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Effects of Some Carbon Sources on Growth and Nitrogen Fixation in the Cyanobacterium *Nostoc linckia*

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Abstract. Glucose, fructose, sucrose, maltose, and lactose stimulated photoheterotrophic growth of *Nostoc linckia* (ROTH.) BORN. as well as its heterocyst frequency, chlorophyll and protein contents, ammoniacal nitrogen uptake and nitrogenase activities. Glucose, fructose and sucrose also supported slow chemoheterotrophic growth. α-ketoglutarate, pyruvate, ribose, succinate, acetate, sorbose and formate were inhibitory.

Although most cyanobacteria are, in general, obligate photoautotrophs, several strains can grow heterotrophically at the expense of various sugars which provide both carbon and energy (KHOJA and WHITTON 1975, LADHA and KUMAR 1977, HAURY and SPILLER 1981).

Diazotrophic mechanism requires a large input of reducing power and ATP for the production of ammonia. In cyanobacteria, energy to form a reductant can come from photosynthesis. In most filamentous cyanobacteria, nitrogen fixation under aerobic conditions occurs in heterocysts (HASELKORN 1978). These forms have developed an exchange mechanism between heterocysts and vegetative cells whereas CO_2 is fixed aerobically in vegetative cells and an unidentified compound is subsequently transferred to heterocysts (WOLK 1968). Such transfer of fixed carbon from vegetative cells to heterocysts prompted us to investigate the possible effects of exogenous carbon sources on phototrophic and chemoheterotrophic growth, heterocyst differentiation, ammoniacal nitrogen uptake and nitrogen fixation in *N. linckia*.

MATERIAL AND METHODS

Nostoc linckia (ROTH.) BORN. was grown axenically in the medium of ALLEN and ARNON (1955), adjusted to pH 7.5 after autoclaving. The medium either lacked combined nitrogen or was supplemented with 5 mM KNO_3, and was buffered with 4 mM of Tris (hydroxymethyl) aminomethane/HCl. Cultures were grown on 14/10 h light/dark cycle at 26 ± 2 °C and 2000 ± ± 200 lx. They were shaken by hand thrice daily.

Growth experiments were carried out in culture tubes by measuring the absorbance of the cultures at 660 nm in a Bausch and Lomb Spectronic 20 co-

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lorimeter. Dark heterotrophic conditions were created by wrapping the tubes with aluminium foils and incubating them in the culture room. Generation time was determined by the growth equation of KRATZ and MYERS (1955). Relative chlorophyll contents were calculated from the absorbance values recorded in a Spectronic 20 colorimeter, by means of the equation of MACKINNEY (1941); the colorimetric values agreed to (within $\pm 7\%$) the values from measurements with a Unicam SP 500 spectrophotometer. Heterocyst frequency was measured as a percentage of the total cells by counting at least 12 filaments (about 1000 cells).

Ammoniacal nitrogen uptake was measured in an Orion Ion Meter using the ammonia electrode. Nitrogenase activity was measured by the acetylene--ethylene assay (STEWART *et al.* 1968).The reaction mixture was analyzed in a CIS Gas Chromatograph (Baroda, India) fitted with a Porapak R column and a hydrogen flame ionization detector. Nitrogen was used as a carrier gas. Protein was determined by the method of LOWRY *et al.* (1951) using lysozyme as a standard.

D-glucose, D-fructose, D-lactose, D-ribose, D-maltose, D-sorbose, α-ketoglutarate and formate were the products of Sigma Chemical Co. (St. Louis, U.S.A). Ethylene was from Matheson Gas Co. (Lyndhurst, U.S.A.). Other gases were from Indian Oxygen Limited (Bombay, India).

RESULTS

Growth of cultures under nitrogen fixing conditions with various carbon sources was measured 10 d after inoculation (Table 1). Glucose showed the maximum growth stimulation under both photoheterotrophic and chemo-

TABLE 1

Growth of *N. linckia* in a medium free of combined nitrogen and containing carbon compounds (20 mM). Growth estimated by absorbances (A) at 660 nm on the 10th day

Compound	Growth			
	Photoheterotrophic		Chemoheterotrophic	
	A	GT [h]	A	GT [h]
None	0.38 ± 0.02	60 ± 2.7	NG	–
Glucose	0.58 ± 0.03	41 ± 2.9	0.22 ± 0.08	92 ± 4.1
Fructose	0.54 ± 0.04	47 ± 2.9	0.16 ± 0.02	95 ± 5.6
Sucrose	0.52 ± 0.01	48 ± 2.7	0.13 ± 0.04	99 ± 5.1
Maltose	0.49 ± 0.05	52 ± 2.9	NG	–
Lactose	0.47 ± 0.01	53 ± 3.0	NG	–
α-ketoglutarate	0.40 ± 0.02	59 ± 3.3	NG	–
Pyruvate	0.38 ± 0.01	60 ± 3.1	NG	–
Ribose	0.42 ± 0.02	58 ± 2.9	NG	–
Succinate	0.41 ± 0.01	59 ± 2.9	NG	–
Acetate	0.37 ± 0.01	62 ± 3.0	NG	–
Sorbose	0.11 ± 0.01	118 ± 4.2	NG	–
Formate	0.14 ± 0.01	98 ± 3.9	NG	–

NG = no growth; all values are means of three replicates ± standard deviation.
GT = Generation time.

TABLE 2

Effects of carbon sources on chlorophyll and protein contents, heterocyst frequency, uptake of ammoniacal nitrogen, and nitrogenase activity. Samples were withdrawn and analyzed on the 8th day after inoculation. Other conditions were the same as for Table 1. (Values are means ± S.D.)

Compound added	Chlorophyll [relative]	Protein [μg ml^{-1}]	Ammoniacal nitrogen uptake [μmol ml^{-1}]	Heterocyst frequency [%]	Nitrogenase activity [nmol C_2H_4 mg^{-1}(prot.) h^{-1}]
None	4.6	28.0 ± 2.6	33.5 ± 4.4	5.6 ± 0.6	58.5 ± 5.1
Glucose	7.4	42.5 ± 3.5	41.2 ± 5.0	10.6 ± 0.9	78.5 ± 5.7
Fructose	6.5	38.3 ± 3.0	39.5 ± 4.6	9.3 ± 8.9	74.3 ± 6.0
Sucrose	5.9	34.6 ± 3.7	37.8 ± 3.9	9.0 ± 0.8	71.6 ± 4.9
Maltose	5.1	32.2 ± 2.8	37.0 ± 3.9	6.0 ± 0.9	62.5 ± 4.9
Lactose	4.8	31.0 ± 2.9	35.8 ± 4.0	5.8 ± 0.6	60.2 ± 5.6
α-ketoglutarate	4.6	27.6 ± 2.8	33.7 ± 3.9	5.6 ± 0.9	56.8 ± 4.6
Pyruvate	4.5	27.0 ± 3.3	33.0 ± 3.8	5.5 ± 0.9	55.3 ± 5.6
Ribose	4.7	28.6 ± 4.5	33.8 ± 4.3	5.7 ± 0.9	58.8 ± 5.1
Succinate	4.6	28.2 ± 4.9	33.3 ± 4.1	5.5 ± 0.8	52.5 ± 6.2
Acetate	4.3	18.7 ± 2.2	18.2 ± 3.5	5.4 ± 0.5	48.2 ± 5.2
Sorbose	1.2	7.2 ± 1.9	5.2 ± 0.8	3.2 ± 0.1	15.6 ± 1.5
Formate	1.4	7.9 ± 1.8	5.8 ± 0.6	3.5 ± 0.1	17.2 ± 2.2

heterotrophic conditions; fructose, sucrose, maltose and lactose, α-ketoglutarate, pyruvate, ribose, succinate and acetate either showed a slight stimulatory effect (not significant) or did not affect growth at all. Sorbose and formate inhibited growth within 72 h: the material became pale yellow. Air-grown culture had a doubling time of about 60 h which could be decreased to 41 h in the presence of 20 mM of glucose and to between 47 and 53 h in the presence of the four other growth stimulating sugars. α-ketoglutarate, pyruvate, ribose, succinate and acetate had no significant effect on the doubling time of organisms. Sorbose and formate prolonged the doubling time as well as the lag phase from 60 h to 118 and 98 h, respectively.

Among the sources tested, only glucose, fructose, and sucrose supported growth in the dark. Generally, growth was very poor in the dark.

The heterocyst frequency was increased by glucose, fructose and sucrose from 5.6% to 10.6, 9.3 and 9.0%, respectively (Table 2). Other carbon sources (except for sorbose and formate which inhibited the frequency) did not significantly increase it. The observed rise in heterocyst frequency points to some effect of these sources even at the level of cellular differentiation. Whereas the low-carbon cultures contained heterocysts with normal polar bodies and thick envelopes, the high-carbon ones had rather long filaments with poorly developed heterocysts; these heterocysts lacked conspicuous polar bodies and had thin walls. In higher concentrations of carbon compounds, most heterocysts were deeply bluish green. Among the compounds tested, only glucose, fructose, maltose and sucrose increased the uptake of ammoniacal nitrogen from the medium. Table 2 also shows the effects of the carbon compounds on the contents of chlorophyll-a (relative values) and proteins, and nitrogenase activity.

The effects of different concentrations of glucose on chlorophyll and protein contents, heterocyst frequency, and nitrogenase activity under

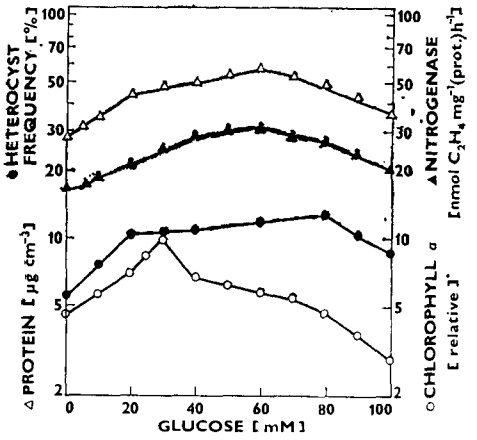

Fig. 1. Effect of glucose supplementation on chlorophyll-a and protein contents, heterocyst frequency, and nitrogenase activity, as determined on the 8th day after inoculation.

phototrophic conditions are shown in Fig. 1. At low initial glucose concentrations, there was an almost exponential increase of chlorophyll and protein contents and nitrogenase activity. Chlorophyll content was maximal at 30 mM of glucose and then dropped with increasing glucose concentrations up to 100 mM. There was no further increase in protein and nitrogenase activity above 60 mM of glucose. The increase in nitrogenase activity at low glucose levels was accompanied by an increase in heterocyst numbers.

DISCUSSION

We have earlier shown that exogenous additions of fructose and glucose stimulate light-dependent growth in wild type *N. linckia* and its propionate--resistant mutant (KUMAR *et al.* 1983). Whereas the generation time of the wild strain grown on glucose in light, with nitrate as the sole nitrogen source, was 41 h, that of the one grown on molecular nitrogen was 60 h.

The heterotrophic potential of any autotrophic organism may be established by simple growth experiments under conditions where the photoautotrophic route is blocked and the organism is compelled to utilize the exogenously supplied carbon sources. We have not conducted any experiments with radio-isotopic compounds, nevertheless we have observed photoheterotrophy and chemoheterotrophy in *N. linckia*, and out of various sources tested, the organism showed maximum growth on glucose, followed by fructose and sucrose. The observed failure of growth stimulation in light by some other carbon sources may be due to that these compounds are not metabolized because of the absence of the required enzymes (see HAURY and SPILLER 1981).

The concentration-dependent stimulation of growth in glucose may be due to a low rate of glucose entry into the cells. It seems probable that higher levels of glucose result in an increase in the rate of assimilation thereby increasing ammoniacal nitrogen uptake. At higher glucose concentrations (30—60 mM), the nitrogenase activity and protein content are maximal, whereas chlorophyll amount decreases. Nitrogen fixation and chlorophyll content are thus inversely related under phototrophic conditions, and in this

respect our results are in agreement with those of HAURY and SPILLER (1981). Loss of chlorophyll at these concentrations of glucose may cause some decline in oxygen evolution and the created microaerobic environment may establish favourable conditions for nitrogen fixation. A similar inverse correlation between high nitrogenase activity in fructose-supplemented cultures of *Anabaena variabilis* and low oxygen evolution has been reported by HAURY and SPILLER (1981).

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REFERENCES

ALLEN, M. B., ARNON, D. I.: Studies on nitrogen fixing blue-green algae. I. Growth and nitrogen fixation by *Anabaena cylindrica* LEMM. — Plant Physiol. **30** : 366—372, 1955.

HASELKORN, R.: Heterocysts. — Annu. Rev. Plant Physiol. **29** : 319—344, 1978.

HAURY, J. F., SPILLER, H.: Fructose uptake and influence on growth and nitrogen fixation by *Anabaena variabilis*. — J. Bacteriol. **147** : 227—235, 1981.

KHOJA, T. M., WHITTON, B. A.: Heterotrophic growth of filamentous blue-green algae. — Brit. Phycol. J. **10** : 139—148, 1975.

KRATZ, W. A., Myers, J.: Nutrition and growth of several blue-green algae. — Amer. J. Bot. **42** : 282—287, 1955.

KUMAR, H. D., MISHRA, D. K., TRIPATHI, A. K.: Isolation and initial characterization of propionate-resistant mutant of the cyanobacterium *Nostoc linckia*. — Plant Nature **1** : 1—9, 1983.

LADHA, J. K., KUMAR, H. D.: Response of a wild type and a non-nitrogen fixing mutant of *Nostoc linckia* to different carbon sources. — New Phytol. **79** : 299—308, 1977.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., RANDALL, R. J.: Protein measurement with folin-phenol reagent. — J. biol. Chem. **193** :263—275, 1951.

MACKINNEY, G.: Absorption of light by chlorophyll solutions. — J. biol. Chem. **140** : 315—322, 1941.

STEWART, W. D. P., FITZGERALD, G. P., BURRIS, R. H.: Acetylene reduction by nitrogen-fixing blue-green algae. — Arch. Microbiol. **62** : 336—348, 1968.

WOLK, C. P.: Movement of carbon from vegetative cells to heterocysts in *Anabaena cylindrica*. — J. Bacteriol. **96** : 2138—2143, 1968.