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Photosynthesis by *in situ* and Isolated Prochloron (Prochlorophyta) Associated with Didemnid Ascidians

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Abstract

Prochloron cells isolated from their symbiotic association with the didemnid ascidian *Trididemnum cyclops* have a light-saturated net photosynthetic rate of 148 μmol O_2 mg chla^{-1} h^{-1}. Light saturated *in situ* photosynthesis occurs at approximately 32% of this rate. Comparable results were obtained with isolated and *in situ Prochloron* associated with small colonies of *Lissoclinum patella* and with discs cut from large colonies. In the latter, prolonged washing (42 hr) reduced *in situ* photosynthesis to 7% of the photosynthetic capacity of corresponding released algal cells. *In situ* photosynthesis, for both associations, incorporates a greater proportion of photosynthetically-fixed carbon into the algal protein fraction (c. 20%) than occurs from photosynthesis of isolated *Prochloron* (<5%). For both associations, approximately 20% of photosynthetically-fixed carbon is transferred to the host, but only in *L. patella* was there significant incorporation into the TCA-insoluble fraction, much of it associated with the test. It is concluded that restriction of *in situ* photosynthesis by the host helps to match algal proliferation to the growth rate of the host.

Keywords: *Prochloron*, photosynthesis, symbiosis, ascidians

1. Introduction

Symbiotic associations between unicellular algae and invertebrate hosts require for their survival a mechanism for controlling the size of the algal populations. This may be achieved by digestion of surplus algae, ejection of surplus algae or restriction of algal cell division (Muscatine and Pool, 1979).

Digestion of algae has been reported for tridacnid clams (Yonge, 1936, 1953), where it takes the form of a slow systematic removal and utilization by amoebocytes of degenerate zooxanthellae from the mantle edge of the clam (Fankboner, 1971). Its significance in maintaining steady-state algal populations is, however, questionable. Extrusion of excess zooxanthellae has been demonstrated for a number of anthozoan species such as zoanthids (Reimer, 1971) and sea anemones (Steele, 1976). It usually follows changes in environmental conditions and involves algal cells in all phases of the life cycle. It has the potential, therefore, to be an efficient regulatory mechanism.

Maintenance of steady-state algal populations by restricting their proliferation is best known for *Hydra viridis* where the control may be exerted through nutrient limitation (suggested by Pool, 1976), through the influence of growth inhibitors secreted by the host (suggested by Pardy and Heacox, 1976) or through a linkage between symbiont and host cell mitosis (suggested by McAuley, 1981). There is, as yet, very little direct evidence for any of these different mechanisms.

We have studied the problem of regulation in the symbiotic association between the prokaryotic green alga *Prochloron* and two ascidian hosts *Lissoclinum patella* and *Trididemnum cyclops*. In these symbiotic systems, the algal cells are located extracellularly, embedded in mucilaginous or fibrous material lining the cloacal cavity of the host colony. Such a location would clearly present special problems of regulation and the mechanisms involved might be expected to be somewhat different from those in associations, such as those referred to above, having closer physical contact between the two partners. We present evidence here that, for two ascidian species, the host regulates *in situ* photosynthesis, and, by implication, the growth rate of their algal symbionts. We also show marked differences between the two species in the nature of the contribution of algal photosynthesis to the animal host.

2. Materials and Methods

The ascidian species, *Lissoclinum patella* (Gottschaldt, 1898) and *Trididemnum cyclops* (Michaelsen, 1921) were chosen because of the ease with which their algal symbionts can be isolated into a buffered sea-water medium (ASW8, Provasoli 1958, buffered with 1.2% HEPES instead of Tris) by gentle squeezing of the host colony. Microscopic examination confirmed that the algal suspension so obtained was free of animal cells or host tissue fragments.

Photosynthesis of *in situ* or isolated *Prochloron* was measured at 25°C either as light-dependent oxygen evolution (using a Clark-type oxygen electrode) or as light-dependent $^{14}CO_2$ uptake from supplied $NaH^{14}CO_3$ (using standard liquid scintillation counting techniques). *In situ* photosynthesis of *Prochloron* associated with *T. cyclops* or with small colonies of *L. patella* was measured using whole colonies either still attached to their substratum or, if removed from the substratum, then allowed a period of recovery (assessed as active pumping through the cloacal aperture). For large colonies of *L. patella*, *in situ* photosynthesis was measured using discs cut from the colony and allowed to recover in flowing sea water. Such discs consist of an upper layer of test (3–4 mm thickness), a middle layer of algal cells (in the cloacal cavity) and a basal test of the same thickness and firm gelatinous texture as the upper test (Kott, 1980).

For comparison of the gross pattern of $^{14}CO_2$ fixation by *in situ* and isolated *Prochloron*, the algae, after the period of incubation in modified ASW8 medium containing $NaH^{14}CO_3$, were extracted in boiling 80% ethanol for 5 min. After a further three washings in cold 80% ethanol, the pellet was extracted with 5% trichloroacetic acid (TCA) at 95°C for 30 min, centrifuged and washed with cold 5% TCA. The ethanol extracts were combined, acidified to remove inorganic ^{14}C and dried down under a stream of warm air. The TCA extracts were also combined and dried down. Radioactivity in these dried extracts and in the TCA-insoluble residue was measured by liquid scintillation spectrometry. Quenching in each fraction was corrected for by an external standard using appropriate quench correction curves. An alternative fractionation procedure using a methanol-chloroform-formic acid (MCF) mixture followed by partitioning into aqueous and organic phase (Kremer et al., 1982) was used to further subdivide the alcohol-soluble compounds.

Radioactive compounds accumulating in the host tissues following *in situ* $^{14}CO_2$ fixation were estimated as previously described (Griffiths and Thinh, 1983) using chlorophyll as a marker for algal contaminants remaining in the host tissue after separation of host and symbiont. Radioactivity in the host tissue, after correction for that in the residual algal contaminants, was estimated for the different fractions as described above. Radioactivity associated with photosynthetic products excreted into the outside medium was measured by scintillation counting after removal of inorganic ^{14}C by acidification and aeration for 12 hr.

Rates of photosynthesis are expressed as μmol O_2 evolved mg chl$a^{-1}h^{-1}$ (oxygen electrode data) or as μmol CO_2 fixed mg chl$a^{-1}h^{-1}$ (^{14}C data).

For the latter, radioactivity in the supplied $NaH^{14}CO_3$ solution was measured by scintillation counting of an aliquot and the total CO_2 in the sea water medium by Gran titration (Mackereth et al., 1978).

3. Results

Photosynthesis by in situ and isolated Prochloron

Net photosynthesis by *in situ* and isolated *Prochloron* associated with *T. cyclops* is shown in Fig. 1a for a range of irradiance levels. Photosynthesis of isolated *Prochloron* saturates at c. 250 μE $m^{-2}s^{-1}$. *In situ* photosynthesis increases with increasing irradiance to a maximum (at c. 2,500 μE $m^{-2}s^{-1}$) which is only 33% of the maximum obtained with isolated *Prochloron* cells (Fig. 1b).

Similar results obtained with *Prochloron* associated with *Lissoclinum patella* are summarized in Table 1 where, to account for high respiratory oxygen uptake by host tissues (especially as excised discs), values for gross photosynthesis are presented. For small (young) colonies light-saturated *in situ* photosynthesis is approximately 20% of maximum photosynthesis of the same *Prochloron* cells after removal from the host. Discs cut from large colonies have a lower rate of *in situ* photosynthesis which declines further with time of washing after cutting. *Prochloron* cells isolated from the same discs, although photosynthesizing at a lower rate than those isolated from small colonies, do not show a similar decline in photosynthetic capacity with time after cutting. Thus, after 42 hr washing, photosynthesis of intact discs is only 7% of that of the corresponding isolated algal cells.

The above results with *L. patella* suggest that if the lower rate of *in situ* photosynthesis of these colonies is due to mutual shading of the algae within the cloacal cavity and/or shading by the test material of the host, the effect cannot be countered by increasing the incident irradiance. Incident irradiance of 750 μE $m^{-2}s^{-1}$ on the surface of a colony of *L. patella* reduces to 530 μE $m^{-2}s^{-1}$ after passage through a 3 mm layer of test to reach the algae below. The colonies clearly have an efficient mechanism for controlling algal photosynthesis even under high irradiance. The mechanism operates without permanently affecting the photosynthetic capacity of the algae. Similarly, with excised discs of *L. patella*, *in situ* photosynthesis after prolonged washing is only a fraction of the full photosynthetic capacity of the released algal cells.

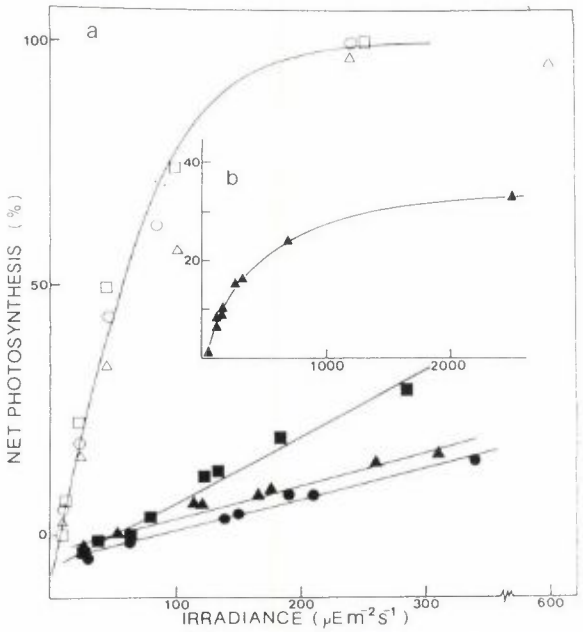

Figure 1. (a) Net photosynthetic oxygen evolution as a function of irradiance by *in situ* (closed symbols) and isolated (open symbols) *Prochloron* associated with *Tridemnum cyclops* (three experiments). Photosynthesis expressed as a percentage of the assimilation ratio for isolated *Prochloron* at saturating irradiance (100% = 148μmol O_2 mg chl$a^{-1}h^{-1}$). The curve fitting the data for isolated *Prochloron* is the hyperbolic tangent function. Average respiratory O_2 uptake by whole colonies 5.36μmol O_2 mg chl$a^{-1}h^{-1}$ (38% due to host respiration, 62% due to algal respiration).
(b) Results for *in situ Prochloron* shown for an extended irradiance range (one experiment only).

Table 1. Photosynthesis of isolated and *in situ Prochloron* associated with small colonies or discs cut from large colonies of *Lissoclinum patella*

	Light saturated gross photosynthesis (μmol O_2mg chl$a^{-1}h^{-1}$)[1]	
	In situ Prochloron (2,120μE$m^{-2}s^{-1}$)	Isolated *Prochloron* (400μE$m^{-2}s^{-1}$)
(1) Small colonies (5–10 mm diam.)		
Experiment 1	30.4	133.9
Experiment 2	29.0	162.5
(2) Discs cut from large colonies		
17 hr after cutting	19.6	77.2
23 hr after cutting	7.1	75.0
42 hr after cutting	6.3	84.4

[1] All values are means of duplicate determinations.

Gross pattern of products of photosynthetic CO_2 fixation by in situ and isolated Prochloron

For both *in situ* and isolated *Prochloron* of *T. cyclops* photosynthetic incorporation of $^{14}CO_2$ into the algal cells was linear over a 4 hr incubation period (Fig. 2). Under 150 $\mu E m^{-2} s^{-1}$ irradiance, the rate of incorporation by *in situ* photosynthesis was c. 21% of that by corresponding isolated *Prochloron*. After 4 hr incubation, a large proportion of the radioactivity fixed from $^{14}CO_2$ appeared in the ethanol-soluble fraction of both isolated and *in situ Prochloron*. The TCA-insoluble (protein) fraction following *in situ* photosynthesis accounted for 19% of the total cellular radioactivity compared with only 4% following photosynthesis by isolated *Prochloron*. *In situ Prochloron* also had a much greater proportional incorporation into the organic phase and into the insoluble residue following extraction with a methanol-chloroform-formic acid (MCF) mixture than was the case with isolated *Prochloron*.

For both isolated and *in situ Prochloron* of *T. cyclops* radioactivity associated with organic compounds excreted to the bathing medium never exceeded 2% of the total fixed. Dark $^{14}CO_2$ fixation for both *in situ* and isolated *Prochloron* was less than 2% of that in the light. Incorporation of radioactivity from $^{14}CO_2$ by *in situ* and isolated *Prochloron* of small colonies of *L. patella* is shown in Fig. 3. The relative rates and patterns of incorporation for *in situ* and isolated *Prochloron* are essentially similar to those recorded above for *T. cyclops* symbionts.

Photosynthetic CO_2 fixation by *Prochloron* isolated from *T. cyclops* or from *L. patella* was unaffected by addition of an aqueous extract of the appropriate host tissue. In neither case was there any change in the extent of excretion of photosynthetic products into the outside medium in the presence of host extract, neither was there any marked effect on the gross pattern of photosynthetic products within the cells.

Transfer of photosynthate from symbiont to host

For the ^{14}C experiments, part of the discrepancy between *in situ* photosynthetic CO_2 fixation and that of isolated *Prochloron* cells may be accounted for by photosynthetic products transferred from symbiont to host. In the experiments summarized in Fig. 4, between 14 and 18% of the total ^{14}C fixed in the light by colonies of *T. cyclops* (4 hr incubation) was associated with the host tissue, similar to that previously reported for this species (Griffiths and Thinh, 1983).

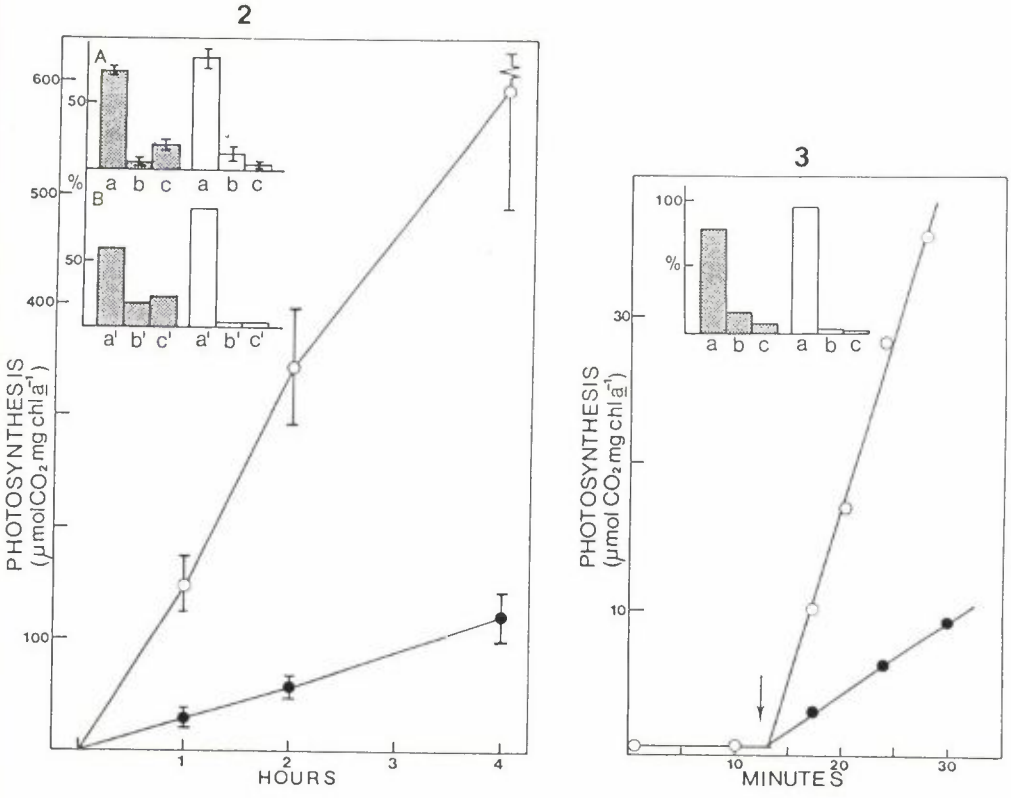

Figure 2. Photosynthetic carbon fixation by *in situ* (•) or isolated (○) *Prochloron* associated with *Trididemnum cyclops*. Irradiance: 150μE $m^{-2}s^{-1}$. Means of three experiments ± standard error; each experiment with duplicate samples, 20–30 colonies (or the equivalent *Prochloron* suspension) per sample. Inset A: % distribution of radioactivity from fixed $^{14}CO_2$ in ethanol-soluble (a), TCA-soluble (b), and TCA-insoluble (c), fractions for *in situ* (closed histograms) and isolated (open histograms) *Prochloron* after 4 hr incubation. Inset B: Similar % distribution into MCF extract, aqueous phase. (a^1), organic phase (b^1) and MCF-insoluble residue (c^1) after 1 hr incubation.

Figure 3. Photosynthetic carbon fixation by *in situ* (•) or isolated (○) *Prochloron* associated with small colonies of *Lissoclinum patella*. Arrow shows start of light period (430μE $m^{-2}s^{-1}$). Means of duplicate samples. Inset: % distribution of radioactivity from fixed $^{14}CO_2$ in ethanol-soluble (a), TCA-soluble (b) and TCA-insoluble (c) fractions for *in situ* (closed histograms) and isolated (open histograms) *Prochloron* for the 30 min samples.

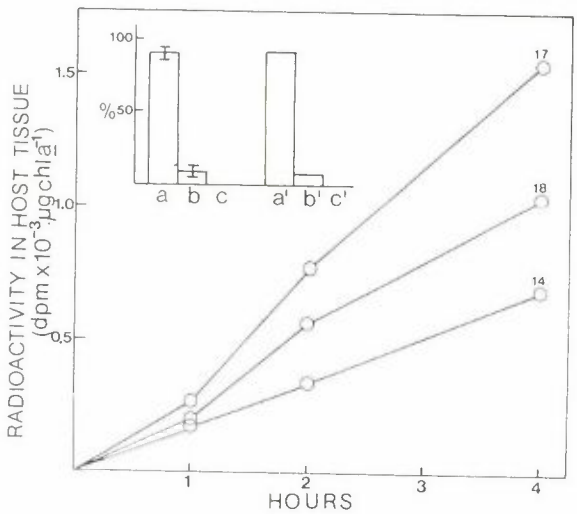

Figure 4. Incorporation of radioactivity (normalized per unit chlorophyll *a* in the intact colonies) from fixed $^{14}CO_2$ into host tissue of the *Prochloron* — *Tridemnum cyclops* association. Three experiments, each with duplicate samples (20–30 colonies per sample). Numbers adjacent to 4 hr sample denote radioactivity in host tissue as a % of total fixed radioactivity. Irradiance: 150μE $m^{-2}s^{-1}$.
Inset: % distribution of radioactivity in ethanol-soluble (a), TCA-soluble (b) and TCA-insoluble (c) fractions (means of three experiments ± standard error) or in MCF extracts, aqueous phase (a^1), organic phase (b^1) and MCF-insoluble residue (c^1) (one experiment) of host tissue after 4 hr incubation.

Most of the radioactivity in the host tissue was in the form of ethanol-soluble compounds or in the aqueous phase of the MCF extract (Fig. 4, inset). There was only low incorporation into the TCA-soluble fraction or the organic phase of the MCF extract and none into the TCA-or MCF-insoluble fraction.

With either small colonies or excised discs from large colonies of *L. patella* there was a similar degree of transfer from algae to host over a 1.5 hr period (but higher levels of transfer with short-term incubation) (Table 2). The pattern of incorporation of photosynthetically fixed carbon into the host tissue was, however, very different. In particular there was significant incorporation into the TCA-insoluble fraction (sometimes in excess of 50% of the total transferred).

Table 2. Incorporation of radioactivity from photosynthetically fixed $^{14}CO_2$ into host tissue in the *Prochloron-Lissoclinum patella* association.
Irradiance: $350\mu Em^{-2}s^{-1}$.

	Radioactivity in host tissue			
	% of total fixed radioactivity	% distribution of radioactivity in host tissue fractions		
		Ethanol-soluble	TCA-soluble	TCA-insoluble
(1) Whole colonies (1.5 hr incubation)				
Colony 1 (5.2)[1]	15	45	15	40
Colony 2 (4.7)	12	25	13	62
Colony 3 (9.5)	17	40	6	54
Colony 4 (16.7)	16	52	3	45
(2) Discs from large colony (7 hr washing)[2]				
10 min. incubation	53	54	19	27
30 min. incubation	26	41	26	33
1 hr incubation	32	44	29	27
1.5 hr incubation	18	39	37	24

[1] Fresh weight of colony (gm)

[2] Mean of duplicate determinations

Periodic excision and analysis of fragments of the bulky test of large colonies of *L. patella* during the course of incubation showed a light-stimulated incorporation of radioactivity from supplied $^{14}CO_2$ into test material (shown by microscopic examination to be free of zooids or algal cells) (Fig. 5a). Incorporation was linear for at least six hours. During darkness there was usually a net loss of radioactivity from the test. Over a 6 hr period of illumination, most of the incorporated radioactivity became associated with the TCA-soluble fraction of the test material but there was significant incorporation into both the ethanol-soluble fraction and the TCA-insoluble residue (Fig. 5b). The greatest loss of radioactivity in the dark was from the TCA-soluble fraction of the test material. Throughout the 26 hr of Experiment I (including 17 hr of darkness) there was a progressively increased incorporation of radioactivity into the TCA-insoluble fraction of the test (Fig. 5b).

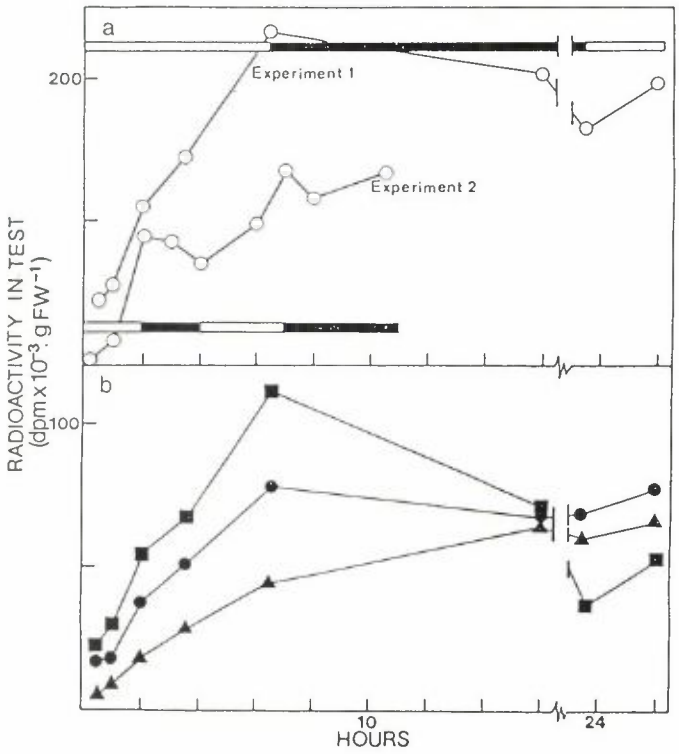

Figure 5. (a) Incorporation of radioactivity from fixed $^{14}CO_2$ into the test of *Lissoclinum patella* in light ($150\mu Em^{-2}s^{-1}$) and darkness (2 experiments). The black bar at the head of the figure indicates the period of darkness for Experiment 1, those at the bottom of the figure refer to Experiment 2.
(b) Incorporation of radioactivity into the ethanol-soluble (•), TCA-soluble (▪) and TCA-insoluble fractions (▲) of Experiment 1 (above).

4. Discussion

In both *T. cyclops* and *L. patella* the *in situ* algal symbionts photosynthesize at rates well below that of which they are capable when separated from the host. Containment within the host cloacal cavity reduces photosynthesis, even at irradiances well above that encountered in the ascidian's natural environment, by approximately 70%. The increased photosynthesis of isolated *Prochloron* is largely directed towards the synthesis of ethanol-soluble products.

The nature of the control exerted by the host may be physical, chemical or both. There is clearly some attenuation of light by the test as is shown by our results with *L. patella* and those of Alberte et al. (1986) who report, for

the same species, a 60 to 80% absorbance of incident light by the host tissues. Further, there would be mutual shading within the algal layer where the cells are densely concentrated into a relatively small volume. Such confinement may also affect the supply of CO_2 and perhaps the build up of photosynthetically produced oxygen. The reduced level of *in situ* photosynthesis of discs of *L. patella* compared with that of colonies of the same species — and its progressive further decline with subsequent wound healing of the discs (Lewin, personal communication) suggests that the rate of circulation of water through the cloacal cavity of the intact colony may affect photosynthesis of the contained algae.

If there is a chemical component to the control of *in situ* photosynthesis, it could not, under the conditions of our experiment, be substituted by an aqueous extract of the host tissue. Such extracts were also without effect on the excretion of photosynthetic products by isolated *Prochloron*. This contrasts with what has been previously described for some endosymbionts like the zooxanthellae isolated from *Tridacna crocea* (Muscatine, 1967) whose excretion of photosynthetically fixed ^{14}C increases in the presence of host tissue homogenate from 2 to 37% of the total fixed.

Whilst excretion from isolated *Prochloron* was always low, that from *in situ Prochloron* (in the form of photosynthetic products transferred to the host) accounted for approximately 20% of the total photosynthesis for both *T. cyclops* and *L. patella*. There was, however, a marked difference between the two hosts in the nature of the translocated products which accumulated in the animal tissues. In particular, the TCA-insoluble host fraction of *T. cyclops* received no contribution from algal photosynthesis whilst in *L. patella* there was appreciable incorporation of photosynthetically fixed carbon into this fraction, much of it associated with the test. The difference between *T. cyclops* and *L. patella* in this respect may be related to the massive nature of the test in the latter species.

Light saturated photosynthesis of isolated *Prochloron* (e.g. 175 μmol CO_2 mg chl$a^{-1}h^{-1}$ for *Prochloron* from *T. cyclops*) is well within the range reported for natural phytoplankton but much lower than the highest values reported by Alberte et al. (1986) for *Prochloron* isolated from *L. patella*. Although we have no direct knowledge of the *in situ* growth rates of *Prochloron* of *T. cyclops*, what indirect evidence we have from a similar species, *Diplosoma virens*, suggests that it is considerably lower than even the slower growing natural phytoplankton. Thus, from our measurement of the rate of proliferation of colonies of *Diplosoma virens* on artificial substrates

(Thinh et al., 1981) we estimate that the colonies (and, by inference, the contained *Prochloron*) have a doubling time of 9 days ($K = 0.033 \log_{10} day^{-1}$). Lewin et al. (1984), from their observations of cell division frequencies, estimated a doubling time of 6 days for *Prochloron* from the same host ascidian ($K = 0.050 \log_{10} day^{-1}$). By expressing the production increments due to *in situ* photosynthesis in the same units as the algal biomass, we can obtain a direct comparison with the growth rates shown above. Thus, a rate of *in situ* photosynthesis of 40μmol C mg chl$a^{-1}h^{-1}$ (see Fig. 2) approximates to 0.144μg Cμg C^{-1} day^{-1} (assuming a 12 hr day and a C:chl*a* ratio of 40) and a K value of 0.058, similar to the *in situ* growth rate constants referred to above.

It is suggested that the ascidian host controls proliferation of its associated alga by restricting *in situ* photosynthesis. The mechanism involved is yet to be determined but a significant component must be attributed to physical constraints of photosynthesis operating within the host colony.

Acknowledgements

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