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An unusual pattern of carbohydrate utilization in *Corallococcus (Myxococcus) coralloides* (Myxobacterales)*

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Abstract. The myxobacterium, *Corallococcus* (*Myxococcus*) *coralloides* strain Cc c127, could not utilize mono- and disaccharides, but maltotriose and the polysaccharides starch, amylose, amylopectin, and pullulan stimulated growth. Radioactive CO_2 was set free from ^{14}C-labeled starch. When starch was degraded, small amounts of maltose and glucose accumulated in the culture supernatant. Maltotriose, however, appeared only temporarily. Outside the cells, the trisaccharide could not be split into glucose and maltose. Pullulan was hydrolyzed exclusively into a trisaccharide which during growth was immediately consumed. Hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and phosphoglucomutase could readily be demonstrated in cell extracts, but fructose-1,6-diphosphate aldolase was present with low activity only. The data suggest that intracellular glucose is metabolized mainly via the pentose phosphate pathway.

Key words: Myxobacteria – Carbohydrate metabolism – Trisaccharide utilization – Starch utilization – Pullulanase

Myxobacteria of the bacteriolytic group (Reichenbach and Dworkin 1981) seem not to be able to grow exclusively on inorganic nitrogen. They rely on peptides and amino acids not only for nitrogen, but also for carbon and energy. It is therefore not always easy to unequivocally prove carbohydrate utilization by these organisms, and the older literature on this topic is full of unclear and contradictory results.

Solntseva (1941) showed that in liquid cultures of *Stigmatella aurantiaca* and *Melittangium boletus* 20% of the added glucose disappeared in the course of 20 days of incubation, which suggested metabolization of glucose by these organisms. This has since unequivocally been proven for *S. aurantiaca* (Reichenbach and Dworkin 1969; Gerth and Reichenbach 1978; Kunze et al. 1984). Glucose and, with some strains, galactose markedly stimulated growth of *Archangium* spp. in a liquid medium containing casein hydrolysate at low concentration (McDonald and Peterson 1962). *Cystobacter* spp. grow on simple media with trace amounts of one or two essential amino acids, some glutamine as the nitrogen source, and glucose as the main carbon and energy source; in this case no acid is produced (Reichenbach 1984). On the other hand, metabolization of glucose and other mono- and disaccharides has definitely been excluded for *Myxococcus* and *Corallococcus* (formerly *Chondrococcus*) strains (Solntseva 1940; Dworkin 1962; Hemphill and Zahler 1968; Bretscher and Kaiser 1978). The inability of *M. xanthus* strain FB to utilize glucose may be explained with a lack of hexokinase and pyruvate kinase (Watson and Dworkin 1968). As several other Embden-Meyerhoff enzymes were present, the pathway seems to operate in the direction of gluconeogenesis. The first two enzymes of the pentose phosphate pathway could also be demonstrated.

Starch degradation has been reported for virtually every species of bacteriolytic myxobacteria including *Myxococcus* and, in particular, *Corallococcus* (Solntseva 1940, 1941; Beebe 1943; Norén 1955; McCurdy 1969). In some cases a pronounced stimulation of growth has been observed with starch (Beebe 1943; Norén 1955), and this although several of the organisms involved could definitely not metabolize glucose and maltose, apparently a contradiction.

We now found a somewhat unusual pattern of carbohydrate metabolism in a bacteriolytic myxobacterium, which may shed some light on the anomaly mentioned above. As reported in this article, *Corallococcus coralloides* strain Cc c127, producer of the new antibiotic corallopyronin (Irschik et al. 1985), does not use glucose or maltose in the medium, but it metabolizes the trisaccharide maltotriose, and its growth is considerably stimulated by starch and related polysaccharides.

Materials and methods

Corallococcus (*Myxococcus*) *coralloides* strain Cc c127 (= *C. coralloides* HI 1) was isolated in 1980 from a soil sample from Gabès, Tunesia. The strain was cultivated at 30° C under shaking (160 rpm) and grew in homogeneous cell suspension. The basic medium was MD1 liquid medium (= *MD1 lm*): 0.3% peptone from casein, tryptically digested (Merck, Darmstadt); 0.2% $MgSO_4 \cdot 7\,H_2O$; 0.05% $CaCl_2 \cdot 2\,H_2O$; pH 7.2, autoclaved. Carbohydrate utilization was tested in MD1 lm with varying peptone and carbohydrate concentrations.

Metabolism of radioactive carbohydrates was studied in the following way. The cells from a preculture in MD1 lm plus 0.3% starch or 0.3% glucose, respectively, were harvested by centrifugation and resuspended in fresh MD1 lm to give an OD_{623} of 3 (optical density at 623 nm, determined in an Eppendorf filter photometer with 1 cm light path; approx. 6×10^9 cells/ml). To 10 ml of these cell

* Prof. Dr. Gerhard Drews gratefully dedicated to his 60th birthday

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Table 1. Effect of carbohydrates on growth of *Corallococcus coralloides* Cc c127 in MD1 lm

Carbohydrate added[a] (0.2%)	Maximal cell density[b] (OD_{623})	Final pH	Doubling time (h)
None, control	1.0	8.2	6.4
Glucose	1.0	8.3	6.4
Maltose	0.85	8.05	
Maltotriose	2.2	7.85	6.4
Dextrin 20	2.0	7.6	
Dextran 4	0.8	8.1	
Dextran 20	0.9	8.35	
α-Cyclodextrin (Schardinger α-dextrin)	0.65	8.5	
Inulin	0.9	8.35	
Starch	1.8	7.6	6.4
Amylose	2.5	7.6	
Amylopectin	2.2	7.8	
Pullulan	2.1	7.9	

[a] The following carbohydrates could also not be used: saccharose, isomaltose, isomaltotriose, α-D-melezitose, α-D-raffinose, D-trehalose, stachyose, α-D-melibiose, gentiobiose, glucose-1-phosphate, glucose-6-phosphate

[b] The cultures were started at an OD of 0.06

Table 2. Influence of starch on the maximal cell yield of *Corallococcus coralloides* Cc c127 at different peptone concentrations

Peptone[a] (%)	Starch (%)	Maximal cell yield (OD_{623})	Final pH
0.1	–	0.55	8.25
0.1	0.1	1.0	7.75
0.1	0.3	1.05	7.65
0.1	0.6	1.1	7.65
0.3[b]	–	1.2	8.25
0.3	0.1	1.85	7.7
0.3	0.3	2.0	7.7
0.3	0.6	2.1	7.7
0.6	–	1.6	8.2
0.6	0.1	2.8	7.8
0.6	0.3	3.25	7.85
0.6	0.6	4.0	7.85
1	–	clumped	8
1	0.1	clumped	8
1	0.3	clumped	8
1	0.6	clumped	8

[a] Peptone from casein, tryptically digested (Merck, Darmstadt)

[b] = MD1 lm

suspensions either 2 μCi of ^{14}C-starch (from NEN; specific activity 1.9 mCi/mg) or of ^{14}C-glucose (from Amersham International; specific activity 269.7 mCi/mM) was added. The production of $^{14}CO_2$ was determined by suspending a small vessel containing 2.5 ml of 25% KOH within the culture flask. In certain time intervalls 50 μl of the KOH was pipetted into 10 ml of scintillation cocktail. The radioactivity was determined in a Packard TriCarb scintillation spectrometer. Aliquots (200 μl) of the cell suspension were centrifuged; 50 μl of the supernatant was used for counting the remaining radioactivity, another 50 μl for performing thin layer chromatography. The pellet was washed once with H_2O, and was resuspended in 200 μl of H_2O. Of this suspension, 100 μl was passed through a membrane filter (0.45 μm pore size; Sartorius, Göttingen). The filter was washed with 20 ml of water and dried at 80° C. After addition of scintillation cocktail, the radioactivity was determined as above.

Thin layer chromatography of carbohydrates was performed on precoated silica gel plates (Merck) with n-butanol/acetic acid/H_2O (60:20:20) as the solvent (Stahl 1964). The carbohydrates were detected by spraying with phenol-sulfuric acid (Adachi 1965). Radioactivity on TL plates was detected with a TL scanner (Berthold, Wildbad: DC-Scanner II LB 2723).

Cell extracts for the measurement of enzyme activities were prepared in the following way. The cells were washed once with 50 mM Tris-HCl buffer pH 7.6 + 3.3 mM $MgCl_2$. Then they were suspended in the same buffer and broken by passage through a French pressure cell at 1,125 kg/cm^2 (= 16,000 psi). After centrifugation at 25,000 × *g* for 15 min the supernatant was collected and used for enzymatic tests. Hexokinase (EC 2.7.1.1), phosphoglucomutase (EC 2.7.5.1), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), and fructose-1,6-diphosphate aldolase (EC 4.1.2.13) were determined according to Bergmeyer and Gawehn (1974), and 6-phosphogluconate dehydrase (EC 3.1.1.12) by the method of McGee and Doudoroff (1954). Phosphorylase activity was investigated by the method of Schwartz and Hofnung (1967).

Results

Utilization of various carbohydrates. If in MD1 lm a carbohydrate was used by *Corallococcus coralloides* Cc c127, a higher cell density resulted and the pH increase of the medium was delayed (ordinarily the medium becomes alkaline because of NH_3 production from amino acids). Table 1 shows that only a few carbohydrates could be metabolized, viz. starch and its components, amylose and amylopectin, certain oligosaccharides derived from starch, and pullulan. Cyclic α-1,4-dextrin (cyclohexaamylose, Schardinger α-dextrin), on the other hand, could not be used. The generation time was not affected if a utilizable carbohydrate was added to MD1 lm.

Influence of different peptone and starch concentrations on growth. At different peptone concentrations the cell yield increased with increasing starch concentration (Table 2). Starch could replace peptone only to a certain extent, and high yields could be obtained only with relatively high peptone concentrations. A peptone concentration of 1% appeared already to be toxic for the organism: The cells clumped together, there was little growth, and the pH rose over 8. At this peptone concentration, starch had no influence on growth.

Degradation of carbohydrates. The supernatant of cultures with various carbohydrates in MD1 lm were taken at different growth stages and analyzed by TLC. While the concentrations of glucose and maltose did not change (Fig. 1a and 1b), maltotriose (0.1%) decreased and disappeared towards the end of bacterial growth (Fig. 1c). Starch was cleaved, and the fragments were further decomposed later on. At an early culture stage there were substantial amounts

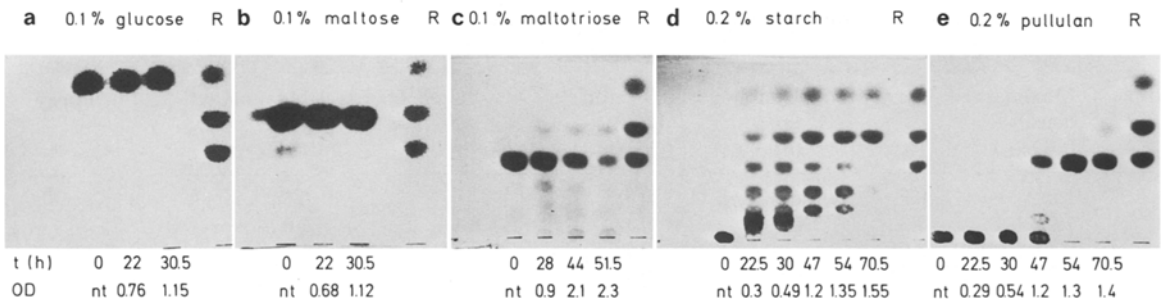

Fig. 1 a—e. Thin-layer chromatograms of culture supernatants of *Corallococcus coralloides* Cc c127 in MD1 lm with different carbohydrates. *R* = reference consisting (from above) of glucose, maltose and maltotriose, *nt* = not tested

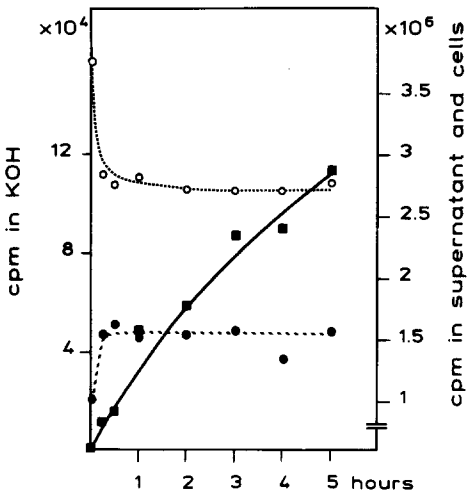

Fig. 2. Radioactivity in different fractions of a culture of *Corallococcus coralloides* Cc c127 in MD1 lm plus ^{14}C-starch. ○----○ Supernatant (total volume 10 ml); ●----● cells (total volume 10 ml); ■——■ $^{14}CO_2$ released and absorbed by 25% KOH solution (total volume 2.5 ml)

of maltose and maltotriose present, but towards the end of the growth phase starch and all oligosaccharides had essentially disappeared and only maltose and glucose were left (Fig. 1 d).

Pullulan (maltotriose units linked by α-1,6 glycosidic bonds; Bender et al. 1959) was cleaved into C-3 units (Fig. 1 d). No maltose and glucose were formed from this polysaccharide.

Utilization of ^{14}C-starch and ^{14}C-glucose. When ^{14}C-starch was added to the culture, radioactive CO_2 was released (Fig. 2). Release of $^{14}CO_2$ went on steadily over several hours. After 5 h incubation, 2.6% of the added radioactivity was found in the CO_2 fraction (Table 4). The radioactivity collected with the cells reached a high level a few seconds after addition of ^{14}C-starch, further increased during the next 15 min, and then remained constant. The radioactivity in the supernatant decreased only to about 70% of the initial value (Fig. 2, Table 4).

The distribution of radioactivity in a TLC of supernatants taken from a starch-containing culture after different incubation times is shown in Fig. 3. Intact starch remains at the start. The diagram shows that the starch became soon cleaved into substances of lower molecular weight, and that these substances disappeared again later

Table 3. Metabolism of U-^{14}C-glucose by *Corallococcus coralloides* Cc c127 in MD1 lm

Incubation time (h)	Radioactivity in		
	supernatant[a] (cpm)	cell suspension[a] (cpm)	absorbed in 25% KOH[b] (cpm)
0[c]	4.8×10^6	15000	0
2	3.82×10^6	31200	450
5	3.76×10^6	31400	1000

[a] Total volume 10 ml
[b] Total volume 2.5 ml
[c] Immediately after addition of ^{14}C-glucose

Table 4. Balance of ^{14}C-starch utilization by *Corallococcus coralloides* Cc c127

Incubation time (h)	Radioactivity in			Recovery (%)
	supernatant (cpm)	cells (cpm)	absorbed in 25% KOH (cpm)	
0[a]	3,761,800	1,044,900	0	100
1	2,814,100	1,531,000	49,000	91
5	2,773,200	1,523,200	114,500	92

[a] Immediately after addition of ^{14}C-starch

on. The radioactivity at $R_f = 0.27$ (maltose) increased for about 2 h and remained then constant. Thus, the experiment confirms the results shown in Fig. 1 d.

As shown in Table 3, ^{14}C-glucose was not metabolized. Release of $^{14}CO_2$ was two orders of magnitude lower than from starch.

Demonstration of enzymes of carbohydrate metabolism. Several enzymatic activities in cell extracts showed that intracellular glucose can be metabolized (Table 5). Hexokinase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were present in approximately identical and high activities. Fructose-1,6-bisphosphate aldolase activity was only about 10% of that of the above mentioned enzymes. Phosphoglucomutase was highly active, whereas phosphorylase and 6-phosphogluconate dehydrase could not be demonstrated.

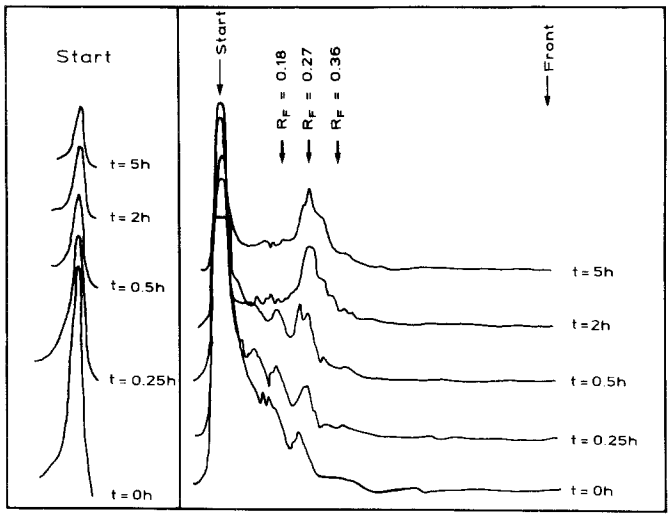

Fig. 3. Scannings of thin-layer chromatograms for ^{14}C-containing substances. The supernatant of a culture of *Corallococcus coralloides* Cc c127 in MD1 lm plus ^{14}C-starch was analyzed after various incubation times. The compound with R_f 0.27 is maltose. The track at the left was recorded at lowered sensitivity of the instrument

Table 5. Enzyme activities in cell extracts

Enzyme[a]	Activity[b]	H acceptor or donor
Hexokinase	29	NADP
Phosphoglucomutase	360	NADP
Glucose-6-phosphate dehydrogenase	38	NADP
Fructose-1,6-diphosphate aldolase	3	NADP
6-Phosphogluconate dehydrogenase	34	NADP

[a] Phosphorylase with starch, maltotriose, or maltose as substrate and NADP as H acceptor, and 6-phosphogluconate dehydrase with NAD as H acceptor or with the semicarbazide reaction were negative

[b] Expressed as nmol H acceptor or donor converted per mg protein and minute

Discussion

Corallococcus coralloides strain Cc c127 utilizes certain carbohydrates. The result is a substantial increase in the cell yield, but not a faster growth rate. The ability of Cc c127 to metabolize carbohydrates seems restricted to some poly- and oligosaccharides, mainly starch and its components and decomposition products. Maltotriose was the smallest oligosaccharide that could be used. Glucose and maltose did not serve as substrates. Certain quantities of these sugars were released during starch hydrolysis and accumulated in the medium.

Dextrans, which are highly branched and consist to a large part of α-1,6-bound glucose, were not used. Pullulan, a polysaccharide consisting of maltotriose units linked by α-1,6 bonds (Bender et al. 1959), was hydrolyzed into a trisaccharide, presumably maltotriose (Fig. 1d). This is characteristic for the action of pullulanase (Lee and Whelan 1971), and Cc c127 seems thus to be another microorganism that produces this enzyme (Morgan et al. 1979). Obviously Cc c127 excretes at least two enzyme activities, one attacking α-1,6- and one cleaving α-1,4-glycosidic bonds. These activities could be found in the supernatant of cultures that had been kept in carbohydrate-free medium for several transfers.

That starch was not only cleaved but also metabolized is shown by the release of $^{14}CO_2$ from ^{14}C-starch at a steady rate over several hours (Fig. 2). As this rate was relatively slow, the carbohydrate is probably used as a building block rather than for energy generation. A considerable part of the small quantity of radioactive starch added to the culture obviously became immediately and strongly bound to the cells, for it could be collected together with the cells by centrifugation (Table 4).

Even at relatively high carbohydrate concentrations the cell yields were clearly limited by the peptone concentration (Table 2), probably because the organism depends on certain amino acids for growth (Bretscher and Kaiser 1978). Still it was possible to double the cell mass at a given peptone level by the addition of starch. In batch cultures without pH control part of this effect may be due to the delayed rise of the pH. In media with 0.3% peptone, the saturation level of carbohydrate was about 0.2%. When maltotriose was added at that concentration, a considerable amount of the trisaccharide remained in the supernatant after growth had ended. When the initial concentration was 0.1%, nearly all of the trisaccharide disappeared. When 0.2% starch was added, all starch was hydrolyzed at the end of growth, and the arising maltotriose was almost completely used up (Fig. 1d). However, only part of the starch has really been metabolized by the cells, because a substantial amount of maltose and some glucose had accumulated in the medium.

When 0.2% pullulan was supplied, no glucose or maltose was produced, and trisaccharide appeared in the medium only after growth had stopped (Fig. 1e). Obviously in this case the activity of the pullulanase was the rate limiting step. The way by which maltotriose is utilized, is not yet clear. It seems that it is not split into useless glucose and maltose outside the cells (Fig. 1c). Also, it is still open whether larger fragments of starch degradation can be taken up by Cc c127.

Determination of enzyme activities in cell extracts showed that glucose can be metabolized as soon as it is inside the cells. Hexokinase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were present in reasonable activities, but the key enzyme of glycolysis, fructose-1,6-diphosphate aldolase, showed only weak activity (Table 5). This suggests that glucose is used mainly via the pentose phosphate pathway to supply ribose, and that only a small fraction of the carbohydrate, at best, may serve as an energy source via oxidative phosphorylation. This is in agreement with the relatively weak CO_2 production from starch mentioned above and may be responsible for the fact that the growth rate is independent of the concentration of added carbohydrate. Thus, the situation in Cc c127 may not be fundamentally different from that in *Myxococcus xanthus* FB (Watson and Dworkin 1968). There, intracellular glucose phosphate can apparently also be metabolized via the pentose phosphate pathway. But while this glucose phosphate has to be synthesized de novo by *M. xanthus*, glucose can be supplied in some way by extracellular oligo- and polysaccharides to Cc c127 and directly be phosphorylated.

There was also very high activity of phosphoglucomutase. This could indicate that starch or maltotriose is degraded by phosphorolysis yielding glucose-1-phosphate. However, phosphorolytic activity could not be demonstrated in cell extracts. Also, hexokinase would not be required

if starch is degraded by phosphorolysis thus bypassing free glucose.

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