. *Meth Biochem Analys* 3:213
- Beauclerk AAD, Smith AJ (1978) Transport of D-glucose and 3-O-methyl-D-glucose in the cyanobacteria *Aphanocapsa* 6714 and *Nostoc* strain mac. *Eur J Biochem* 82:187–197
- Beudeker RF, Cannon GC, Kuenen JG, Shively JM (1980) Relations between D-ribulose-1,5-bisphosphate carboxylase, carboxysomes and CO₂-fixing capacity in the obligate chemolithotroph *Thiobacillus neapolitanus*, grown under different limitations in the chemostat. *Arch Microbiol* 124:185–189
- Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt Biochem* 72:248–254
- Cripps RE (1973) Microbial metabolism of thiophen-2-carboxylate. *Biochem J* 134:353–366
- Dawes EA, Ribbons DW (1963) Endogenous metabolism and survival of *Escherichia coli* in aqueous suspension. *J Applied Bacteriol* 26:VI
- Dawes EA, Senior PJ (1973) The role and regulation of energy reserve polymers in micro-organisms. *Advanc Microb Physiol* 10:136–266
- Doolittle WF (1979) The cyanobacterial genome, its expression, and that control of that expression. *Advanc Microb Physiol* 20:1–102
- Fairbairn NJ (1953) A modified anthrone reagent. *Chemistry and Industry* 1953:86
- Goa J (1953) A microbiuret method for protein determination; determination of total protein in cerebrospinal fluid. *Scand J Clin Lab Invest* 5:218
- Herbert D (1961) The chemical composition of microorganisms as a function of their environment. In: Meynell GG, Gooder H (eds) *Symp Soc Gen Microbiol*, Vol 11. University Press, Cambridge, England, pp 391–416
- Kuenen JG, Veldkamp H (1973) Effects of organic compounds on growth of chemostat cultures of *Thiomicrospira pelophila*, *Thiobacillus thioparus* and *Thiobacillus neapolitanus*. *Arch Microbiol* 94:173–190
- Lehmann M, Wöber G (1976) Accumulation, mobilization and turnover of glycogen in the blue-green bacterium *Anacystis nidulans*. *Arch Microbiol* 111:93–97
- Linton JD, Cripps RE (1978) The occurrence and identification of intracellular polyglucose storage granules in *Methylococcus* NCIB 11083 grown in chemostat culture on methane. *Arch Microbiol* 117:41–48
- Matin A, Rittenberg SC (1971) Enzymes of carbohydrate metabolism in *Thiobacillus* species. *J Bacteriol* 107:179–186
- Matin A (1978) Organic nutrition of chemolithotrophic bacteria. *Ann Rev Microbiol* 32:433–469
- Oren A, Shilo M (1979) Anaerobic heterotrophic dark metabolism in the cyanobacterium *Oscillatoria limnetica*: sulphur respiration and lactate fermentation. *Arch Microbiol* 122:77–84
- Pelroy RA, Rippka R, Stanier RY (1972) Metabolism of glucose by unicellular blue-green algae. *Arch Mikrobiol* 87:303–323
- Postgate JR (1969) Viable counts and viability. In: Norris JR, Ribbons DW (eds) *Methods in microbiology*, Vol 1. Academic Press, New York London, pp 611–628
- Sarkissian GS, Fowler MW (1974) Interrelationship between nitrate assimilation and carbohydrate metabolism in plant roots. *Planta (Berl)* 119:335–349
- Schade HAR (1973) On the staining of glycogen for electron microscopy with polyacids of tungsten and molybdenum. I. Direct staining of sections of osmium fixed and Epon embedded mouse liver with aqueous solutions of phosphotungstic acid (PTA). In: Wisse E, Daems WTh, Molenaar I, Duyn P van (eds) *Electron microscopy and cytochemistry*. Elsevier North Holland Publishing Company, Amsterdam, pp 263–266
- Sietsma JH, Eveleigh DE, Haskins RH (1969) Cell wall composition and protoplast formation of some Oomycetes species. *Biochim Biophys Acta* 184:306–317
- Smith AJ, Hoare DS (1977) Specialist phototrophs, lithotrophs and methylotrophs: a unity among a diversity of prokaryotes. *Bacterial Rev* 41:419–448
- Somogyi M (1952) Notes on sugar determination. *J Biol Chem* 195:19–23
- Van Houte J, Jansen HM (1970) Role of glycogen in survival of *Streptococcus mitis*. *J Bacteriol* 101:1083–1085
- Vishniac W, Santer M (1957) The thiobacilli. *Bacteriol Rev* 26:168–195
- Wilkinson JF (1959) The problem of energy-storage compounds in bacteria. *Exptl Cell Res Suppl* 7:111–130
- Wood AP, Kelly DP, Thurnston CF (1977) Simultaneous operation of three catabolic pathways in the metabolism of glucose by *Thiobacillus* A2. *Arch Microbiol* 113:265–275
- Zevenhuizen LPTM (1966) Function, structure and metabolism of the intracellular polysaccharide of *Arthrobacter*. Doctoral thesis, Wageningen

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