AUTOTROPHIC MICROBIOLOGY AND ONE-CARBON METABOLISM

ADVANCES IN AUTOTROPHIC MICROBIOLOGY AND ONE-CARBON METABOLISM

Vol 1



Autotrophic Microbiology and One-Carbon Metabolism

Edited by

GEOFF A. CODD The University, Dundee, Scotland

LUBBERT DIJKHUIZEN University of Groningen, The Netherlands

and

F. ROBERT TABITA Ohio State University, Columbus, Ohio, USA



Kluwer Academic Publishers Dordrecht / Boston / London ISBN-13: 978-94-010-7384-4 e-ISBN-13: 978-94-009-1978-5 DOI: 10.1007/978-94-009-1978-5

Published by Kluwer Academic Publishers, P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

Kluwer Academic Publishers incorporates the publishing programmes of D. Reidel, Martinus Nijhoff, Dr W. Junk and MTP Press.

Sold and distributed in the U.S.A. and Canada by Kluwer Academic Publishers. 101 Philip Drive, Norwell, MA 02061, U.SA.

In all other countries, sold and distributed by Kluwer Academic Publishers Group, P.O. Box 322, 3300 AH Dordrecht, The Netherlands.

printed on acid-free paper

All Rights Reserved © 1990 by Kluwer Academic Publishers Softcover reprint of the hardcover 1st edition 1990 No part of the material protected by this copyright notice may be reproduced or utilized in any form or by any means, electronic or mechanical including photocopying, recording or by any information storage and retrieval system, without written permission from the copyright owner.

Table of Contents

Preface	vii
1. The Biology of the Prochlorales T. BURGER-WIERSMA and H.C.P. MATTHIJS	1
 Inorganic Carbon Transport and Accumulation in Cyanobacteria A.G. MILLER 	25
 Hydrogenases in Lithoautotrophic Bacteria B. FRIEDRICH and C.G. FRIEDRICH 	55
4. Denitrification by Obligate and Facultative Autotrophs L.A. ROBERTSON and J.G. KUENEN	93
 Formate Dehydrogenase: Microbiology, Biochemistry and Genetics J.G. FERRY 	117
 The Biochemistry and Genetics of C₁ Metabolism in the Pink Pigmented Facultative Methylotrophs P.M. GOODWIN 	143
 C₁ Metabolism in Anaerobic Non-Methanogenic Bacteria J.H.F.G. HEIJTHUIJSEN and T.A. HANSEN 	163
 Biochemistry and Applications of Alcohol Oxidase from Methylotrophic Yeasts J.R. WOODWARD 	193
Index	227

Preface

Autotrophic and methylotrophic microorganisms are able to grow at the expense of one-carbon compounds (e.g. carbon dioxide, formaldehyde) as the principal carbon sources for the synthesis of cell material, using light, inorganic compounds or one-carbon compounds as energy sources. The study of the special adaptations required in aerobic and anaerobic microorganisms to sustain an autotrophic or methylotrophic mode of life is a fascinating field of research for scientists from various disciplines. Current research efforts not only focus on fundamental aspects, i.e. metabolic pathways and their regulation, ecology, energy conversion and genetics, but also the possible application of these organisms, in waste water treatment, degradation of xenobiotics, single-cell protein production, as biocatalysts for the production of fine chemicals, draws strong attention.

The aim of this series is to provide annual reviews on the biochemistry, physiology, ecology, genetics, and application of microbial autotrophs and methylotrophs. The scope of the series includes all aspects of the biology of these microbes, and will deal with phototrophic and chemolithotrophic prokaryotic autotrophs, carboxydobacteria, acetogenic-, methanogenic- and methylotrophic bacteria, as well as methylotrophic eukaryotes.

The exciting advances made in recent years in the study of these organisms is reflected in the chapters of this first volume which have been written by experts in the field. We would like to express our sincere thanks to all the contributors for their stimulating and comprehensive chapters.

G.A. Codd L. Dijkhuizen F.R. Tabita

1. The Biology of the Prochlorales

T. BURGER-WIERSMA and H.C.P. MATTHIJS

Laboratorium voor Microbiologie, Universiteit van Amsterdam, Nieuwe Achtergracht 127, 1018 WS Amsterdam, The Netherlands

Introduction

The organisms belonging to the Prochlorophyta (Lewin 1976) are oxygenic phototrophic prokaryotes which contain chlorophylls *a* and *b*. Until recently (Lewin 1975) this combination of pigments was thought to be restricted to eukaryotic cells only. Due to this combination of eukaryotic and prokaryotic features their taxonomic position has been subject to discussion for several years. In 1986 Florenzano et al. proposed a new order, the *Prochlorales*, in the class *Photobacteria* (Gibbons and Murray 1978) to accommodate organisms with the aforesaid properties. This prompted Burger-Wiersma et al. (1989) to refer to them as oxychlorobacteria instead of prochlorophytes.

At present, the discovery of three oxychlorobacterial species has been reported, i.e. the marine symbiont *Prochloron didemni* (Lewin 1977), the freshwater planktonic *Prochlorothrix hollandica* (Burger-Wiersma et al. 1986), and a marine picoplanktonic strain (Chisholm et al. 1988). The discovery of these new prokaryotes has stimulated speculation about their phylogeny (e.g. Lewin 1984; Walsby 1986; Cox 1986b) and many studies are focussed on the relatedness of the oxychlorobacteria to cyanobacteria on the one hand and green chloroplasts on the other.

Habitat

The three oxychlorobacteria inhabit fairly different habitats. The oxychlorobacterium described by Chisholm et al. (1988) has been detected in the Pacific and North Atlantic Oceans. Pigment analyses suggested that similar organisms may inhabit the Banda Sea, Indonesia (Gieskes et al. 1988). In all instances maximal abundance appeared to be restricted to the deeper layers of the euphotic zone. However, Gieskes and Kraay (1983) reported an unusual pigment composition in the surface waters of the tropical Atlantic Ocean, which might be associated with oxychlorobacteria also.

Up to now, Prochlorothrix hollandica has only been isolated from the

shallow, highly eutrophic Loosdrecht Lakes system in The Netherlands (Burger-Wiersma et al. 1986, 1989). There is now evidence of significant numbers of this species in several comparable lakes in The Netherlands (L. Van Liere personal communication; J. Van den Does personal communication; T. Burger-Wiersma unpublished). The Loosdrecht Lakes system originated from peat excavation. Due to shallowness of the lake, no thermal stratification develops and the phytoplankton is homogeneously mixed throughout the entire water column. In summer *Prochlorothrix* is one of the dominating species besides several filamentous cyanobacteria (Van Liere et al. submitted). The organism is most abundant in those parts of the lake where the availability of phosphorus is relatively more limiting for phytoplankton growth.

During *Prochlorothrix* blooms the pH varies between 8 and 10, and the water temperature is 15-25 °C (Burger-Wiersma et al. 1989). Based on the optimum temperature curve for growth, these authors proposed that the need for elevated temperatures to exhibit substantial growth might restrict the habitat of *Prochlorothrix* to shallow systems where complete mixing of the entire water column takes place throughout the year. This allows inoculation from the sediments when temperature increases, a situation almost never encountered in deeper lakes.

Thus far Prochloron didemni is exclusively found as an extracellular symbiont of marine tropical and subtropical sessile tunicates, the ascidians (e.g. Lewin 1981; Müller et al. 1984; Cox 1986a). It can occupy the external surface, the test or the cloacal cavity of its host, and this may have implications for the nature of the symbiosis. Cox (1986a) discriminates three types of symbiosis based on the aforesaid locations of the *Prochloron* cells: the association with cells inhabiting the external surface of the hosts is clearly not obligate for the host, but appears to be obligate for *Prochloron*. The larvae of the ascidians do not have special modifications to carry the symbiont, infection must be accomplished by *Prochloron* cells carried by seawater. The ascidian species with *Prochloron* embedded in the test are never observed without their symbionts. Therefore, the association is likely to be obligate for both partners. The larvae of this group are especially equipped for transmitting Prochloron cells to daughter colonies. The ascidians with cloacal cavity-dwelling Prochloron cells may be found without their symbionts, although their larvae have special mechanisms to accommodate the symbiont in order to inoculate the daughter colonies. However, no phylogenetic differences between strains of *Prochloron* isolated from different hosts could be shown using molecular techniques like 16S ribosomal RNA sequence homology (Stackebrandt et al. 1982) or DNA-DNA reassociation (Stam et al. 1985).

The difference in habitats is primarily expressed by the temperature and light climate encountered by the three oxychlorobacteria.

At the depth where the latest discovered oxychlorobacterium occurs most

abundantly, the temperature ranges from 10 to 15 °C (Chisholm et al. 1988). Maximal increase in numbers of *Prochlorothrix* is found when the ambient water temperature is 20 to 25 °C (T. Burger-Wiersma, unpublished results). This coincides nicely with the growth rate vs temperature relationship which was reported for this organism (Burger-Wiersma et al. 1989). *Prochloron* is abundant in tropical waters where seasonal variation in temperature is low and ambient temperatures of approximately 30 °C are customary. However, the organism is also found in areas with a more dynamic seasonality (McCourt et al. 1984, Müller et al. 1984). In these areas a close correlation between water temperature and the number of *Prochloron*-containing colonies was shown (McCourt et al. 1984). This observation agrees well with the sensitivity of photosynthesis at low temperatures (Thinh and Griffiths 1977; Alberte et al. 1986, 1987).

There is only little light attenuation in the tropical waters where *Prochloron* is found; the ambient photon flux density may range from 1000 to 2500 μ mol m⁻²s⁻¹ (Thinh and Griffiths 1977; Pardy 1984; Alberte et al. 1986, 1987). However, the real light climate experienced by *Prochloron* may be much lower in the case of the organism being embedded inside its host. Alberte et al. (1986) determined a decrease in photon flux density of 60 to 80% attributable to the animal tissue. Contrasting to *Prochloron, Prochlorothrix* is found in rather turbid waters characterized by a steep light gradient. Due to mixing of the entire water column, the organisms are exposed to rapidly changing photon flux densities ranging from complete darkness to levels of incident irradiance. Further studies may elucidate whether these light conditions are advantageous for the abundant presence of *Prochlorothrix*. There is very little information on the light levels encountered by the deep ocean species. The ambient photon flux density reported for this strain is 1 to 10 % of the incident irradiance (Chisholm et al. 1988).

Morphology and Ultrastructure

The morphology of the three oxychlorobacterial species reported so far is rather diverse. *Prochloron didemni* is a spherical unicell with a diameter ranging from 10 to 25 μ m (Cox 1986a). The size appears to be independent of the sampling station, host species and location in or on the host, although the larvae may carry smaller cells (Cox 1986a). In contrast, *Prochlorothrix hollandica* is filamentous with long cylindrical cells (Burger-Wiersma et al. 1986). The individual cells are 3 to 10 μ m long and 0.5 to 1.5 μ m in diameter, but unfavourable growth conditions may increase both cell length and diameter (Burger-Wiersma et al. 1989). The straight, undifferentiated, sheathless trichomes are generally composed of 5 to 25 cells, but trichomes consisting of more than 100 cells have been observed also. The oceanic freeliving species is unicellular and coccoid to rod-shaped, but much smaller than *Prochloron*, i.e. 0.6 to 0.8 μ m (Chisholm et al. 1988). Transmission electron micrographs of the oxychlorobacteria reveal their prokaryotic nature (Lewin 1975; Schulz-Baldes and Lewin 1976; Burger-Wiersma et al. 1986, Chisholm et al. 1988).

The electron-dense layer surrounding the cytoplasm of the organisms indicated the presence of peptidoglycan in the cell walls. This was confirmed by cell wall analysis of *Prochloron* (Moriarty 1979; Stackebrandt and Kandler 1982) and *Prochlorothrix* (Jürgens and Burger-Wiersma 1989).

Polyhedral bodies have been observed in all species. Immuno-electronmicroscopy has revealed that the polyhedral bodies in *Prochloron* and *Prochlorothrix* are carboxysomes since they contain the CO₂-assimilating enzyme of the Calvin cycle, ribulose 1,5-bisphosphate carboxylase/oxygenase [RuBisCO] (Berhow and McFadden 1983; Codd 1988; Hawthornthwaite and Codd 1988). The carboxysomes in *Prochlorothrix* are generally located in the central cytoplasmic region, though mainly in the vicinity of the thylakoid membranes. In *Prochloron* they usually occur in the peripheral region of the cells, either singly or in small clusters (Griffiths et al. 1984; Cox 1986a). Different opinions have emerged on the presence or absence of membranes surrounding the carboxysomes in *Prochloron*. Schulz-Baldes and Lewin (1976) reported a lack of such a membrane, Griffiths et al. (1984) observed proximity to an adjacent thylakoid membrane and Cox and Dwarte (1981) described a bounding tripartite membrane and suggested it to be a modified thylakoid membrane.

Gas vesicles have been reported to be present in *Prochlorothrix* at the cell poles (Golecki and Jürgens 1989). Pressure nephelometry showed that the constituent gas vesicles had a mean critical pressure of approximately 9 bar, which is within the range encountered in phytoplanktonic cyanobacteria (A.E. Walsby, personal communication).

In general, the thylakoids of the oxychlorobacteria are arranged in parallel layers at the periphery of the cytoplasm around a thylakoid-free central area (Cox 1986a; Burger-Wiersma et al. 1986; Chisholm et al. 1988). In *Prochloron* they may also occupy the central region of the cells (Schulz-Baldes and Lewin 1976). In the latter case the thylakoids are either randomly distributed and separated by so-called thylakoidal sacs (Griffiths et al. 1984; Thinh 1978; Thinh et al. 1985; Cox 1986a), or the cells are packed with thylakoids (Cox 1986a). This morphological difference may be caused by light conditions since the two types of cells were found on the external surface and in the cloacal cavity, respectively. This would agree with the observation, that in *Prochlorothrix* a low growth irradiance induced an increase in stacking and number of thylakoids (H.C.P Matthijs et al., unpublished).

The ultrastructure of *Prochloron* has been studied in greater detail than that of the other two species (Griffiths et al. 1984; Thinh et al. 1985; Cox 1986a). Different results on the location of DNA in *Prochloron* have been reported. Several authors described its presence in the central region of the cells (Schulz-Baldes and Lewin 1976; Whatley 1977; Cox 1986a). This agrees with the position of DNA in the other two oxychlorobacteria (Burger-Wiersma et al.

1986; Chisholm et al. 1988). One report mentioned the uncommon aggregation of DNA in Prochloron (Whatley 1977) and Coleman and Lewin (1983) observed its peculiar disposition in areas between the thylakoid membranes. Especially intriguing is the presence of the large crystalline bodies in Prochloron obtained from different, but not all, hosts (Griffiths et al. 1984; Thinh et al. 1985). These bodies are significantly larger than the carboxysomes. They are composed of regularly arranged sub-structures and appear to be closely related to the thylakoid membranes, either merging with them or emerging from them. The authors suggest that the production of these large crystalline bodies may represent the response of the organism to less favourable conditions. These conditions have been shown to be accompanied by the formation of excessive amounts of phenolic compounds and coagulation of proteins (Fall et al. 1983; Barclay et al. 1987). The latter process may induce changes in the ultrastructure of the cells. To some extent this may explain unusual features like the formation of large crystalline bodies, thylakoidal sacs and DNA disposition or aggregation.

The oxychlorobacteria demonstrate tight packing of the thylakoid membranes and differ in this respect from cyanobacteria. This may be simply due to the absence of steric restraint of phycobilisomes or merely be a quasimechanical consequence of the presence of chlorophyll b in these organisms (Walsby 1986). The functional reasons for thylakoid membrane organization into appressed membrane stacks is not very well understood (Miller and Lyon 1985). Although chlorophyll b containment by an organism goes hand in hand with thylakoid membrane appression, chloroplasts of barley mutants depleted in chlorophyll b nevertheless demonstrate thylakoid membrane stacking and so-called lateral heterogeneity of photosystems 1 and 2 (Miller and Lyon 1985). Such a localization of the 2 types of photosystems in different patches of the thylakoid membrane very interestingly has also been documented for both Prochloron (Giddings et al. 1980) and Prochlorothrix (Miller et al. 1988). This suggests that, in marked contrast to cyanobacteria, 'true' stacking may occur in these organisms. The presence of chlorophyll b in these differently organized thylakoids is of great interest for a better understanding of any additional role for this pigment besides light-harvesting (Barber 1986).

Physiology and Ecology

Growth Characteristics

In contrast to earlier attempts, Chisholm and co-workers have succeeded in growing the deepsea oxychlorobacterium in laboratory cultures (S.W. Chisholm personal communication). Details on optimum growth conditions must await further studies.

Thus far, there has been only one account of successful cultivation of *Prochloron* in laboratory cultures (Patterson and Withers 1982). In this study

the cells were grown in a mineral seawater medium and several organic nitrogen and carbon sources were tested for their growth promoting abilities. Of all the organics tested only L-tryptophan and a combination of indole and serine affected the growth of *Prochloron* in a positive way. The latter two compounds can act as precursors in the synthesis of tryptophan. Further tests revealed that the organism might be deficient in anthranilate synthase. These results might indicate the nature of the obligatory relationship between *Prochloron* and its host. Growth was further maximized by an initial pH of 5.5 (Patterson and Withers 1982). The latter result is rather surprising since several studies emphasize importance of a pH 8-buffered system for isolated cells in order to keep them photosynthetically active (Thinh and Griffiths 1977; Critchley and Andrews 1984; Alberte et al. 1986). In spite of the growth promoting measures applied by Patterson and Withers (1982) less than four doublings could be achieved in these cultures.

Lectins have been detected in the association of *Prochloron* and its host (Müller et al. 1984). Lectins might be fruitful in the propagation of isolated *Prochloron* based on results obtained in the culturing of certain Pseudomonads symbiotic with sponges.

As opposed to *Prochloron*, *Prochlorothrix* can be easily grown in a mineral medium without organic supplements, although all efforts to grow the strain in axenic cultures have failed thus far. Based on these observations, Burger-Wiersma et al. (1989) suggested that growth of *Prochlorothrix* might be dependent on substrates provided by the contaminating heterotrophic bacteria. Optimum growth was found at pH 8.4, significantly different from that found for *Prochloron* (Patterson and Withers 1982). Maximal growth rate occurred at 25 °C, rapidly decreasing at temperatures below 20 °C (Burger-Wiersma et al. 1989). Growth of *Prochlorothrix* was inhibited by NaCl at concentrations exceeding 25 mM. At 100 mM NaCl or its equivalent seawater concentration growth ceased completely. This could be explained by the inability of the strain to synthesize organic osmotica when it was subjected to these osmotic upshocks (Burger-Wiersma et al. 1989; R.H. Reed personal communication).

Carbon Metabolism

A general consensus on the operation of C3-type carbon dioxide fixation has been arrived at by measurements of the early carbon fixation products and detection of appreciable RuBisCO and phosphoribulokinase activity in cellfree extracts of *Prochloron* (Akazawa et al. 1978; Berhow and McFadden 1983; Kremer et al. 1984). The operation of the C3 pathway has also been made very likely for *Prochlorothrix* (Hawthornthwaite and Codd 1988).

Excretion of glycolic acid by isolated cells of *Prochloron* after exposure to light has been reported (Fisher and Trench 1980). This could be indicative for photorespiration to occur in *Prochloron*. Especially with regard to the symbiotic nature of *Prochloron*, initial products of carbon dioxide fixation

have been analyzed (Akazawa et al. 1978, Fisher and Trench 1980, Kremer et al. 1982). These products included 3-phosphoglycerate, sugar-phosphates, polyglucose, maltose, glucose, fructose, glutamate, aspartate and glycolate; sucrose was not detected. None of these products has been earmarked to fulfill a role in the translocation of fixed carbon compounds between *Prochloron* and its host. The photosynthesis products very much point to a type of intermediary metabolism commonly found in prokaryotic cells, illustrated by the lack of sucrose as a primary product of photosynthesis. The finding of substantial amounts of α -1,4- glucan in *Prochloron* may indicate a special type of secondary metabolism possibly occurring because of the interaction with the host (Akazawa et al. 1978). Fredrick (1980, 1981) also speculated about the meaning of the presence of α -1,4 glucan and concluded that this branched polycarbohydrate did not help in further establishing the phylogenetic position of *Prochloron*.

Measurements of enzyme activity in *Prochloron* have frequently been reported to be hampered by the unusual high content of phenolic compounds in isolated cells and the coagulation of proteins. Until now they are limited to the successful assay of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, phosphoribulokinase and RuBisCO (Fall et al. 1983, Barclay et al. 1987). Nevertheless, Andrews et al. (1984) and Berhow and McFadden (1983) elegantly succeeded in the characterization of the RuBisCO enzyme from *Prochloron*. The K_m for CO₂ (9 μ M) was an order of magnitude higher than that normally found for carboxylase from chloroplasts, but corresponds very well to the usually encountered values in cyanobacteria (Berhow and McFadden 1983; Andrews et al. 1984). These observations point to a close relatedness of the *Prochloron* enzyme and cyanobacterial RuBisCO. Berhow and McFadden (1983) obtained active enzyme with a final specific activity of 1.5 units per mg protein (comparable to the activity of enzyme obtained from other organisms) from Prochloron cells that were lyophilized directly after harvest. The subunit composition of 57.5 (large, L) and 18.8 (small, S) kDa and the sedimendation position on isopycnic sucrose gradients corresponded to the normally observed molecular mass ranges of occurrence of this enzyme with a L and S subunit stoichiometry of 8 to 8 (L8S8) (Codd 1988). Andrews et al. (1984) succeeded in cross-reconstitution of the Prochloron and cyanobacterial enzymes, a result which further demonstrated the close relationship between *Prochloron* and cyanobacteria.

Nitrogen Metabolism

Prochlorothrix can use either ammonium or nitrate ions as nitrogen source (Burger-Wiersma et al. 1989). As in most photoautotrophs, growth is inhibited at higher ammonium levels, probably due to uncoupling of photosynthetic electron transfer.

Parry (1985) showed that *Prochloron* cells in intact associations incorporated ammonium but not nitrate ions. The absence of nitrate uptake

is explained by the ammonium-rich microhabitat of the *Prochloron* cells in the cloacal cavities of their hosts. In such an environment, nitrate reductase, necessary for the assimilation of nitrate ions, is absent and its induction may take several hours. Although intact colonies were incubated, no nitrogen incorporation in the ascidian was detected. Therefore, the author's conclusion that the ascidian acquires usuable nitrogenous compounds is unjustified.

Low but definite nitrogenase activity was shown only in associations of *Prochloron* and its host *Lissoclinum patella* (Paerl 1984). Other associations collected at the same location and at the same period were unable to fix dinitrogen. This may explain why the *Lissoclinum patella/Prochloron* association is most abundant in the oligotrophic regions of its habitat. The capability to fix molecular nitrogen might be restricted to intact colonies since no nitrogenase activity was detected in either isolated *Prochloron* cells or in ascidians free of *Prochloron*. In spite of the extreme oxygen sensitivity of nitrogenase, light enhanced nitrogenase activity. Paerl (1984) assumed a very low oxygen content of the seawater inside the cloacal cavity of the host because of a high oxygen consumption rate of the cloacal tissue. It is however questionable whether the decrease in oxygen in the microhabitat of the cloacal cavity is adequate to guarantee a sufficiently low oxygen level within the *Prochloron* cells.

Parry's assumption (1985) that his results, in which ammonium uptake was demonstrated, conflict with those on the occurrence of dinitrogen fixation (Paerl 1984) is disputable. Dinitrogen-fixing cells switch to ammonium uptake whenever ammonium is available since growth on ammonium is more efficient. Furthermore, omission of direct measurement of the ambient ammonium levels invalidates the claim that the ammonium levels within the cloacal cavity of the host would be too high to allow nitrogen fixation.

Attempts to induce nitrogenase activity in *Prochlorothrix* were unsuccessful indicating that this organism is unable to fix molecular dinitrogen (Burger-Wiersma et al. 1989). A new free amino acid, 3-(N-methylamino)glutaric acid, was identified in extracts of *Prochloron* isolated from different, but not all, hosts and appeared to be present in isolated host material also (Summons 1981). This amino acid was not detected in *Prochlorothrix* (Volkman et al. 1988).

Symbiosis of Prochloron

The relationship between host and symbiont has been subjected to many studies, most of them focussing on the exchange of metabolic intermediates.

Low molecular weight products were shown to be transported following $^{14}CO_2$ fixation in the intact association of host and *Prochloron* (Fisher and Trench 1980; Griffiths and Thinh 1983). The identity of the translocated components was not established. Isolated cells of *Prochloron* were found to excrete glycolic acid (Fisher and Trench 1980), which accounted for seven percent of the CO₂ fixed. This would be insufficient to account for the 50%

photosynthate translocation reported by Griffiths and Thinh (1983), but agrees well with other translocation estimates of 7% (Akazawa et al. 1978) and 15 to 19% (Alberte et al. 1986). Interestingly, Berhow and McFadden (1983) concluded that the presence and activity of RuBisCO relative to the chlorophyll content are in normally encountered ranges. This indicates that factors other than CO₂ fixation must determine the need for symbiosis of *Prochloron*. Addition of extracts from the ascidian host did not improve the excretion of photosynthates from isolated *Prochloron* cells. In this respect the results differ from those obtained in other host/zooxanthellae relationships (Fisher and Trench 1980).

Although the symbiosis between *Prochloron* and its host is obviously obligatory for *Prochloron* (Lewin 1981) there are only a few reports indicating that the organism actually benefits by the association. Kremer et al. (1982) reported that the photosynthetic rates in *Prochloron* were decreased by a factor of three after isolation of the cells. Müller et al. (1984) indicated the presence of a cytostatic compound made by the host, which was active in mouse lyphoma cells but which did not inhibit proliferation of *Prochloron*. Its proposed function is to help in maintaining a unique environment for *Prochloron* and to keep out other bacteria from the host. Otherwise the production of lectins by the host only in presence of *Prochloron* may specifically contribute to a proper environment for the symbiont. The lectins were shown to bind to the glycoproteins of *Prochloron*, as to greatly reduce the tendency for strong aggregation of the *Prochloron* colonies.

In some cases the exchange of metabolites and nutrients may provide a logical explanation for phenomena observed in naturally occurring associations of *Prochloron* and its host. Unfortunately, these speculations are almost never substantiated. Olson (1983) observed that the larvae of Didemnum molle can carry low-light adapted cells of Prochloron. He suggested that this was necessary to ensure a high photosynthetic activity after settlement of the larvae since the juveniles were dependent on the translocation of photosynthates. In a later study Olson (1986) showed a relationship between light intensity and growth rate of Prochloron/Didemnum molle associations. This agrees well with the results of Bachmann et al. (1985), who observed an increasing colony-size with increasing light intensities; the smallest colonies were found at depths over 25 m and were devoid of *Prochloron* cells. The size of the colonies was suggested to depend on the translocation of photosynthates from Prochloron to its host. According to these authors, this would be indicated by the fast death of the colonies after being deprived of light. This speculation, however, is in contradiction with their own conclusion that the association is facultative for the host. More probably, the fast death of the association was an artifact since Olson (1986) has described the survival of Prochloron/Didemnum molle associations after nine days' exposure to darkness.

Parry (1985) suggested the translocation of ammonium ions from the host to the symbiont. He based this proposal on a combination of observations.

The oxychlorobacterial cells showed ammonium and no nitrate assimilation, whereas the ammonium content of the tropical reef water was very low.

Photosynthesis

The maximal rates for carbon dioxide fixation in the light in *Prochloron*, ranging from 100 to 1000 μ mol CO₂/mg chl/h (Fisher and Trench 1980; Berhow and McFadden 1983; Critchley and Andrews 1984; Kremer et al. 1984, Alberte et al. 1986) correspond to the rates commonly observed in cyanobacteria and chloroplasts. Carbon dioxide fixation rates in the dark were less than 5 % (Fisher and Trench 1980) or 1 % (Alberte et al. 1986) of those in the light. From the data of Burger-Wiersma and Post (1989) we calculated a range of 280 to 620 μ mol CO₂/mg chl/h. In this study the differences between rates were due to different growth irradiances.

The variation in observed carbon fixation rates in Prochloron were far too large to be attributed to the different ambient light conditions. Most likely they were caused by the method of isolating the cells from their host. Usually the cells are isolated by gently squeezing the colonies. In this process however, the acid-containing vesicles of the hosts may be ruptured, thereby liberating sulphuric acid into the isolation medium. Thinh and Griffiths (1977) reported a complete loss of photosynthetic activity after isolation of the cells in a nonbuffered system. Collection of the isolated cells into a buffered system (pH 7.5) still resulted in a 50-75 % loss of photosynthesis. Apparently, Alberte et al. (1986, 1987) observed an increase in photosynthetic activity after isolation of the cells. They reported a maximal oxygen evolution rate of 912-1188 μ mol O₂/mg chl/h for cells isolated from *Lissoclinum patella* (Alberte et al. 1986); a value high in the range usually found for cyanobacteria and green algae. Whole colonies of the same species showed a maximal oxygen evolution rate of 26.4-40.8 μ mol O₂/mg chl/h (Alberte et al. 1987) which is low as compared to those reported in other studies (Thinh and Griffiths 1977; Pardy 1984). Therefore, caution should be taken in interpreting the results of Alberte et al. (1987), the more so as these authors stated that the values of maximal oxygen evolution rate in isolated and *in hospite* cells were roughly the same.

In general, all phototrophic organisms show the same adaptation pattern when grown in different photon flux densities, i.e. increased photosynthetic pigment levels at low photon flux densities in order to sustain optimum photosynthesis. The response of *Prochloron* and *Prochlorothrix* conforms to this general adaptation pattern: Burger-Wiersma and Post (1989) report a fivefold increase in chlorophyll content in *Prochlorothrix* when comparing cells grown at 200 and 8 μ mol photons m⁻²s⁻¹. In spite of the pigment increase at low photon flux densities a decrease in light utilization efficiency was found. Self-shading of chlorophyll molecules in the tightly packed thylakoids may have caused this decrease.

Alberte et al. (1986) observed a twofold increase in chlorophyll levels in *Prochloron* cells isolated from *Lissoclinum*-colonies growing at ambient

photon flux densities of 400 and 2200 μ mol m⁻²s⁻¹, respectively. The concurrent twofold increase in the efficiency of light utilization in *Prochloron* growing at the low irradiance suggests that the increase in pigments principally benefits antenna function. The enhancement of antenna function was further supported by the larger increase of chlorophyll *b* relative to chlorophyll *a* and by the simultaneous decrease of the number of reaction centres I and II per cell. Apparently, the antenna size is enlarged at the expense of the number of reaction centres.

Two strategies of light-shade adaptation based on the concept of photosynthetic units (PSU) can be distinguished. A PSU is defined as the number of photosynthetic pigment molecules involved in the production of one molecule of oxygen. Either the PSU changes in size due to changes in the amount of light harvesting antennae relative to the reaction centres, or the number of PSU's is altered upon a change in ambient photon flux density. Green algae are known to change both PSU size and number, whereas cyanobacteria show enlarged PSU sizes at low ambient photon flux densities, mainly due to increased phycobiliprotein levels. Similar to green algae, Prochloron and Prochlorothrix were shown to change both PSU size and number (Alberte et al. 1986; Burger-Wiersma and Post 1989). PSU size can be expressed either as the number of chlorophyll molecules per reaction center (RC) I or as the number of chlorophyll molecules per RC II. Highlight-grown Prochloron and Prochlorothrix cells had significantly smaller PSU sizes, based both on RC I and RC II values, than lowlight cells. However, the decrease in PSU/RC II in Prochlorothrix of 60% was large compared to that in *Prochloron* (25%) and to that of the decrease in PSU/RC I in both organisms (20-30%). From these measurements it can be concluded that Prochlorothrix adapts to low light by increasing the ratio RC I/RC II, an adaptation pattern usually observed in cyanobacteria. By contrast, the RC I/RC II ratio remained more or less constant in *Prochloron*, as is commonly found for eukaryotes (c.f. Alberte et al. 1986).

The maximal rate of oxygen evolution is determined by the number of RC II and by the rate at which the RC II can reopen after performing the primary reactions. This is related to the capacity of electron flow and thus by the rate at which electrons are transferred in linear electron transport. From the data of Alberte et al. (1986) we calculate a time constant of 3.4 ms for *Prochloron*, slightly lower than the 4 ms reported for *Prochlorothrix* (Burger-Wiersma and Post 1989). In both cases, this time constant appeared to be independent of ambient photon flux density suggesting that the rate of photochemistry is not affected by light-limited growth. These time constants for photosynthetic electron transport are well in the range found for green algae and diatoms, whereas for cyanobacteria longer time constants (ca. 10 ms) are reported (c.f. Burger-Wiersma and Post 1989).

Molecular Assembly

Pigments

Chlorophylls. The oxychlorobacteria characteristically contain both chlorophylls a and b. This observation was based on separation by chromatography (TLC and HPLC) followed by identification by spectroscopy (Lewin and Withers 1975; Burger-Wiersma et al. 1986). Additional fluorescence excitation and emission spectroscopy at 77 °K revealed peaks that were in accordance with those reported for isolated chlorophylls a and b, (chl a Exc. 429, Em.678 and chl b Exc. 478, Em 658) (Thorne et al. 1978; H.C.P. Matthijs unpublished). Freezing and thawing of the host animals before isolation of *Prochloron* cells was shown to be harmful to the chlorophylls in that an extensive conversion to phaeophytins a and b was noticed. This relates to the reports on phenolic and acid compounds being present in Prochloron under stress conditions (Fall et al. 1983; Barclay et al. 1987).

In the case of the recently described species from the deep ocean, both chlorophyll a and b appeared to be slightly modified to their bivinyl chlorophyll derivatives (Chisholm et al. 1988; S.W. Chisholm personal communication).

The pathways of chlorophyll synthesis in *Prochlorothrix* have been revealed to involve formation of d-aminolevulic acid from glutamate via a pathway which, in contrast to higher plants, did not depend on RNA as has been found for cyanobacteria (Rieble and Beale 1988).

Chlorophyll *a* is common to all oxygenic phototrophs and is present in both antennae and reaction center pigment beds. Chlorophyll b is known to be restricted to the light-harvesting antennae. The stoichiometry in chl a/b in light harvesting complex 2 of green chloroplasts is about unity, and more than four in the light harvesting complex of photosystem 1. The overall chlorophyll a to b ratio usually reflects the light conditions in which an organism grows. Low light induces a need for extended antennae, which in the case of green plant chloroplasts results in an increase of chlorophyll b relative to a and by consequence in a lowered chlorophyll a to b ratio. In comparison to chloroplasts with a chlorophyll *a* to *b* ratio of about three, the various findings of Prochloron demonstrate a ratio of 3 to 20 (Thorne et al. 1977; Paerl et al. 1984; Alberte et al. 1986), the ratio in *Prochlorothrix* amounts from 7 to 18 (Burger-Wiersma and Post 1989). The low light-adapted planktonic oxychlorobacterial species isolated from the deep euphotic zone of the ocean demonstrated a chlorophyll *a* to *b* ratio of 1 (Chisholm et al. 1988). These rather divergent numbers may be species-dependent or could be caused by the light conditions during growth.

Usually, a decrease of the ratio of chlorophyll a to accessory pigments is observed upon transfer of phototrophs from high to low photon flux densities due to the increased production of light harvesting pigments relative to

reaction centres. Different observations on whether low light conditions do give rise to a lowered ratio of chlorophyll a to b in the oxychlorobacteria have emerged. On the one hand, a relative increase in chlorophyll b content and a lower a/b ratio was reported in low light- adapted cells of *Prochloron* (Olson 1983, Bachmann et al. 1985; Alberte et al. 1986). Olson (1983) even showed a small but significant difference in chlorophyll a/b ratio between the top and bottom halves of *Prochloron*/ascidian associations. On the other hand, such a relationship could either not be demonstrated (Paerl et al. 1984) or was found to be even reversed (Thorne et al. 1977). Matthijs et al. (unpublished) have observed a decrease of the chlorophyll a/b ratio as a result of low light adaptation in *Prochlorothrix* grown in continuous cultures. However, an opposite adaptive pattern was also reported for Prochlorothrix (Burger-Wiersma and Post 1989). In these experiments *Prochlorothrix* increased its chlorophyll a/b ratio when grown at low photon flux densities. For *Prochlorothrix*, these controversial results may be explained by the difference in the growth techniques applied: in the experiments of Burger-Wiersma and Post (1989) the cultures were continuously illuminated, whereas Matthiis et al. grew the cultures at 16:8 light/dark cycles. Further experiments should disclose whether the above described differences in adaptative patterns for *Prochlorothrix* are in some way related to a possibly different organization of the photosynthetic apparatus, or to abnormalities in the composition of its constituting chlorophyll protein complexes (Matthijs et al. 1989).

Carotenoids. The carotenoid and xanthophyll type pigments determined in *Prochloron* and *Prochlorothrix* typically resemble those characteristically classified in cyanobacteria. A number of studies have revealed the presence of two major compounds, β , β -carotene and zeaxanthin. These compounds have been shown to make up more than 50 (up to 71) and 20 to 40 % of the total carotenoid pool, respectively (Withers et al. 1978a; Burger-Wiersma et al. 1986; Foss et al. 1987). The cryptoxanthin content amounts to about 5% on average. In addition mutachrome, echinenone, isocryptoxanthin and β , β -carotene monoepoxide have been regularly identified in trace amounts of less than 1 % of the total carotenoid content (Withers et al. 1978b; Foss et al. 1987).

No evident differences in the carotenoid distribution ranges in *Prochloron* and *Prochlorothrix* species have been found. Different carotenoid contents and differences in their relative presence have been determined in *Prochlorothrix* in response to photon flux density during growth (H.C.P. Matthijs unpublished). The carotenoid content of *Prochloron* with regard to the presence of β , β -carotene and zeaxanthin varies widely between different batches. No evident link to differences in irradiant light energy could be arrived at (Hiller and Larkum 1985; Alberte et al. 1986). The carotenoid synthesis pathways clearly differ from the ones found in green plants, i.e. alenic, ϵ -type carotenoids, carotenoid epoxides and glycosidic type carotenoids which are normally encountered synthesis products in chloroplasts were

14 T. Burger-Wiersma and H.C.P. Matthijs

lacking in Prochloron (Foss et al. 1987).

Omata et al. (1985) have separated the cell and thylakoid membranes of *Prochloron*. Zeaxanthin was nearly completely recovered in the cell membrane fraction. The thylakoid membrane contained most of the β , β -carotene, very much like in cyanobacteria. Interestingly however, the β , β - carotene content of the cell membrane fraction from *Prochloron* was distinctly higher than the one encountered in cell membranes from cyanobacteria. It was suggested that the cell membrane of *Prochloron* in this respect resembles the chloroplast envelope which normally contains β , β -carotene (Omata et al. 1985). The physiological function of β , β carotene (additional light-harvesting and/or protection against photooxidation), can be correlated with its linkage to the thylakoid membrane. The association of zeaxanthin with the cell membrane points to a light-shielding function only.

Components of the Photosynthetic Electron Transfer Chain

The light-harvesting complexes of the oxychlorobacteria were thought to be analogous to LHC2 of green chloroplasts, with a chl a to b ratio of about unity (Withers et al. 1978a). More recently, deviating a to b ratios of 2.4 for Prochloron (Hiller and Larkum 1985) and about 4 for Prochlorothrix have been reported (Bullerjahn et al. 1987). A further indication of the difference of the chlorophyll *a/b* complexes from *Prochlorothrix* and the LHC2 from chloroplasts is found in the lack of the typical negative deflection at about 650 nm, normally detected in CD spectra of LHC2 (Matthijs et al. 1989). Furthermore, biochemical analysis of the complexes has indicated a molecular mass of the major polypeptide of the chlorophyll a/b complexes of 31 to 34 kDa (Hiller and Larkum 1985; Schuster et al. 1984; Bullerjahn et al. 1987) which is high compared to a similar range of estimates for LHC2 (24 to 29 kDa). A lack of immunological cross-reactivity of the polypeptides from the chlorophyll *a/b*-protein complexes of both *Prochloron* and *Prochlorothrix* with antibodies raised against LHC2 from various plants and green algae was also reported. These data point to major differences between the green plant chloroplast LHC2 complexes and the chlorophyll a/b protein complexes of oxychlorobacteria. The observed differences may have an impact on the physiological role of the chlorophyll a/b -protein complexes. In the case of green plant chloroplasts, light energy captured by chlorophyll b contributes to photosynthesis mostly via photosystem 2. However, a significant contribution to photosystem 1 activity has been shown for *Prochloron* (Hiller and Larkum 1985) and *Prochlorothrix* (Bullerjahn et al. 1987; G.S. Bullerjahn et al. 1990; A.F. Post personal communication). Green plant chloroplast LHC1 also contains a minor amount of chlorophyll b. No data on a possible analogy between LHC1 and the chlorophyll protein complexes from the oxychlorobacteria are available at present.

Optimal transfer of electrons requires continuous adjustment of the light energy distribution to photosystems 1 and 2. To achieve this, the model for