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DIFFERENCES BETWEEN CULTURED AND FRESHLY ISOLATED CYANOBIONT FROM *PELTIGERA*—IS THERE SYMBIOSIS-SPECIFIC REGULATION OF A GLUCOSE CARRIER?

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Abstract: The cyanobiont (*Nostoc*) of the lichen *Peltigera horizontalis* was isolated using a procedure of repeated grinding, differential centrifugation and partitioning in an aqueous two-phase system of dextran/polyethylene glycol. A yield of 22% was calculated on the basis of chlorophyll recovery from the thallus. Freshly isolated *Nostoc* differed little from that kept in prolonged culture with respect to the relative activities of a number of enzymes of carbohydrate metabolism in the soluble fraction. However, much higher specific activities of the disproportionating transglycosylase and starch phosphorylase, higher intracellular concentrations of glucose, maltooligosaccharides and glycogen and a lower rate of glucose uptake were evident in freshly isolated *Nostoc*. These activities and pool sizes, as well as CO_2-fixation capability, stayed roughly constant for at least 5 h after isolation; glucose release, which is characteristic for the symbiotic *Nostoc*, dropped sharply within 1 h. These observations suggest the transport of glucose out of the cell, rather than that the activities of cytoplasmic enzymes may be a point of early regulation during transition of *Nostoc* from the lichenized to the free-living state.

Introduction

Differences between lichen photobionts in culture and in symbiosis have been noted in morphology, reproductive characteristics, cell surface properties and physiological qualities (Galun & Bubrick 1984; Bubrick 1988). The ability of the photobiont to release carbohydrates in the symbiotic stage and the loss of this trait upon cultivation is already known (Drew & Smith 1967*a*; Green & Smith 1974) but the causes for this change are unclear. The functioning of this transition obviously is essential for establishing unhindered growth of the isolated photobiont and, in the reverse direction, for successful lichen formation from the free-living partners.

In *Peltigera polydactyla* (Drew & Smith 1967*b*; Hill & Smith 1972) and other lichens having cyanobacteria as photobionts (Richardson *et al.* 1968), glucose is transferred to the fungus. An α-glucan is intermediate in the formation of this sugar (Hill 1972, 1976; Meindl & Loos 1990) and some enzymes that are possibly involved have been demonstrated in a cultured *Peltigera* cyanobiont (Bogner *et al.* 1993). In the present work, for a better understanding of the special glucose-releasing ability of the cyanobiont, the amount and activity of several enzymes of carbohydrate metabolism have been compared between *Nostoc* freshly isolated from *P. horizontalis* and *Nostoc* grown in prolonged

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culture. In addition, the changes in pool sizes (e.g. of glucose) and in rates of glucose uptake were measured within a few hours of isolation, a time span during which a severe drop in glucose release is observed (Green & Smith 1974). From these kinetics, the biochemical processes that may regulate the early transition from the symbiotic to the free-living state are dicussed.

Materials and Methods

Plant material

Peltigera horizontalis was collected from the wooded fringes of the Danube valley south-west of Regensburg, Germany, and, if necessary, stored overnight at 18°C in a moist atmosphere. *Nostoc* sp. originating from *P. horizontalis* (Meindl & Loos 1990) was grown in liquid mineral medium BG11 (Stanier *et al.* 1971) in which $NaNO_3$ was replaced by NaCl (1 mM); illumination, gassing and temperature were as described previously (Fischer *et al.* 1989). *Nostoc* filaments were harvested by centrifugation 12–18 days after inoculation in the early stationary phase (Bogner *et al.* 1993); filaments that were 6–8 days old were used for glucose uptake experiments.

Preparation of isolated *Nostoc*

Thalli of *P. horizontalis* were freed from adhering earth, moss, etc. and, after several washings, floated in tap water. After removal of rhizinae, most of the lower hyphal layer was pulled off with fine tweezers and discarded. The remaining thallus fragments were kept in ice water until grinding; all further steps were carried out at 0–4°C. Typically, 5 g of thallus fragments and 10 ml of homogenizing solution containing 50 mM HEPES/NaOH buffer, pH 7·0, 0·25 M sorbitol, 1% polyvinylpyrrolidone 40 000 (Sigma) and 0·25% bovine serum albumin were ground (250 strokes) in a mortar. To sediment larger thallus debris, the homogenate was quickly brought just up to 700 g and the centrifuge was switched off (no braking). The supernatant was set aside, the pellet suspended with 5 ml fresh homogenizing medium and again subjected to grinding, followed by brief centrifugation as above. The supernatants were combined and the supernatants obtained in two further cycles of suspending/grinding/centrifuging were added. The combined supernatants were centrifuged for 10 min at 1200 g. The supernatant was discarded, but the olive-coloured pellet was suspended in fresh homogenizing medium and mixed (25 inversions) with the ten-fold volume of a phase mixture consisting of 50 mM HEPES/NaOH buffer, pH 7·0, 0·25 M sorbitol, 8·3% (by weight) polyethylene glycol 4000 (Sigma) and 8·3% (by weight) dextran T500 (Pharmacia). After centrifuging for 10 min at 1200 g, fungal debris was contained mainly in the dextran-rich bottom phase and *Nostoc* cells were enriched at the interface. The interface was withdrawn with a pipette, diluted to approximately 10 ml with homogenizing medium and centrifuged (1200 g). The pellet was suspended in 2–3 ml fresh homogenizing medium and treated with the phase mixture as above. The *Nostoc* cells that formed a sharp band at the interface (Fig. 1A) were diluted and washed again with homogenizing medium and finally suspended in the desired medium. The wet-weight yield of packed cells was between 0·5% and 1% of the thallus fragment fresh weight. The final suspension of the isolated cyanobiont was obtained 2·5–3 h after the first grinding step. The percentage of intact cells was estimated by staining the dead cells with phenosafranine (Widholm 1972) in a total of 50 cells.

Measurement of enzyme activities in the soluble cell fraction

Freshly isolated *Nostoc* cells were suspended in 0·02 M imidazole/HCl buffer, pH 6·8, and broken in an ice bath by intermittent sonication (8–10 times for 30 s each) with the microtip of a Branson sonifier. The homogenate was centrifuged (3 min, 11 000 g) and the supernatant dialysed at 0–4°C against 0·02 M imidazole/HCl buffer, pH 6·8. The soluble cell fraction from the cultured cyanobiont was obtained similarly (Fuchs *et al.* 1994). Enzyme tests were run at 30°C with incubation times ranging from 15 to 60 min; linear rates of activity were sustained throughout these incubation periods. ADP-glucose pyrophosphorylase was assayed by the formation of ADP-[^{14}C]glucose (Levi & Preiss 1976); the labelled sugar nucleotide was recovered and counted according to Ghosh & Preiss (1966). Glycogen synthase was measured by incorporation of labelled glucose from ADP-[^{14}C]glucose into glycogen following the procedure

of Levi & Preiss (1976) except that glycogen from oyster (Type II, Sigma) was used as the acceptor. In the assay for phosphoglucomutase the reaction was monitored via NADPH absorption in the presence of an excess of glucose-6-phosphate dehydrogenase as described by Levi & Preiss (1976). Hexokinase activity was determined similarly with the glucose test system of Bergmeyer *et al.* (1974) in which the normal addition of hexokinase was omitted but 1·4 mM glucose was included as a substrate. Activity of amylase was measured as the formation of reducing groups according to Bernfeld (1955) as modified by Bogner *et al.* (1993). Starch phosphorylase was assayed with the coupled optical test based on NADP reduction (Steup 1990). Disproportioning transglycosylase (D-enzyme) was determined as glucose release with maltotriose as substrate (Fuchs *et al.* 1994). Reaction products were checked by thin-layer chromatography (TLC) as described elsewhere (Fischer *et al.* 1989); maltopentaose and glucose proved to be the only primary products. Tests for maltase were done exactly as those for D-enzyme but with 10 mM maltose as substrate. Phosphatase was assayed in 0·02 M imidazole buffer, pH 6·8, with 5 mM *p*-nitrophenyl phosphate as substrate. The reaction was stopped by addition of twice the volume of 0·2 M sodium borate buffer, pH 9·8, free *p*-nitrophenol was measured at 405 nm and the activity was calculated using an absorption coefficient of 18 500 $l\ mol^{-1}\ cm^{-1}$.

Measurement of $^{14}CO_2$ assimilation, released material and glucose uptake

For measurement of $^{14}CO_2$ assimilation and of released labelled material, between 3 and 5 mg (wet weight) of freshly isolated cyanobiont was shaken for 1 h at 28°C in 0·6 ml of 50 mM citric acid/trisodium citrate buffer, pH 4·8, in the presence of $^{14}CO_2$ (0·9%, by vol.) under the illumination of fluorescent light tubes (Osram Dulux L, 60·2 $W\ m^{-2}$). Further experimental details and processing of the samples have been outlined previously (Fischer *et al.* 1989), along with TLC analysis of the released material. Glucose uptake was followed by circular shaking (240 rpm) of *Nostoc* at 28°C in the dark in 50 mM citric acid/trisodium citrate buffer, pH 4·8, in the presence of [^{14}C]glucose (5 mM, 33–100 kBq $nmol^{-1}$). At intervals, 200–300 µl samples were withdrawn, filtered by vacuum on membrane filters and washed twice with 2 ml citrate buffer. The filters with the cyanobacteria were hydrolysed (2 M trifluoroacetic acid, 120°C, 2 h), an aliquot was counted and the remainder was used for protein determination, which was based on an assay of total amino acids. This was effected by hydrolysis in 6 M HCl (105°C, 18 h), removal of HCl in an airstream and photometric evaluation of amino acids with a ninhydrin reagent (Sigma) using L-leucine as a standard. Since the samples from the *Nostoc* suspension (especially the cultured form) contained different amounts of cells due to uneven size and distribution of filamental clumps, all values of radioactivity incorporated from [^{14}C]glucose were based on protein content.

Determination of cellular contents of glucose, glucose-6-phosphate, maltooligosaccharides and glycogen; determination of chlorophyll

Nostoc cells suspended in 0·02 M imidazole/HCl buffer were broken by ultrasonic treatment as described above. The homogenate was centrifuged (10 min, 48 000 g) and the soluble fraction containing most of the glycogen (Bogner *et al.* 1993) was heat-treated (5 min, 96°C), cleared by centrifugation and mixed with a fourfold volume of ethanol. After standing overnight at −20°C the precipitate formed was spun down and the glycogen content determined enzymatically (Keppler & Decker 1974). Ethanol was removed from the supernatant and the maltooligosaccharides were measured in the same way; free glucose and glucose-6-phosphate were determined after the method of Bergmeyer *et al.* (1974). In some preparations the soluble components were extracted principally according to Bieleski (1982). For this purpose the cells were briefly sonicated in an ice bath in a mixture of CH_3OH:$CHCl_3$:H_2O:HCOOH=12:5:2:1 (by vol.) and incubated overnight at −20°C. By adding $CHCl_3$ and H_2O the ratio $CHCl_3$:CH_3OH:H_2O:HCOOH was made up to 18:12:5:1 (by vol.); after mixing and phase separation the contents of glucose and glucose 6-phosphate in the aqueous phase were determined as above.

For calculation of intracellular concentrations the volume bounded by the cytoplasmic membrane was estimated as the volume inaccessible to D-glucosamine, a compound that was not taken up significantly either by symbiotic or by cultured *Nostoc*. For this purpose 0·5 ml cell

suspension in 50 mM citrate/trisodium citrate buffer, pH 4·8, containing 37 kBq of carrier-free D-[^{14}C]glucosamine and 45 kBq of $^{3}H_2O$ was incubated for 3 min at 0°C and centrifuged. The radioactivity due to ^{14}C and ^{3}H in the pellet and in the supernatant was measured in a scintillation spectrometer; counting efficiencies for either isotope in each fraction were determined and taken into consideration. The volume of the D-glucosamine-inaccessible room was calculated as given by Rottenberg (1979); it amounted to 30·7 ± 2·1% and 22·7 ± 0·9% of the packed cell volume of newly isolated and cultured *Nostoc*, respectively (mean of three and four determinations, respectively).

Chlorophyll was determined spectrophotometrically at 663 nm in 80% acetone (by vol.) according to MacKinney (1941).

Results

Isolation of *Nostoc* from *P. horizontalis*

Preparation of *Nostoc* from *P. horizontalis* proceeded in three principal steps: (i) pulling off most of the lower hyphal layer from the thallus, resulting in what are called 'thallus fragments'; (ii) repeated grinding of the thallus fragments combined with differential centrifugation; (iii) enrichment of the cyanobiont at the interface of a polyethylene glycol/dextran phase system (Fig. 1A). Microscopic examination of the *Nostoc* preparations disclosed occasional hyphal fragments and cells of more or less ovoid shape (maximal length 11 μm), which occurred singly or as aggregates of two and more cells (Fig. 1B); this agrees well with the description of *Nostoc* isolated from *P. polydactyla* (Drew & Smith 1967*a*). The activity of phosphatase, an enzyme known to be located on fungal cell walls (Tonino & Steyn-Parvé 1963; Calleja *et al.* 1980), was considered an indicator of preparation purity. Whereas this enzyme activity was easily measurable in the fungal fractions, it was not detectable with whole cells of the isolated photobiont (data not shown).

The yield of *Nostoc* cells was based on chlorophyll recovered in the isolated cyanobiont as compared to thallus chlorophyll content. A yield of 22% was found on average from six preparations (range 10 to 36%). Of the freshly isolated cells 94–96% were estimated to be intact using the phenosafranine staining method (Widholm 1972). Incubation in diluted buffers promoted cell rupture in some cases; therefore, the homogenizing medium and media for prolonged incubations were osmotically stabilized.

In Fig. 1C cultured *Nostoc* cells are shown for comparison. These grew as tightly wound filaments of elliptical cells together with loose filaments made up of more cylindrical cells. Such different growth forms of *Nostoc* have been observed previously (Boissière *et al.* 1987; Meindl & Loos 1990). No attempts were made to separate them since no correlation was evident between the rate of glucose release and preponderance of the one or the other cell type (data not shown). In any case the cell size was clearly smaller than that of the freshly isolated cyanobiont (Fig. 1B).

Distribution of enzyme activities and rates of glucose uptake in cultured and freshly isolated *Nostoc*

Enzymes of glycogen metabolism were assayed from the two cell types and related arbitrarily to the activity of hexokinase (=100%). The newly isolated cyanobiont differed clearly from the cultured one by (i) the presence of phosphorylase and (ii) a more than 20-fold relative activity of D-enzyme

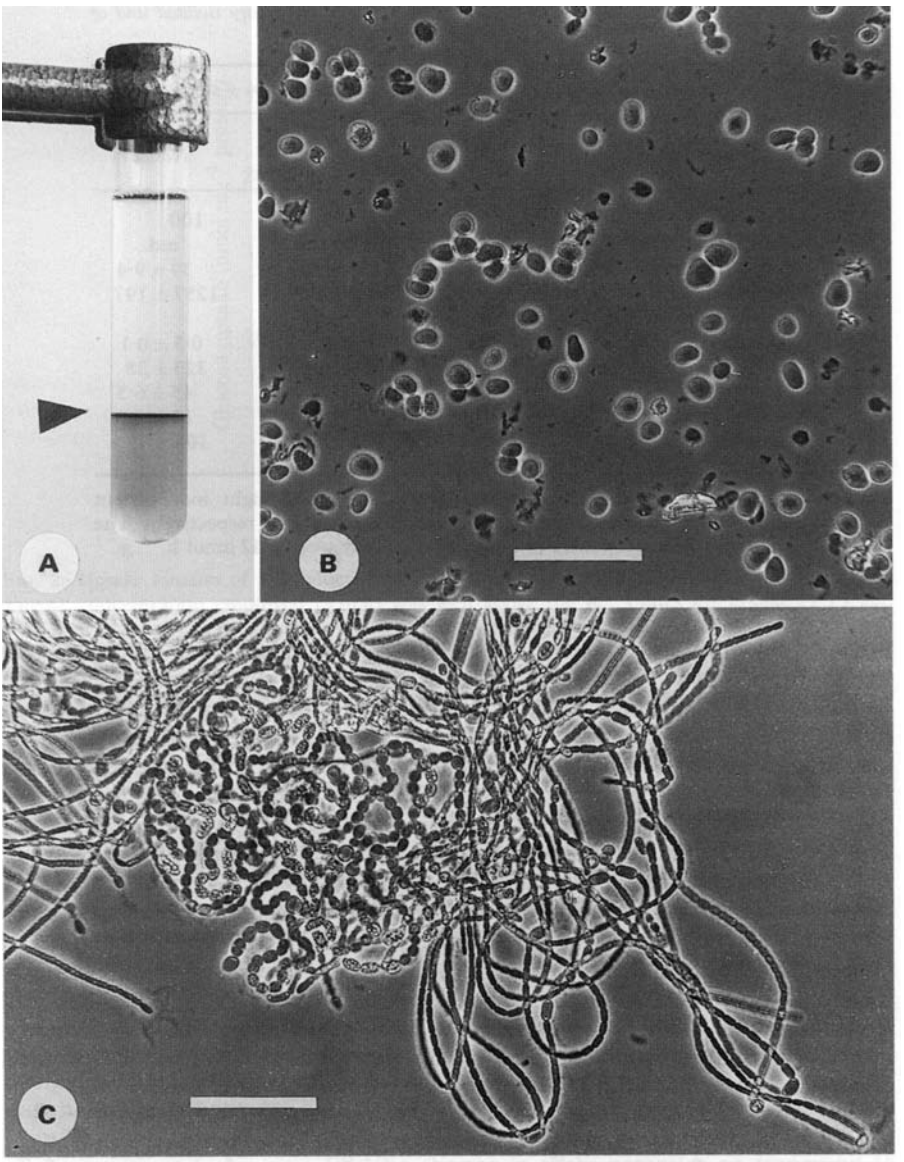

FIG. 1A–C. *Nostoc* freshly isolated from *Peltigera horizontalis* and after prolonged cultivation. A, Freshly isolated cells assembled at the interface (arrowhead) of a polyethylene glycol/dextran phase system. B, Phase contrast micrograph of these cells, C, *Nostoc* filaments in liquid culture. Scale bar=50 μm.

(Table 1). Results for amylase activity were ambiguous; in three *Nostoc* preparations equally high activity was encountered, and in three others consistently approximately sevenfold lower activity was found relative to that of the cultured form. With most other enzymes tested (phosphatase,

TABLE 1. *Enzyme distribution in the soluble fraction of freshly isolated and of cultured* Nostoc

Enzyme	Activity (% ± S.E.M.)	
	Freshly isolated *Nostoc*	Cultured *Nostoc*
Hexokinase	100	100
Phosphorylase	19 ± 2·6	n.d.
Disproportionating transglycosylase	1148 ± 289	39 ± 9·4
Amylase	1476 ± 589 187 ± 36	1257 ± 197
Maltase	n.d.	0·3 ± 0·1
Phosphoglucomutase	384 ± 92	323 ± 28
Phosphatase	58 ± 12	45 ± 6·3
ADP-glucose pyrophosphorylase	6·2 ± 2·0	12 ± 2·3
Glycogen synthase	156 ± 67	104 ± 12

The data are average values from two to six and eight independent preparations from freshly isolated and cultured *Nostoc*, respectively. The respective absolute values of hexokinase activity were 3·42 μmol h^{-1} g^{-1} wet weight and 2·30 μmol h^{-1} mg^{-1} protein.
n.d. = not detected.

phosphoglucomutase, ADP-glucose pyrophosphorylase and glycogen synthase) there were no gross differences in activity distribution (Table 1). Maltase activity, although very low, was observed only in the cultured cells. In freshly isolated cells no such activity was detected with the usual enzymatic test. In a more sensitive radiochemical assay no formation of labelled glucose from [^{14}C]maltose could be found either. Obviously, free glucose is not formed by maltase action; rather the especially high activity of D-enzyme may provide this sugar in appreciable amounts by the disproportionation of maltooligosaccharides.

Glucose excretion may be influenced not only by a set of cytosolic enzymes but also by a membrane-bound glucose uptake system. Therefore, rates of glucose uptake were compared in the two types of *Nostoc* cells. The cultured form had a nine- to 15-fold higher rate of glucose uptake than *Nostoc* prepared from the thallus (Fig. 2). Average values were 32·2 and 3·14 nmol h^{-1} mg^{-1} protein originating from seven and five independent experiments for cultured and symbiont forms, respectively.

Kinetics of CO_2 fixation, glucose release, glucose uptake and enzyme activities of the freshly isolated cyanobiont

When the freshly isolated *Nostoc* was allowed to assimilate $^{14}CO_2$, 22–29% of the total fixed ^{14}C was recovered in the medium [*cf.* Fig. 3 (top), time zero]; the average from five experiments was 25%. In TLC analysis of the released material only one radioactive peak was detected at the position of glucose. After treatment with glucose oxidase, all radioactivity migrated as far as did gluconic acid (Meindl & Loos 1990). From this, glucose is concluded to be the sole released organic product of the photobiont of *Peltigera horizontalis*

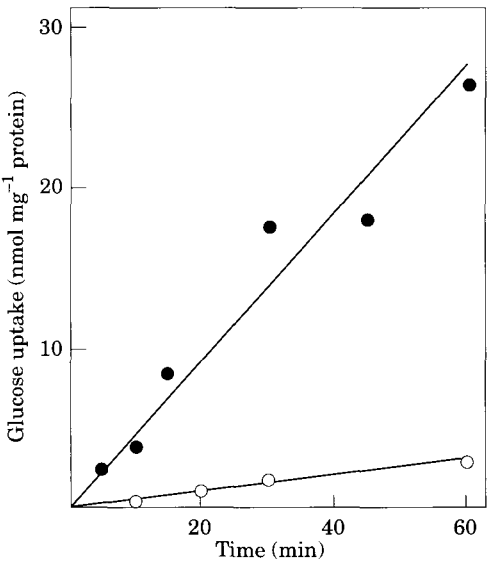

FIG. 2. Uptake kinetics of [^{14}C]glucose by freshly isolated (○) and cultured (●) *Nostoc*. The glucose concentration employed (5 mM) was well above the K_m values for uptake (1·15 and 0·8 mM, respectively, data not shown). For experimental details see Materials and Methods.

(Richardson *et al.* 1968; Meindl & Loos 1990). Based on the specific activity of $^{14}CO_2$, absolute rates of glucose release were calculated that amounted to 0·57 μmol h^{-1} g^{-1} wet weight on average (five experiments). To follow the kinetics of CO_2 assimilation and glucose release, samples from a stock suspension were taken at intervals and tested. As shown in Fig. 3 (top), between 0 and 5 h there were no major changes in $^{14}CO_2$ fixation, whereas the amount of released material dropped severely within 1 h representing only 6% of total ^{14}C fixed after 3–5 h. Two further experiments gave similar results.

To see whether the decrease in glucose release was due to the appearance of a more efficient glucose uptake system (*cf.* Fig. 2), glucose uptake was measured over the same period of time; the rate of uptake, however, stayed more or less constant at a low level (Fig. 3, bottom). The activities of phosphorylase and D-enzyme, which had been found to be much higher in the freshly isolated cells (Table 1), did not change in the course of 5 h either, nor did the amylase activity (Fig. 3, bottom). The rapid drop in glucose release cannot be due to an increase in rate of glucose uptake or to a decrease in activity of the mentioned enzymes. For the change in these activities on transition to the cultured status obviously a much longer adaptation time is required.

Intracellular concentrations of glucose and related metabolites in *Nostoc* immediately and 3 h after isolation and in cultured *Nostoc*

Determination of the intracellular concentrations of glucose and glucose-6-phosphate revealed for all cell stages at least an 18-fold greater concentration of the free sugar than that of the corresponding 6-phosphate

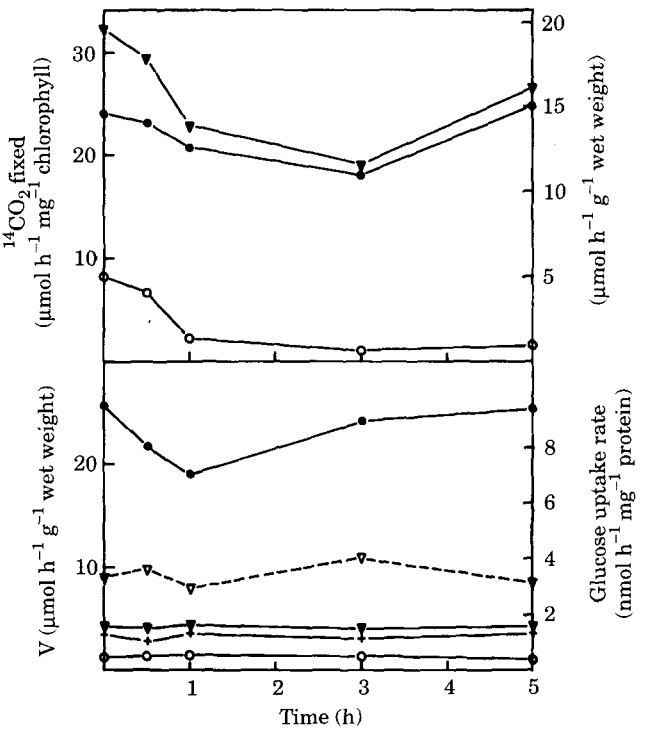

FIG. 3. Kinetics of CO_2 assimilation and glucose release (top) and of glucose uptake and enzyme activities (bottom) by the freshly isolated cyanobiont from *P. horizontalis*. Top: ▼, total $^{14}CO_2$ fixed; ●, ^{14}C in cells; ○, ^{14}C released (=glucose). Bottom: ▽, rate of glucose uptake; ●, D-enzyme; ▼, hexokinase; +, amylase; ○, phosphorylase. A stock suspension of freshly isolated *Nostoc* was shaken at 28°C in BG11 medium (Stanier *et al.* 1971) containing 0·1 M KCl under an illumination of 22·6 W m^{-2} and gassed with a 2% CO_2–air mixture. At the indicated times samples were withdrawn and used for the various tests as described in Materials and Methods. The data on glucose uptake, D-enzyme and phosphorylase are the averages from two preparations; those on amylase and hexokinase are from one and three preparations, respectively. All values are corrected for the percentage of living cells which was determined with the phenosafranine method (see Materials and Methods), and which decreased, for example, from 96% to 63% within 5 h.

(Table 2A). The latter was at the limits of detectability especially with the non-cultured cells, which were available in relatively smaller amounts. In freshly isolated *Nostoc*, a rather high glucose concentration of 17 mM was determined, which in the course of 3 h dropped by 24%. In another set of experiments (see Table 2B), somewhat lower concentrations of glucose+ glucose-6-phosphate were measured (probably due to the different extraction procedure) and within 3 h of isolation of slight decline (by 14%) was again observed. This was in contrast to the pronounced decrease in glucose release over the same time span (by 80%; *cf.* Fig. 3, top), which conclusively was not due to depletion of the internal glucose pool.

Possible precursors of intracellular glucose might be maltooligosaccharides and a glucan, which has been termed glycogen on the grounds of its large

TABLE 2. *Intracellular concentrations of glucose and related compounds in* Nostoc *immediately and 3 h after isolation and from culture*

Metabolite	Isolated *Nostoc* Time after isolation 0 h	Isolated *Nostoc* Time after isolation 3 h	Cultured *Nostoc*
A	Concentration (mM ± S.E.M).		
Glucose	16·8 ± 0·52	12·8 ± 0·76	2·60 ± 0·22
Glucose-6-phosphate	0·2 ± 0·12	0·7 ± 0·30	0·13 ± 0·08
B	Concentration (g l^{-1})		
Glucose+ Glucose-6-phosphate	1·79 (=9·9 mM)	1·54 (=8·6 mM)	0·13 (=0·7 mM)
Maltooligosaccharides	2·15	2·72	0·55
Glycogen	15·4	13·6	2·77

A: Extraction essentially according to Bieleski (1982); average values from three experiments. B: Procedure employing cell breakage and extraction/precipitation with ethanol. Details are given under Materials and Methods, including determination of intracellular volumes.

molecular mass, its sensitivity to amyloglucosidase and the occurrence of glycogen in other cyanobacteria (Bogner *et al.* 1993); circumstantial evidence exists for its degradability also by endogenous amylase in *Nostoc* homogenates (Bogner *et al.* 1993). The contents of the mentioned carbohydrates did not show any major changes within the first 3 h of cyanobiont isolation; distinctly lower concentrations of these metabolites and also of glucose were found, however, in cultured *Nostoc*. They amounted to only one quarter or less of those of the freshly isolated photobiont (Table 2).

Discussion

Nostoc from *P. horizontalis* was isolated with a yield of 22%, which clearly exceeds that obtained in previous work on two related *Peltigera* species (6–7%; Drew & Smith 1967*a*; Green & Smith 1974), but still is far from complete recovery as reported for cephalodial *Nostoc* from *P. aphthosa* (Millbank & Kershaw 1969). A merit of the method employed here seems to be the relatively low contamination with mycobiont particles. This has been achieved by combining differential centrifugation with partitoning in an aqueous two-phase system. With the latter technique, specific accumulation of whole cells, for example of certain *Chlorella* strains at the interface has been shown by Albertsson (1958, 1971).

Several enzymes of carbohydrate metabolism were studied in the freshly isolated cyanobiont and in the cultured form to understand the formation of glucose, which is the transfer carbohydrate in the intact thallus (Drew & Smith 1967*b*; Hill & Smith 1972; Richardson *et al.* 1968). In newly isolated *Nostoc* maltase activity was not detected and therefore obviously plays no role in glucose formation. In free-living cyanobacteria this enzyme also seems to be missing (Martel *et al.* 1992) or present with very low activity (Table 1; Fuchs

et al. 1994). Of minor importance in providing free glucose is an α-glucosidase from the cultured cyanobiont cleaving *p*-nitrophenyl-α-D-glucoside (Bogner *et al.* 1993); this enzyme evidently is identical with an isomaltase of low activity (Fuchs *et al.* 1994). Formation of glucose from an α-1,4-glucan, suggested by the drastically elevated level of disproportionating transglycosylase (D-enzyme) in the freshly isolated *Nostoc* is a further possibility and would involve the following reaction sequence: (1) the glucan is broken down by amylase largely to maltooligosaccharides; (2) these are disproportionated by D-enzyme to free glucose (to be released from the cell) and maltodextrins; (3) the maltodextrins are broken down again amylolytically. Such a scheme would agree with the proposed role of D-enzyme as a means of providing free glucose (Fuchs *et al.* 1994). The reason for the absence of measurable phosphorylase activity in the cultured cyanobiont and its presence in the freshly isolated *Nostoc* cells remains unclear. As compared to the much higher amylase activity and regarding its exo-type of action, phosphorylase should hardly contribute to the formation of maltooligosaccharides from higher maltodextrins.

After isolation of *Nostoc* from the thallus, around 25% of total fixed ^{14}C was recovered as glucose released to the medium, which was half as much as was found by Drew & Smith (1967*a*) but much more than the 3·4% calculated from the data of Green & Smith (1974). In all cases glucose release decreased with time, a severe drop occurring after 1 h (in the present work), after 36 h (Drew & Smith 1967*a*) or in less than 3 h (Green & Smith 1974). These quantitative discrepancies may well be due, at least in part, to the different cell preparation techniques employed and the different species of *Peltigera* used.

Absolute values for glucose release were calculated on the basis of the specific radioactivity of the $^{14}CO_2$ used. To estimate also glucose originating from non-labelled pools, different determination methods (e.g. an enzymatic one) have to be employed. After preliminary experiments we feel that glucose values that are too high are obtained in this way due to partial lysis of cells, especially after longer times of incubation. Appropriate corrections appear difficult at the moment.

The decrease in glucose release (at least of labelled glucose) might be caused by changes in enzyme activities in glucan and glucose metabolism, and, as consequence, of a diminished intracellular concentration of free glucose (see Chambers *et al.* 1976). No kinetic correlation, however, was found between this concentration and the drop in glucose release. Nor was there such a correlation between release and the activities of amylase, D-enzyme and phosphorylase, these latter two enzymes being much reduced or lacking only after prolonged culture. Alternatively, rapid loss of glucose-releasing capability might be caused by the appearance of an effective glucose uptake system. In fact, glucose is taken up by the cultured cyanobiont much more efficiently than by the immediately isolated one (see Fig. 2), as indicated also in the work of Drew & Smith (1967*a*). But again, the kinetic behaviour does not fit the picture: no increase in rate of glucose uptake occurred within 5 h of isolation (see Fig. 3). Therefore the fast decline in glucose release obviously is not due to changes in intracellular glucose concentration, possibly relevant enzyme activities or glucose uptake rate. To follow the long-term changes of these

characters during transition to the cultured state seems difficult since several hours after cyanobiont isolation bacterial contamination becomes a problem in liquid culture.

Thus, our observations do not favour cytoplasmic components as points of regulation for glucose release. Further investigations should, therefore, be aimed at regulatory mechanisms affecting the cytoplasmic membrane or its components. Chemical or mechanical signals might be involved in such a mechanism as discussed by Smith (1974, 1975) and Chambers *et al.* (1976). Perhaps a carrier responsible for glucose efflux is down-regulated after isolation of *Nostoc* from the lichen; a different carrier system should be involved in glucose uptake since this process stays constant for at least 5 h after isolation. An understanding of the decrease in glucose release of the freshly isolated cyanobiont will perhaps help to elucidate the physiologically important shut-down of glucose transfer occurring in intact thalli with incomplete hydration (Tysiaczny & Kershaw 1979; MacFarlane & Kershaw 1981).

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