PHYSIOLOGY AND BIOCHEMISTRY OF AUTOTROPHIC BACTERIA

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1. INTRODUCTION

The three years since the previous International Symposium on Microbial Growth on C_1 Compounds have seen numerous advances in our knowledge of bacterial autotrophy. Significant advances have been made in the recognition and characterization of "new" groups of autotrophs and in the understanding of the pathways of CO_2 assimilation, including the enzymes involved. Several routes of CO_2 assimilation in prokaryotes are recognised. The reductive tricarboxylic acid cycle in the green sulphur bacterium *Chlorobium* is well established [1], and a variant of this cycle, in which phosphoenolpyruvate carboxylase is replaced by pyruvate carboxylase, has been found in the aerobic obligate autotroph, *Hydrogenobacter thermophilus* [2]. The total synthesis of acetyl-CoA from CO_2 and H_2 is achieved by a linear pathway in the anaerobic, autotrophic bacterial methanogens, acetogens and most sulphate-reducers [3]. Advances on these organisms, and with autotrophic sulphate-reducing bacteria and sulphur-reducing archaeabacteria which assimilate CO_2 via a reductive citric acid cycle, are fully discussed elsewhere [3, Fuchs this symposium].

The Calvin cycle does not operate in the above organisms, but is responsible for CO_2 assimilation in most other known groups of autotrophic prokaryotes, besides algae and plants [4, 5]. Interest in the Calvin cycle in microbes continues since its regulation at the physiological and molecular levels varies between bacterial groups. Furthermore, the facility with which the key enzymes of the cycle can be studied and manipulated at the molecular and genetic levels, may be useful in attempts to alleviate constraints on higher plant productivity. Here we briefly review the diversity and physiological spectrum of CO_2-fixing bacteria and consider the occurrence and operation of the Calvin cycle in prokaryotes. Details of the regulation of the Calvin cycle at the molecular and genetic levels are considered elsewhere in these proceedings [Dow, Bowien, Friedrich, Tabita]. We provide an overview of molecular and genetic advances on the key enzymes, inorganic carbon accumulation and the compartmentation of CO_2 fixation in Calvin cycle bacteria. Finally, we briefly consider patterns of physiological regulation of the Calvin cycle in obligate and facultative autotrophs and some ecophysiological aspects. For a detailed discussion of physiological aspects the reader is referred to two excellent reviews by Dijkhuizen and Harder [6,7].

2. DIVERSITY AND THE PHYSIOLOGICAL SPECTRUM AMONG THE CO_2-FIXING BACTERIA

Among the aerobic and anaerobic litho-(autho)trophs as well as among the photo-(auto)trophs we see a spectrum of physiological types with respect to their energy source and/or their carbon source. Many organisms use CO_2 as the exclusive or major carbon souce under all growth conditions. Organisms in this category are also obligately dependent on light or their lithotrophic energy source. In a second category with a facultative metabolism, the energy source and/or carbon source can be replaced by organic compounds. Among the lithotrophs we find a third category in which CO_2 cannot be used as primary carbon source, but only in heterotrophic (anaplerotic) carbon metabolism. Among the phototrophic microbes the only organisms in this category are the halophilic bactaeria which use bacteriorhodopsin for energy generation. The spectrum has been long known for many lithotrophs and phototrophs and as knowledge increases, more and more microbial families or genera turn out to harbour these different metabolic types. For example, the complete spectrum is known for the aerobic sulphur oxidizers [8], nitrifying bacteria [9] iron oxidizers, and also for aerobic H_2 bacteria [10, 11]. Recently facultatively autotrophic *Beggiatoa* species have been described [12] as well as obligately and facultatively autotrophic methane producers [13], hydrogen/sulphur autotrophs [14, Stetter, this symposium] and autotrophic sulphate reducers [15,16]. Many of the anaerobic phototrophs are facultative autotrophs, for example the Rhodospirillacae. However, all known Chromatiaceae and Chlorobiaceae have had a very limited organic substrate range, while the known *Chlorobium* spp. cannot grow without light [17]. The importance of organic compounds in the metabolism of the facultative organisms may vary significantly. Many of the facultative lithotrophs grow much faster on organic compounds, i.e. heterotrophically or mixotrophically, than lithoautotrophically. By contrast the facultative photoautotrophs are all primarily phototrophs, which grow faster in the light, autotrophically or mixotrophically. In the latter group, heterotrophic potential merely seems to serve as a survival mechanism [17, 18].

3. OCCURRENCE OF THE CALVIN CYCLE IN AUTOTROPHIC BACTERIA

There is abundant evidence that the Calvin cycle is used by the colourless sulphur-oxidizing bacteria and carboxydotrophic bacteria among the chemolithoautotrophs [5, 6]. The nutritional capabilities of filamentous sulphide oxidizers such as *Beggiatoa* are beginning to be clarified. Freshwater *Beggiatoa* strains have not been grown autotrophically [19, W.R. Strohl pers. commun.] However, autotrophic growth by marine strains of *Beggiatoa* and the presence of the carboxylating enzyme of the Calvin cycle, ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCo) has been shown in this organism [12, Nelson pers. commun.] It seems, therefore, likely that these are Calvin cycle organisms. Chemolithoautotrophic growth of the iron-oxidizer *Gallionella* has also been obtained recently. The

presence of adequate levels of phosphoribulokinase (PRK) and RuBisCo in *G. ferruginea* suggests that this organism can fix CO_2 via the Calvin cycle [20, D. Hanert pers. commun].

Besides the purple sulphur bacteria, purple non-sulphur bacteria and cyanobacteria among the phototrophs the Calvin cycle appears to operate in the chlorophyll b-containing prokaryote *Prochloron* [21, 22]. The unequivocal demonstration of autotrophy in this organism has been prevented by the inability to obtain growth without its animal host. However, the recent discovery of a free-living filamentous chlorophyll-b containing oxygenic prokaryote which grows well in laboratory culture [23] provides exciting prospects for the study of CO_2 fixation and photosynthesis in these organisms of evolutionary importance. Although low PRK and RuBisCo activities were initially found in the green non-sulphur bacterium *Chloroflexus* [see 5], recent attempts to measure these enzymes in autotrophic cultures were negative and a novel CO_2 fixation pathway with acetyl-CoA as an intermediate has been indicated [24].

4. CALVIN CYCLE ENZYMES IN AUTOTROPHIC BACTERIA

Research on bacterial Calvin cycle enzymes has mainly concentrated on the 2 enzymes which are unique to, and essential for CO_2 fixation by this route: RuBisCo and PRK. In contrast to RuBisCo, PRK has received little attention until recently, although this enzyme is a major control point *in vivo*.

4.1 Phosphoribulokinase

PRK catalyzes the phosphorylation of ribulose 5-phosphate to produce the CO_2-acceptor, ribulose 1,5-biphosphate (RuBP). Allosteric regulation of PRK is chemolithoautotrophic and purple photosynthetic bacteria (activation by NADH; inhibition by AMP and/or PEP) is well established and consistent with the production of NADH during chemolithotrophy and anoxygenic photosynthesis [see 5]. In contrast, algal and plant chloroplast PRK's lack a requirement for NAD(P)H but their activities are increased in the dark by reduced sulphydryl compounds and by ferredoxin/thioredoxin photoactivation. Cyanobacterial PRK's are of particular interest in view of the postulated origin of chloroplasts from ancestral endosymbiotic cyanobacteria. The PRK's from the cyanobacteria *Chlorogloeopsis fritschii* and *Nostoc muscorum* (*Anabaena* 7119) resemble chloroplast PRK's in regulatory characteristics [25,26]. This apparent regulatory dichotomy can be viewed against the structural properties of the few PRK's so far purified (Table 1).

All of the prokaryotic PRK's are of high M_r and contain one class of subunit in a hexameric or octomeric enzyme. The eukaryotic PRK's are all low M_r dimers (Table 1). It will be of interest to know whether the *C. fritschii* PRK structure is typical for cyanobacteria and whether a molecular evolution of this enzyme in microbial autotrophs can be discerned. Heterologous DNA hybridization using probes to PRK genes will be of interest and a synthetic DNA probe to part of the *Alcaligenes eutrophus* H16 PRK [37] provides good opportunities for this approach. This probe hybridises with chromosomal and megaplasmid pHG1 DNA from *A. eutrophus* H16 indicating the dual location of PRK genes in this

organism. Powls et al [38] have shown that light-activated *Scenedesmus* PRK is derived from a single higher M_r protein which shows latent activites of PRK and NADPH-glyceraldehyde 3-phosphate dehydrogenase, thus raising the possibility of a common genetic origin for these enzymes in this alga.

Table 1. Molecular properties of phosphoribulokinase

Source	Native enzyme M_r	Subunit (Su) M_r	Proposed quaternary structure	Ref
A. PROKARYOTES				
Chromatium D	240,000	-	-	27
Rhodopseudomonas capsulata	220,000	36,000	6 Su	28
Rhodopseudomonas acidophila	248,000	32,000	8 Su	29
Alcaligenes eutrophus	256,000	33,000	8 Su	30
Chlorogloeopsis fritschii	230,000	40,000	6 Su	25
B. EUKARYOTES				
Chlamydomonas reinhardtii	-	42,000	-	31
Scenedesmus obliquus	84,000[a]	42,000	2 Su	31
Bryopsis maxima	90,000	41,000	2 Su	33
Tobacco	90,000	46,000	2 Su	34
Spinach	90,000	44,000	2 Su	35
Wheat	83,000	42,000	2 Su	36

[a] a latent 470,000 M_r hexadecamer also occurs in this alga [32].

4.2 Ribulose 1,5-bisphosphate carboxylase/oxygenase

RuBisCO's have been purified and characterized to varying extents from at least 40 prokaryotes [5] and recombinant DNA technology has been adopted for their study. The topic of RuBisCO is very large; entire symposia are devoted to it periodically [e.g. 39], and comprehensive coverage of RuBisCO research is not possible here. As in eukaryotes, most bacterial RuBisCO's are high M_r (ca. 500,000) proteins containing 8 large (L; 55,000-64,000 Daltons) plus 8 small (S; 10,000-16,500) subunits [5]. In all cases, RuBisCO is a bifunctional enzyme, catalyzing the carboxylation and oxygenation of RuBP, the latter being an apparently wasteful and currently unavoidable process. O_2 is a linear competitive inhibitor of carboxylation and vice versa [5,39, 40]. All RuBisCO's can exist in an inactive and active form, activation proceeding via an ordered reversible binding of a CO_2 at lysine residue 201 on the L subunit, followed by a divalent metal cation. These findings, plus the location of the single catalytic site for carboxylation and oxygenation at lysine 175 of the L subunits of apparently all enzymes [5, 39, 40] suggests a uniform mechanism of RuBisCO function throughout the Calvin cycle autotrophs. This is

consistent with the high degree of amino acid sequency homology among L subunits of diverse prokaryotes and eukaryotes [see 41] except *Rhodospirillum rubrum.* The *R. rubrum* enzyme, the only known 2L RuBisCO, may have taken a different evolutionary course.

S subunits are clearly not needed for activation and catalysis of the *R. rubrum* enzyme, or of 6L RuBisCO's from *Rhodopsendomonas sphaeroides* [42] and *Rps. capsulata* [43]. However, an essentiality for S subunits exists among 8L8S RuBisCO's. 8L "catalytic cores" from 3 cyanobacterial enzymes and *Chromatium vinosum* RuBisCO show negligible catalysis, although this is restored fully by reconstitution with homologous S subunits and partially by heterologous S subunit hybridization [44-46]. These findings, and differences in the activation and catalytic characteristics between 6L and 8L8S enzymes from *Rps. sphaeroides* [47] indicate a functional role of S subunits in maintaining the conformation of the active site on the L subunits. Strategies to improve plant RuBisCO should involve attempts to modify both subunits.

Several bacteria contain 2 forms of RuBisCO. *Rps. sphaeroides, Rps. capsulata* and *Rps. blastica* contain 8L8S enzymes besides the 6L form [42, 43, 48]. Differences in kinetic properties [42, 43], enzyme synthesis regulation [49, 50] and in L subunit structures in the 2 forms [51] strongly suggest that the L subunits of the different RuBisCO's in a single organism are different gene products. Multiple forms of RuBisCO have been reported in nitrifying bacteria and *Rhizobium* [see 52, 41] and the location and number of the respective genes in these organisms will be of interest.

The localization, cloning and heterologous expression of RuBisCO genes has been achieved from several purple bacteria, cyanobacteria and hydrogen bacteria [e.g. 30, 41, 53-57]. We have constructed a gene bank for *Chlorogloeopsis fritschii,* cloned the RuBisCO genes into Charon 4A and obtained expression of the enzyme in *Escherichia coli* with high activity [56]. Principles to emerge from the cloning and expression of the microbial 8L8S RubisCO's are that the L and S subunit genes are linked, they are under the control of a single promoter and are co-transcribed. Heterologoous expression of 8L8S bacterial RuBisCO's appears to be possible without special assembly factors, in contrast to the chloroplast enzyme. Foundations have been laid for the application of bacterial RuBisCO genetics to the study of enzyme assembly, subunit interaction and enzyme modification.

The possibility of achieving a 50% increase in net plant photosynthesis by selective abolition of the oxygenase reaction [58] is an attractive, though uncertain prospect. The oxygenase reaction is a feature of all extant RuBisCO's and clearly arose early during the evolution of the enzyme [5, 39]. The oxygenase reaction may be an inevitable and unavoidable feature of RuBisCO [40]. However, differential effects of metal ions and temperature on the carboxylase and oxygenase activities of the *R. rubrum* [59, 60] and *Euglena* enzymes [61] and variations in carboxylase/oxygenase (C/O) specificities between C_3 plants, C_4 plants and phototrophic microbes [62] indicate that manipulation of the enzyme in favour of carboxylation may be possible . Initial attempts to change the C/O specificity of *Alcaligenes eutrophus* RuBisCO by mutant selection were unsuccessful [63]. However, autotrophic bacteria, including extremophiles, provide a diverse resource and further attempts by traditional selection and site-directed mutagenesis would appear worthwhile.

5. CO_2-CONCENTRATION MECHANISMS AND CARBONIC ANHYDRASE IN CALVIN CYCLE BACTERIA

The oxygenase reaction of RuBisCO would not have imposed a constraint on the earliest Calvin cycle prokaryotes which developed in anaerobic environments. Indeed, the C/O specificities of RuBisCO's of extant purple bacteria are considerably lower than those of higher plants [62]. The increase in O_2 tensions due to the evolution of oxygenic cyanobacteria would have provided a selection pressure to increase the C/O specificity of RuBisCO itself. This has occurred to some extent in C_3 plants [62]. However, other strategies to reduce photorespiration caused by the oxygenase reaction have been used. The development of the C_4 pathway by increasing CO_2 supply to RuBisCO occurred in some plants, but this does not account for the low photorespiration rates in cyanobacteria and algae [5]. The C/O specificies of the few cyanobactaerial RuBisCO's examined for this ratio are lower than in C_3 and C_4 plants [60, 62]. It is well established that cyanobactaeria and microalgae have developed "CO_2-concentrating mechanisms" which increase photosynthetic efficiency and minimise photorespiration at CO_2 concentrations which would otherwise be limiting [5, 64].

An active inorganic transport mechanism has been identified in several cyanobacteria [5, 64]. Recently a membrane potential-driven active transport of CO_2 has been found in the chemolithoautotroph *Thiobacillus neapolitanus* (Y.A. Holthuizen, F.F. M. Dissel-Emiliana, J.G. Kuenen, W.N. Konings, in prepn.). The cyanobacterial photosystem I-driven system involves special proteins in the cytoplasmic membrane. Omata and Ogawa [65, 66] have identified a 42,000 Dalton polypeptide which is synthesized in the plasmalemma of *Anacystis nidulans* when cells are transferred from high to low CO_2 growth conditions and evidence suggests that this is involved in active inorganic carbon transport. Several microalgae and cyanobacteria can use CO_2 and bicarbonate for growth and Aizawa and Miyachi [64] have summarized the evidence that carbonic anhydrase (CA) also functions in inorganic carbon concentration and the suppression of cyanobacterial and algal photorespiration. CA activities in microalgae tend to be higher in low CO_2- than high CO_2-grown cultures. Among cyanobacteria, CA has been found in extracts of *Microcoleus lyngbyaceus*, 3 *Anabaena variabilis* strains, *Anacystis nidulans, Coccochloris peniocystis* and an *Oscillatoria* sp [67-69]. CA activities were higher in extracts of low CO_2-, than high CO_2- grown cultures and were detected in broken cell homogenates but not in whole cells. Soluble (cytoplasmic) CA's have been inferred in *A. variabilis* ATCC 29413 [68]. *A. nidulans, C. peniocystis* and *Ocillatoria* sp. [69]. The *A. variabilis* strain M-2 and M-3 CA's are both cytoplasmic and associated with cell membranes [68].

Air-grown *Chlorogloeopsis fritschii* cultures show high CA activity and the possibility was considered that this enzyme is associated with carboxysomes [70] for which a CO_2-concentrating function has been considered [71, 72]. Although more than 95% of the *C.fritschii* CA was partculate *in vitro*, the enzyme was not associated with the carboxysomes or thylakoids. Recently, we have shown that the *C. fritschii* CA is associated with the cell surface. The specific activity of this enzyme is increased between 2.6 and

6.8 times by transferring cultures from growth on 5% CO_2 in air, to air, and inhibition by the CA inhibitors ethoxyzolamide, acetozolamide and sulphanilamide occurs.

This is the first report of a cell surface CA in the cyanobacteria and a role for this enzyme in the uptake of bicarbonate by this organism has been indicated. Inhibition of the external CA results in a 60-80% decrease in bicarbonate-dependent photosynthesis by low CO_2-grown *C. fritschii* [A.M. Hawthornthwaite, K. Okabe and G.A. Codd, in prepn.]. External CA as in *C. fritschii* and several green algae may be particularly importan t in neutral and alkaline pH waters [64]. The presence of CA in other Calvin cycle bacteria jhas been little studied. The enzyme occurs in *Rhodospirillum rubrum* [73], *Nitrosomonas europea* [74] and *Thiobacillus thiooxidans* [75]. CA activities were high in phototrophic cultures and undetectable in chemoheterotrophic cells of *R. rubrum* [73]. The increased activity in low CO_2-, versus high CO_2-grown *N. europea* [74] further indicates a role for CA in the growth of these organisms on inorganic carbon.

6. CARBOXYSOMES

Polyhedral bodies occur in some but not all members of the colourless sulphur-oxidizing, nitrite-oxidizing and ammonia-oxidizing bacteria and in all cyanobacteria examined. These bodies have been isolated from members of the above groups and shown to contain RuBisCO, a fact recognized by their renaming as carboxysomes [71,76, 8]. Initial demonstrations of the presence of RuBisCO in carboxysomes depended upon isolation of the organelles and enzyme localization *in vitro*. This approach has been complemented recently by immunoelectronmicroscopy of cyanobacterial cell sections using gold-labelled RuBisCO antibodies [77]. Polyhedral bodies, though of diverse shape, size and number, also exist in *Prochloron* strains and cyanelles, photosynthetic endosymbionts of free-living cyanobacterial origin [71]. *In vitro* RuBisCO localization and cell section immunogold labelling has shown that the polyhedral bodies from these phototrophs are also carboxysomes (Table 2).

PRK is not present in the carboxysomes of *C. fritschii* [77, 78] *Prochloron*, cyanelles (Table 2) or *Thiobacillus neapolitanus* [79, 80]. The absence of PRK from carboxysomes indicates that RuBP would have to enter the organelles if carboxysomal RuBisCO participates in CO_2 fixation *in vivo* RuBP-dependent CO_2 fixation into 3-phosphoglyceric acid has been obtained using intact *T. neapolitanus* carboxysomes *in vitro* [79, 80] although whether this occurs *in vivo* is unknown. An active role for carboxysomes in CO_2 fixation is one of several possibilities which also include RuBisCO protective and storage roles [71, 72]. Evidence for and against all of these possibilities exists and identification of the several crypic peptides in these organelles, in addition to their most abundant protein RuBisCO, is currently limiting advances in the elucidation of carboxysome function.

Table 2. Localization of Calvin cycle enzymes in *Prochloron* and the cyanelles of *Cyanophor paradoxa*

Organism	Enzyme	Fraction	Sp. Act[a]	% Distribution	EM Gold labelling of cell section[c]
Prochloron	RuBisCO	Cytoplasm	0.30	79	+
		PB[b]	0.08	21	+
	PRK	Cytoplasm	0.29	100	+
		PB	0.00	0	-
Cyanophora cyanelle	RuBisCO	Cytoplasm	0.44	47	+
		PB	0.50	53	+
	PRK	Cytoplasm	0.57	93	+
		PB	0.04	7	-

a. specific activity moles substrate transformed min^{-1} mg $protein^{1}$. b, polyhydral body fraction. c, methods as in 77 and E. Mangeney, A.M. Hawthhornthwaite, G.A. Codd, S.P. Gibbs in prepn.).

7. PHYSIOLOGICAL CONTROL OF CO_2 FIXATION

Autotrophic CO_2 assimilation is a very energy expensive process and in order to control this process economically the facultative autotrophs need an accurate system to gear the rate of CO_2 fixation to their requirements. Control of the Calvin cycle takes place primarily at the level of the two key enzymes, RuBisCO and PRK. In particular, the latter is subject to allosteric control by the redox status and the energy charge of the cell. Metabolic intermediates which may play an important role in the (de)repression of the two key enzymes are likely to be among the first products of the Calvin cycle, for example, phosphoglycerate and phophoenolpyruvate, or related metabolites [5, 6].

In the obligate autotrophs, control takes place primarily by modulation of existing pathways. However, a general pattern is that obligate autotrophs will respond to limitation by CO_2 by an increase in the concentration of RuBisCO. For example, in *T. neapolitanus*, a 3-5 fold increase occurs which is accompanied by a similar increase in the number of carboxysomes [72]. This is also true for *T. ferrooxidans* W. Hazeu, P. Bos and J.G. Kuenen unpublished].

In both the phototrophs and lithotrophs CO_2 limitation, and in general a high O_2/CO_2 ratio, may lead to increased oxygenase activity of RuBisCO, which may result in glycollate production/excretion [5]. In these organisms one finds no, or hardly any response of RuBisCO levels to the presence of organic compounds in the growth medium [8].

The response of facultative autotrophs to changes in their nutritional environment is generally much more elaborate and flexible than that found among the obligate autotrophs. While the principles of control may be very similar in most facultative autotrophs, the details of reponse and enzyme adjustment

are very dependent on the metabolic strategy of the organisms. They respond to CO_2 limitation in a way similar to the obligate autotrophs [81, 82]. The facultative autotrophs often show diauxic responses when presented with excess concentrations of mixtures of organic compounds and the lithotrophic energy source, resulting in complete repression of RuBisCO, But this is not seen under growth limitation by these mixtures. For example, in *Thiobacillus versutus* (formerly A2), grown on a mixture of acetate and thiosulphate, one observes a very accurate tuning of CO_2-fixing capacity to need [8]. This applies to most other facultatives [6]. However, with certain organic carbon sources; fructose in *Alcaligenes eutrophus* [82], and oxalic acid in *Pseudomonas oxalaticus* [6]. CO_2 fixation capacity may not be repressed, or may be only partly repressed, by the organic compound. Also in phototrophs (*Rhodopseudomonas* spp.) a similar pattern can be seen [81]. An interesting situation exists in *Chromatium* and *Chlorobium* species, in which acetate can almost entirely replace CO_2 as carbon source, but CO_2 fixation capacity is not repressed. In fact, when presented with excess sulphide and acetate, *Chlorobium* consumes sulphide preferentially, implying that it must fix CO_2 in the presence of excess acetate [83].

8. ECOPHYSIOLOGICAL ASPECTS

With the exception of the oxygenic cyanobacteria, all other phototrophic and chemolithotrophic bacteria are limited to environments where there is a reduced energy and/or electron donor available in addition to light for the phototrophs [84, 17]. Of course, many of the chemolithotrophs require the simultaneous presence of oxygen as terminal electron acceptor. Such habitats are found at the interface of aerobic and anaerobic environments. These interfaces may be relatively stable when there is a continuous supply of both the inorganic source of energy reducing power in the dark, e.g. around submarine volcanic springs, but in most known cases they are highly dynamic, due to diurnal or tidal rhythms. Examples are tidal mudflats with algal mats, or interfaces of stratified water bodies.

Studies by Loogman and Mur, and Riegman and Mur have shown that cyanobacteria and green algae are extremely well adapted to changes in the light-dark cycle. These organisms adjust the rate of glycogen synthesis during the light period in such a way that in the dark a growth rate can be maintained at the expense of reserve material to equal the rate of growth in the light [85].

Facultative chemolithotrophs appear to be well equipped to survive in environments where mixtures of organic and inorganic carbon and/or energy sources are available, or in the case of phototrophs, where organic compounds and light are simultaneously present. The specialized, obligate autotrophs may become dominant in environments where fluxes of the reduced inorganic energy source, or light in the case of phototrophs, are high relative to the flux of organic substrates. This has been shown to be the case among the sulphur-oxidizing bacteria [8]. Few studies have been made of the metabolic flexibility of the facultative organisms, i.e. how fast they can switch from heterotrophic to autotrophic growth. *T. versutus* [8] can grow well under alternating supply of organic and inorganic substrates, but with increasing length of the heterotrophic periods its autotrophic potential is repressed progressively,

resulting in long lags before autotrophic growth can be resumed. This implies that such organisms are primarily competitive under environmental conditions during which mixtures of organic and inorganic substrates are available. A somewhat similar case has been investigated by Wijbenga and Van Gemerden [cf. 17] who found that *Rhodopseudomonas capsulata* behaved in a manner analogous to that of *T. versutus* during alternating supply of sulphide and acetate and lost in competition with *Chromatium* under these conditions. Further information on the ecophysiological aspects of autotrophic growth can be found in refs.17,85, 86.

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