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STRATEGIES FOR GROWTH AND EVOLUTION OF MICRO-ORGANISMS IN OLIGOTROPHIC HABITATS

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INTRODUCTION

The strategy of microbes to adapt to a particular environment occurs both at the phenotypical and at the genotypical level. In general phenotypic responses may be needed to cope with temporary changes, whereas genetic adaptations may be needed for long lasting changes in the environment.

The enormous diversity of microorganisms in nature illustrates the equally enormous diversity in ecological niches to harbour these organisms. Within the limits of biological feasibility, organisms have adapted to physical, chemical, and biological changes or stress. Examples are the adaptation to different temperatures, to low nutrient environments, to low light intensities, to the supply of single or mixed substrates, to the continuous or discontinuous availability of nutrients, and to environments with aerobic-anaerobic changes.

The genetic adaptation for these changes may have resulted in the development of organisms which became either highly specialized or remained highly versatile; in other words, evolution led to organisms with either a low or a high potential for phenotypic changes. Irrespective whether we talk about a genetic or a phenotypic change, in each case it is the competition and the subsequent selection in the environment which determines the value of a certain change. A better understanding of competition and selection thus will teach us more of the strategies and mechanisms of adaptations in microorganisms. These selection processes can be assumed to have taken place in both freshwater and marine habitats. In the NATO meeting attention was focussed on the sea, but relevant information can be deduced as well from the many studies with freshwater organisms. So far, no essential difference with respect to the principles of selection and competition have been encountered in marine and freshwater bacteria, and it seems admissible to include studies with both groups of organisms.

In addition, for a proper judgement of the selection pressure exerted on organisms living under oligotrophic conditions (i.e., low population densities, low substrate concentrations, but high turnover rates) a comparison with more eutrophic habitats may be very rewarding. This holds in particular for studies in which substrate limitation occurs. Perturbation of the established balanced growth provides us with extremely useful information on an organism's capacity to assimilate excess nutrients. Such culture systems are technically much easier to handle than strict oligotrophic systems, yet both systems share a number of characteristics. In the study of selection and competition between microorganisms the continuous culture ("chemostat") has been proven to be a most useful tool. This culture method allows us to artificially, and specifically, amplify the selective pressure exerted on bacteria. In general, organisms show a saturation-type of growth-rate response to increasing substrate concentrations. For bacteria, the relation between the specific growth rate (μ) and the concentration of the substrate (s) under conditions of balanced growth initially has been described by Monod (1942) in analogy to the Michaelis-Menten kinetics of enzyme systems.

In Monod's description the specific rate of growth at a given substrate concentration is determined by the organism's kinetic parameters μ_{max} and K_s, according to

$$\mu = \mu_{max} \frac{s}{K_s + s}$$

in which μ_{max} is the maximum growth rate attainable in the presence of excess substrates and K_s is the saturation constant numerically equal to s at $\mu = {}^1/2\ \mu_{max}$.

In later modifications, the maintenance energy concept and the effects of inhibitory substrates were included in the mathematical description of balanced growth.

Organisms which are able to grow relatively rapidly at low nutrient concentrations are usually said to have a high affinity for the substrate. However, the term affinity is poorly described and certainly is not only related to the K_s value. Indeed, in many cases organisms with a high affinity for a substrate were found to have a low K_s value, but erroneously a low K_s value is often interpreted as equivalent to a high affinity. An organism with a high μ_{max} value may have a high K_s value compared to another organism with a lower

μ_{max} value, but still be able to grow faster at low substrate concentrations.

The specific rate of growth at the prevailing substrate concentration is the decisive factor. A mere comparison of K_s values makes sense only for organisms with similar μ_{max} values. In order to compare different organisms with respect to their affinity of the same substrate, the slope of the μ/s curve at a given low concentration of s (not exceeding K_s) can be calculated according to

$$\frac{d\mu}{ds} = \mu_{max} \frac{K_s}{(K_s + s)^2}$$

The organism with the highest $d\mu/ds$ value then is said to have the highest affinity (Zevenboom 1980; see also Healey 1980; Brown and Molot 1980).

When substrate concentrations are low compared to the K_s values, as usually is the case during carbon and energy limitation, the ratio μ_{max}/K_s can be used to compare the affinity for a substrate. However, it should be realized that threshold concentrations, and the minimum growth rate (discussed later) may complicate the interpretation.

In the chemostat, low substrate concentrations can be maintained for long periods of time, and the continuous cultivation thus is an elegant and indispensible tool in the study of competition and selection. When discussing the "strategies" of microorganisms we mean a way of life which by selection has shown survival value during the evolution.

ADAPTATION TO LOW NUTRIENT CONCENTRATIONS

Affinity

Physiologically very similar organisms may occupy different niches on the basis of slight differences in the kinetic parameters K_s and $_{max}$ for a given growth-rate limiting substrate. This can be demonstrated with the aid of continuous cultures inoculated with natural samples; the operation of chemostats at different dilution rates often results in the selective enrichment of different organisms. These phenomena have been extensively studied by Jannasch in Woods Hole, U.S.A. and the group of Veldkamp in Groningen, The Netherlands. Jannasch (1967) demonstrated the reproducible enrichment of several marine bacteria by varying the dilution rate of sea water supplemented with a nutrient (lactate). The dominant species were isolated and grown in chemostats at various dilution rates in order to estimate the kinetic parameters K_s and μ_{max}. Thereafter, competition experiments under similar conditions were performed with mixtures of the two pure cultures. The outcome of these selection experiments could be predicted on the basis of the pure culture responses to various concentrations of the substrate (various dilution rates) according to Fig. 1.

In another example (Kuenen et al. 1977), two closely related obligate chemolithotrophic sulfur bacteria (Thiobacillus thioparus and the spirillum-shaped Thiomicrospira pelophila) were isolated from the same sample taken from an intertidal mud flat of the Waddensea (Holland). Their carbon and energy metabolism was remarkably similar (Kuenen and Veldkamp 1973) but they had different sulfide tolerances (Kuenen and Veldkamp 1972).

The small spirillum grew better at higher sulfide concentrations than the rod-shaped Thiobacillus. The magnitude of the sulfide concentrations employed (300 ml seawater on top of 100 ml agar containing 2 to 20 mM sulfide) and the ambiguous results under thiosulfate limitation suggested that the success of the spirillum at higher concentrations of sulfide was not explained by a better affinity for sulfide, but rather by some other factor related to the high sulfide concentrations. It was postulated that the actual selective factor was the concentration of iron. At high sulfide concentrations iron is precipitated as ferrous iron with an extremely low solubility product. Subsequently, the μ/Fe curves of the two isolates were determined and found to cross. Competition experiments performed at dilution rates of 0.05-0.25 h^{-1} resulted in a dominant population of the spirillum; a further increase in the dilution rate resulted in the selective enrichment of the Thiobacillus.

Similarly, different chemo-organotrophic bacteria became dominant in phosphate-limited continuous cultures inoculated with natural samples and run at either low or high dilution rates (Kuenen et al. 1977). After five volume changes a spirillum had become dominant in the culture run at a dilution rate of 0.03 h^{-1}.

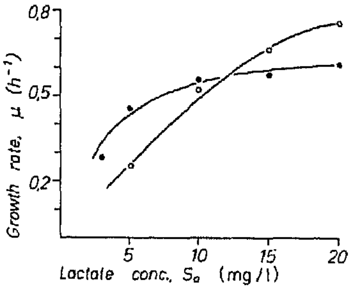

Figure 1. Relation between the specific growth rate of Pseudomonas (201) (O--O) and Spirillum (101) (●--●) and the substrate lactate. From Jannasch (1967).

Table 1. Kinetic parameters of organisms selected in continuous culture under phosphate limitation (from Kuenen et al. 1977).

Organism	μ_{max} (h^{-1})	$K_s(PO_4^{3-})$ ($nmol \cdot L^{-1}$)	Dilution Rate of Enrichment (h^{-1})
Rod-shaped bacterium	0.48	66	0.03
Spirillum sp.	0.24	27	0.3

The parallel experiment at a dilution rate of 0.3 h^{-1} yielded a dominant culture of a rod-shaped bacterium. When the respective organisms were isolated by plating and assayed for their kinetic parameters, they were found to differ in both K_s and μ_{max} value (Table 1). The extremely low K_s values (10^{-7}, 10^{-8} M) reflect actual concentrations found in the habitat of the organisms.

Assuming the absence of interactions other than the competition for phosphate, the spirillum will outcompete the rod-shaped organism at phosphate concentrations below 0.01 μM (the crossing point). At that concentration, the specific growth rate of both organisms is 0.07 h^{-1}, well below the maximum specific growth rate of the spirillum (0.24 h^{-1}). The spirillum thus can be said to be best adapted to low phosphate concentrations. Interestingly, experiments with other growth-rate limitations showed that its high affinity is not restricted to phosphate. It was found that the spirillum outgrew the rod-shaped bacterium at low dilution rates as well under limitations of K^+, Mg^{2+}, NH_4^+, aspartate, succinate, and lactate. For all carbon sources, the specific growth rate of the rod-shaped bacterium was higher than that of the spirillum. Thus it appears that spirillum is well adapted to low nutrient concentrations in general and its good affinity does not seem to be a specific property related to one or two nutrients.

A very intriguing case of crossing μ/s curves was reported by Harder (1969; Harder and Veldkamp 1971). An obligately psychrophilic *Pseudomonas* and a facultatively psychrophilic *Spirillum* were both isolated from samples taken from the North Sea in the winter and the early spring using lactate as the carbon and energy source (Harder and Veldkamp 1968). On the basis of μ_{max} determinations of pure cultures and the outcome of competition experiments at various dilution rates, the μ/s curves at different temperatures as shown in Fig. 2 were derived.

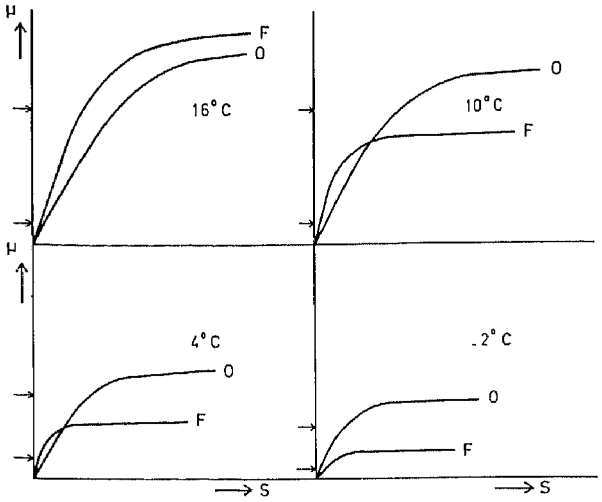

Figure 2. Specific growth rates of an obligately psychrophilic *Pseudomonas* sp. (O), and a facultatively psychrophilic *Spirillum* sp. (F) as a function of the lactate concentration at different temperatures. The curves are schematic and based on the measurements at the growth rates indicated. From Harder and Veldkamp (1971).

At 10°C, the obligate psychrophile was unable to outcompete the *Spirillum* at low lactate concentrations; lowering the temperature to 4°C restricted the competitive advantage of the *Spirillum* to even lower concentrations. A still further reduction in the temperature to -2° resulted in the competitive exclusion of the *Spirillum* irrespective of the dilution rate. The opposite was found true at a temperature of 16°C. These data suggest that mineralization in permanently cold ocean waters proceeds through the action of obligately psychrophilic bacteria.

Surface to Volume Ratio

One question emerging from the results discussed above is why certain organisms exhibit higher affinities for substrates present in low concentrations. And also, why can such organisms not express their supremacy at higher substrate concentrations as well? Or, in other words, why does an organism not combine a high μ_{max} with a high affinity?

The first point, the scavenging potential, may be related to the organism's surface to volume ratio (S/V ratio). In general, this ratio is higher in organisms which become dominant at lower substrate concentration. Table 2 shows some relevant data. These figures suggest that in general a genetic adaptation is required for

Table 2. Surface to volume ratios of different spiral- and rod-shaped bacteria grown at limiting substrate concentrations (adapted from Keunen et al. 1977).

Organism	Limiting Substrate	Specific Growth Rate (h^{-1})	Average Length (μm)	Average Diameter (μm)	Surface/ Volume (μm^{-1})
Pseudomonas sp.[a]	lactate	0.1	2.9	1.1	4.3
Spirillum sp.	lactate	0.1	3.5	0.55	8.0
Pseudomonas sp.[b]	lactate	0.16	2.26	0.80	5.66
Spirillum sp.	lactate	0.16	2.21	0.56	7.82
Unidentified rod[c]	phosphate	0.2	2.8	1.1	4.3
Spirillum sp.	phosphate	0.2	3.8	0.6	7.2
Thiobacillus thioparus[c]	thiosulfate	0.1	2.0	0.4	11
Thiomicrospira pelophila	thiosulfate	0.1	2.5	0.2	21

[a] Harder and Veldkamp unpublished
[b] Matin and Veldkamp 1978
[c] Kuenen et al. 1977

obtaining a high S/V ratio and that such an adaptation is related to the potential to grow well at low concentrations of the nutrient.

Adaptations to the uptake of nutrients present in low concentrations by virtue of an increase in the S/V ratio are not only controlled at the genetic level. A phenotypic response was reported by Matin and Veldkamp (1978).

A *Spirillum* and a *Pseudomonas*, both freshwater isolates, were shown to have crossing μ/lactate curves, the *Pseudomonas* outgrew the *Spirillum* at dilution rates exceeding 0.29 h^{-1}, but the converse occurred at lower D values. Both organisms were found to increase the S/V ratio at low dilution rates, the ratio in *Spirillum* remained the highest, however (Table 3). This might explain the success of this organism at low substrate concentrations.

The formation of prosthecae also increases the S/V ratio. At very low nutrient concentrations the prosthecae of *Ancalomicrobium* are fully developed, whereas the opposite is true under nutrient-rich conditions. In other species the occurrence of prosthecae is not so easily explained (Dow and Whittenbury 1980). Oligotrophic

Table 3. Surface to volume ration of two chemoorganotropic bacteria at various specific growth rates (from Matin and Veldkamp 1978).

Specific Growth Rate	Surface/Volume (μm^{-1}) *Spirillum*	 *Pseudomonas*
0.06	8.05	6.24
0.16	7.82	5.66
0.35(μ_{max})	6.27	
0.64(μ_{max})		4.59

environments often contain microbial forms with very long stalks or appendages which do not necessarily improve the rate of nutrient uptake but may instead be a reflection of a reduced growth rate under near starvation conditions (Dow and Whittenbury 1980).

However, it has been demonstrated in *Hyphomicrobium* that organisms with long stalks have a higher affinity for methanol than organisms with short stalks. Long stalks were observed at low specific growth rates whereas short stalks were found at high growth rates (Table 4; Meiberg et al., unpublished). Phenotypic changes are usually smaller than genetically controlled modifications. The organisms can apparently cope with small variations in the environment by phenotypic responses, but for larger changes in the S/V ratio a genetic change is needed.

The second question, why does not an organism combine fast growth at saturating substrate concentrations (high μ_{max}) with relatively fast growth at low concentrations (high affinity), is hard to answer. It may be that at the phenotypic level the "elasticity" of the system is often too small to allow dramatic changes in the S/V ratio. A genetic adaptation seems to be required to change the S/V ratio by more than 20-30%. As a consequence, the price to be paid could be a lower μ_{max}. This may be rationalized by arguing that the synthesis of cells with relatively large amounts of membrane (high S/V ratio), cannot proceed at the same high speed as that of cells which have more protein per unit dry weight (low S/V ratio) available for biosynthetic processes (growth).

It is obvious that the phenomenon just described as "limited elasticity" is one of the constraints put upon the evolution of microorganisms. It should be realized that much of this "limited

Table 4. Affinities for methanol of Hyphomicrobium X (DSM 1869) at different dilution rates (from Meiberg et al., unpublished).*

Specific Growth Rate (h^{-1})	Stalk Length	Apparent K_s (Methanol) (M)
0.01	long	10^{-7}
0.1	short	10^{-6}

*The organism's μ_{max} is approximately 0.18 h^{-1}.

elasticity" in all probability is to be traced back to the purely biochemical level, for example to the properties and limitations of single enzymes, or membranes, or of the total machinery for protein synthesis.

Growth vs. Storage

The underlying principle of the dilution-rate dependent outcome of competition experiments is the apparent inconsistency of a high μ_{max} with a high affinity. As a consequence organisms with different attributes, resulting in crossing μ/s curves, become dominant at different substrate concentrations. Non-crossing μ/s curves would have resulted in the competitive exclusion of one of the organisms, irrespective of the dilution rate imposed. It must be pointed out that this refers to balanced growth only; that is, the nutrient is added continuously and at a constant rate. As a rule, such conditions do not occur in nature for long periods of time. In addition, the decisive influence of the S/V ratio would suggest that uptake mechanisms always are the rate-limiting factor.

The importance of other parameters can nicely be demonstrated by the outcome of competition studies between two species of phototrophic bacteria, Chromatium vinosum and Chromatium weissei (van Gemerden 1974). C. vinosum measures on the average 2 x 4 μm, resulting in a S/V ratio of 2.4 μm^{-1}; C. weissei measures on the average 4 x 8 μm, resulting in a S/V ratio of 1.2 μm^{-1}. However, the μ/sulfide curves of the two organisms do not cross: C. vinosum exhibits a higher μ_{max} value for sulfide and has a higher affinity as well. As a consequence, C. weissei is outcompeted at all dilution rates. However, the addition of substrate to non-growing pure cultures resulted in a faster uptake by C. weissei, the organism with the lower S/V ratio.

Under these conditions, the oxidation of sulfide results in part in the formation of sulfate and in part in the formation of sulfur. The latter compound is accumulated inside the cells. Also, the glycogen synthesized from CO_2 is stored intracellularly. Thus, the substrate sulfide is depleted virtually without the synthesis of structural cell material.

The specific rate of glycogen synthesis was found to be similar in the two organisms. However, _C. weissei_ produced less sulfate and more sulfur than _C. vinosum_. This hoarding of the substrate is a competitive advantage under fluctuating conditions. Both sulfur and glycogen were utilized for growth once sulfide was depleted. In fact, balanced coexistence of the two organisms was observed under fluctuating conditions; the relative abundance of the two organisms was dependent on the amount of sulfide accumulated in the dark period (Table 5).

A last point of particular importance is that the specific rate of CO_2 fixation exhibited by _C. weissei_ is higher than its maximum specific growth rate. _C. weissei_ was found to oxidize sulfide and

Table 5. Abundancy of _Chromatium vinosum_ and _Chromatium weissei_ during balanced coexistence in relation to the light regimen (from van Gemerden 1974).*

Light Regimen	Organism	Relative Abundance (% Biovol.) Inoculum	 Balanced Coexistence
continuous light	_C. vinosum_	10	100
	C. weissei	90	0
18 h light	_C. vinosum_	30	100
6 h dark	_C. weissei_	70	0
6 h light	_C. vinosum_	20	63
6 h dark	_C. weissei_	80	37
4 h light	_C. vinosum_	60	30
8 h dark	_C. weissei_	40	70

* Sulfide-limited continuous cultures were illuminated as indicated. In the dark periods the addition of the substrate was continued resulting in the accumulation of sulfide in the culture vessel, followed by its rapid oxidation during the next light period. Experiments were performed at $D = 0.011\ h^{-1}$.

sulfur at a rate that would permit the organisms to grow at a μ of 0.05 h^{-1}, whereas the organisms μ_{max} is only 0.04 h^{-1}. This implies that even if the substrate were available at elevated concentrations for a somewhat longer period of time, it would only be advantageous for a limited period.

Once the organisms are "stuffed" with sulfur and glycogen, the rate of sulfide oxidation has to slow down. Thus, a high uptake capacity is of particular relevance if fluctuating concentrations of substrates are encountered in the organism's habitat.

Specialism vs. Versatility

It can be deduced from the previous discussion that no organisms can combine more than a limited number of attributes. This implies that organisms evolving in one or another direction are under the continuous challenge to either give up an old property in order to hold the new one, or to discard the newly acquired trait. This "give-and-take" principle will be illustrated by comparing a highly specialized autotroph with a very versatile mixotroph. Both bacteria are representative of the colorless sulfur bacteria; these organisms are not encountered in the open ocean, however, the principle may have a wide application.

The specialist is the obligately chemolithotrophic Thiobacillus neapolitanus, able to grow rapidly in thiosulfate + CO_2 media. This organism cannot grow at all heterotrophically and even under mixotrophic conditions (thiosulfate + organic compounds) little of the cellular carbon is derived from the organic carbon supplied in the medium (Kuenen and Veldkamp 1973).

The versatile organism is Thiobacillus A2, able to grow autotrophically (thiosulfate + CO_2), heterotrophically (20 or more different organic compounds) and mixotrophically (thiosulfate + organic compounds). Two substrates can be used simultaneously by this organism, provided both substrates are at limiting concentrations.

In a comparison of these species, we first focus on the μ_{max} exhibited under various conditions (Table 6). The specialized Thiobacillus neopolitanus shows a very high μ_{max} in thiosulfate + CO_2 media and the addition of acetate did not enhance this value. The versatile Thiobacillus A2 has a much wider choice of carbon and energy sources. However, it seems that the price to be paid is that it cannot grow rapidly on any substrate and that its heterotrophic μ_{max} is not enhanced by the addition of thiosulfate.

Relevant information on the organism's affinity for thiosulfate could be deduced from the outcome of competition experiments. Under autotrophic conditions (thiosulfate limitation), Thiobacillus neapolitanus outcompeted Thiobacillus A2 at dilution rates of

Table 6. Maximum specific growth rates of two specialists (Thiobacillus neapolitanus, Spirillum G7) and a versatile bacterium (Thiobacillus A2) on thiosulfate, acetate, or both (from Kuenen 1980).

Organism	Thiosulfate $\mu_{max}(h^{-1})$	Thiosulfate and Acetate $\mu_{max}(h^{-1})$	Acetate $\mu_{max}(h^{-1})$
T. neapolitanus	0.35	0.35	no growth
T. A2	0.10	0.22	0.22
Spirillum G7	no growth	0.43	0.43

0.075 h^{-1}, 0.050 h^{-1}, and 0.025 h^{-1}, indicating a superior affinity for thiosulfate of the specialist. The fact that the versatile organism was not completely eliminated but maintained at a level of 5-10% of the total cell number is explained by the excretion of organic compounds (glycollate) by Thiobacillus neapolitanus. The latter organism is unable to re-assimilate the glycollate, whereas this compound is efficiently utilized by Thiobacillus A2.

The low but persistent numbers of the versatile Thiobacillus A2 at dilution rates of 0.025 h^{-1} and higher, point to the importance of organic compounds for the survival of this facultative chemolithotroph. The inclusion of increasing amounts of glycollate or acetate in the media resulted in balanced coexistence with increasing numbers of Thiobacillus A2. The outcome of a series of such experiments performed at a dilution rate of 0.07 h^{-1} is shown in Fig. 3 (Gottschal et al. 1979). Media containing 7.8 mM acetate or glycollate in addition to 40 mM thiosulfate resulted in 90% of the versatile Thiobacillus A2 and 10% of the specialist Thiobacillus neapolitanus (Gottschal et al. 1979). Similarly, the inclusion of glucose in the thiosulfate medium resulted in a rapid domination of the versatile organism and the virtual exclusion of Thiobacillus neapolitanus which previously made up about 90% of the biomass (Smith and Kelly 1979). These data clearly demonstrate that the capacity for mixotrophy is of selective advantage to the versatile organism in its competition with obligate chemolithotrophic species. The phenomenon appears to be a general one; similar competition experiments were conducted between the versatile Thiobacillus A2 and a heterotrophic specialist, the Spirillum G7 (see Table 6 for μ_{max} values). With acetate as the sole growth-limiting substrate, Thiobacillus A2 was excluded at dilution rates of 0.07 h^{-1} and 0.15 h^{-1}. However, increasing concentrations of thiosulfate (0-10 mM) in the acetate

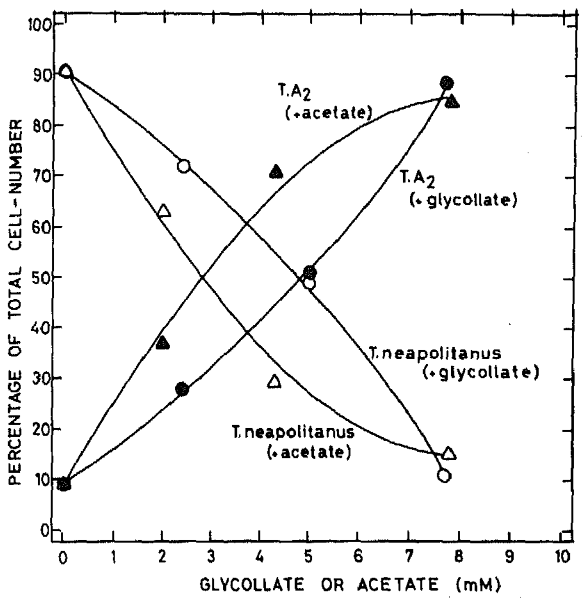

Figure 3. Effect of different concentrations of organic substrate on the outcome of the competition between Thiobacillus A2 and T. neapolitanus for thiosulfate. Chemostats were run at a dilution rate of 0.07 h^{-1}. The inflowing medium contained thiosulfate (40 mM) and either acetate or glycollate at concentrations ranging from 0 to 7 mM. Cell numbers were determined after steady states were established. From Gottschal et al. (1979).

(10 mM) medium eventually led to the elimination of the heterotrophic specialist (Gottschal et al. 1979) (see Fig. 4).

The general validity of these observations is indicated by the fact that facultatively chemolithotrophic thiobacilli indeed could be enriched from various freshwater environments by feeding continuous cultures with mixtures of acetate and thiosulfate. Surprisingly, similar experiments inoculated with samples from tidal mud flats resulted in a mixed culture of a heterotroph and a specialized chemolithotroph (Gottschal and Kuenen 1980).

Another example of competition between a specialist and a more versatile organism for mixed substrates was reported by Laanbroek et al. (1979). The specialist, Clostridium cochlearium, was found to outcompete the less specialized Clostridium tetanomorphum during growth on glutamate as the only carbon and energy source. Upon the inclusion of glucose in the reservoir solution, the two organisms were able to coexist. Glutamate can be utilized by both organisms but the specialist is unable to grow on glucose. Conceivably, the

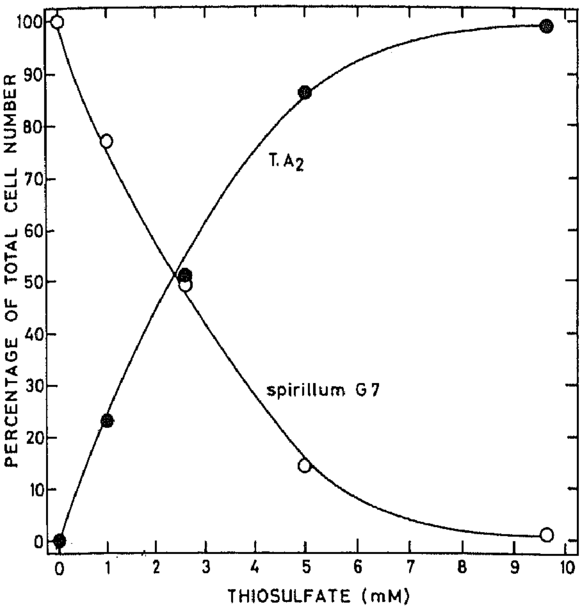

Figure 4. Effect of different concentrations of thiosulfate on the outcome of the competition between *Thiobacillus* A2 and *Spirillum* G7 for acetate. Chemostats were run at dilution rates of $0.07\ h^{-1}$. The inflowing medium contained acetate (10 mM) together with thiosulfate in concentrations ranging from 0 to 9.7 mM. Cell numbers were estimated after steady states were established. From Gottschal et al. (1979).

versatile organism grew on the glutamate in addition to the glucose in the mixed culture.

Summarizing this section, it may be concluded that specialists have an advantage over versatile organisms when the turn-over rate of a single substrate is high. Versatile organisms may be expected to flourish when two (or more) substrates are turned over at comparable speeds. Based on this information, one could expect that versatile organisms are more abundant in natural ecosystems. However, as will be discussed in the next section, an organism's reactivity is of utmost importance when substrates are available at limited and, in particular, at fluctuating concentrations.

It can be argued that in general specialists, not being flexible, have no need for inducible pathways for their carbon sources and energy sources. Such organisms may even have some metabolic "lesions" in pathways which are not functional in the organism's metabolism. For example, in the case of the specialist *Thiobacillus neapolitanus*, it is clear that it does not need a cyclic oxidation of acetyl-CoA; thus the fact that its TCA cycle is incomplete is not a disadvantage. Being a specialist, depending for growth and survival on one substrate, it can be expected to be equipped with excess capacity to oxidize that substrate.

On the other hand, the versatile organisms cannot permit themselves to have excess capacity for all the different compounds which might be utilized when the excess capacities are not needed. Instead, the full potential is not switched on in the absence of the corresponding nutrient; in other words these organisms will show a strong adaptation to different growth conditions. Thus, Thiobacillus A2 can fix CO_2 when necessary; its ability to oxidize thiosulfate is substrate-inducible and is repressed by organic compounds (Gottschal et al. 1981b). Similarly, in Rhodopseudomonas capsulata, a versatile phototroph, the utilization of acetate results in a low potential rate of sulfide oxidation; this is in contrast to the more specialized Chromatium vinosum (Wijbenga and van Gemerden 1981; Beeftink and van Gemerden 1979).

These phenomena have two consequences, both of which lead to a lower ability of the versatile organisms to react when compared to the specialists. First of all, in an environment where the concentration of substrates fluctuate - which rather is a rule than an exception - the versatile organisms may be unable to respond quickly to a sudden deviation from zero concentration because its potential is repressed. In the resulting lag period, its specialist competitor with a constitutive system is strongly favored. Secondly, when there is a sudden increase in the supply of a nutrient that has been available in low concentrations, the nutrient will be oxidized more slowly by the versatile organism because even under fully induced conditions it has a lower capacity than the specialist. Under fluctuating conditions of organic and inorganic compounds, the versatile Thiobacillus A2 is at a competitive disadvantage compared to the specialist Thiobacillus neapolitanus and the specialist Spirillum G7. Its continuous adaptation causes the versatile organism to be slow in its response to a single substrate (Gottschal et al. 1981a). Likewise, under fluctuating conditions the specialist C. vinosum has a competitive advantage over the versatile R. capsulata (Wijbenga and van Gemerden, unpublished). Some characteristic differences between metabolically versatile organisms and highly specialized organisms are listed in Table 7.

In conclusion, the price to be paid for being versatile is to be less reactive; in other words, a strategy directed at versatility is incompatible with a strategy aimed at reactivity. To some extent we can understand in a functional sense why such a choice can or even must be made. Again, one should realize that this strategy applies not only to intact organisms, but is also a part of the basic properties of subcellular structures.

Table 7. Differences between metabolically versatile and specialized organisms extrapolated from studies with *Thiobacillus* species.

Specialist	Versatile
1. Few substrates utilized	1. Many substrates utilized
2. High specific growth rate on a single substrate	2a. Low specific growth rate on a single substrate
	2b. Relatively high specific growth rate on mixed substrates
3. High excess capacity to respire substrates	3. Low excess capacity
4. Constitutive enzymes for energy and carbon metabolism	4. Inducible enzymes
5. High reactivity for few substrates	5. Low reactivity for many substrates
6. Metabolic lesions	6. Many pathways, often overlapping
7. Low endogenous respiration	7. High endogenous respiration
8. Very resistant to starvation	8. Probably less resistant

Adaptations of Enzyme Systems

One way for an organism to be able to continue to grow at decreasing concentrations of a nutrient, is to increase the content of the critical enzyme. Another way is to modulate the enzyme's activity, for example, by allosteric control. A third way is the *de novo* synthesis of special low-affinity enzymes.

The effect of the dilution rate on the synthesis of various enzymes in carbon-limited continuous cultures has recently been summarized by Matin (1979). Out of 51 responses reported, the majority showed an increased activity with decreasing dilution rate. Such a response was observed for almost all the catabolic enzymes examined. An increased activity with increasing dilution rate was found only in enzymes which are involved either in biosynthetic reactions or with the respiratory chain. The simplest explanation for the latter phenomenon is that induction increases with the increasing substrate concentration encountered at higher dilution rates. Likewise, increasing enzyme synthesis with decreasing dilution rates can be explained by a release of catabolite repression as the result of decreasing intracellular pools of metabolites in organisms grown at lower dilution rate (Matin 1979).

The increased synthesis of catabolic enzymes in slower growing organims is understandable if one realizes that the steady-state substrate concentrations are well below the K_m of the enzymes involved. If however such enzymes are inducible rather than derepressible, a low substrate concentration would not, or not sufficiently, initiate the synthesis of that enzyme. This shortage of enzyme would automatically result in a rise in the substrate concentration unless another organism were present that contained the enzyme constitutively. Thus, at low substrate concentrations, organisms with inducible enzyme systems can be expected to be outcompeted by those that contain such an enzyme constitutively. Alternatively, the enzyme can be derepressable rather than inducible so that at low metabolite concentrations it can be present at sufficient levels. The competitive exclusion of E. coli wild type by mutants which are constitutive for the hydrolyzing enzyme β-galactosidase under lactose-limitation may illustrate this phenomenon (Matin 1979).

Another way to cope with low nutrient concentrations is the preferential synthesis of enzymes with improved affinity. The best example is the combined action of glutamine synthetase (GS) and glutamine-oxoglutarate-amidotransferase (GOGAT) at low concentrations of NH_4^+ which replaces the glutamate dehydrogenase (GDH) operative at high concentrations of $NH4^+$ (Tempest et al. 1970). In Aerobacter aerogenes the $K_mNH_4^+$ of GDH is about 10 mM, while the corresponding value of GS is about 0.5 mM NH_4^+; however, in the latter system 1 ATP is consumed in the synthesis of 1 glutamic acid. In many organisms these two systems are inducible and can be repressed. However, in a species of Caulobacter studied by Ely et al. (1978) GDH is lacking and GS is present at a constitutive level. The obvious advantages for the organism are that it does not have to carry the genetic information for other assimilation pathways, and that NH_4^+ can be taken up without any time lag. The disadvantage of such a "strategy" is that at higher levels of NH_4^+ the organism still must use the system which requires ATP, and, even worse, is inhibited at high concentrations of NH_4^+. The latter phenomenon may ultimately even result in the complete inhibition of growth. This may be considered an extreme case of adaptation (specialization) to low nutrient concentrations.

Generally, at higher concentrations, caulobacters are inhibited by a wide variety of organic and inorganic nutrients. This nutrient-concentration sensitivity is probably related to the balance of nutrients in general and not to the concentration of a single compound (Poindexter 1981).

The existence of low and high affinity enzyme systems is not restricted to the assimilation of NH_4^+ and such systems have been reported as well for organic nutrients as glycerol and glucose (Neijssel et al. 1975; Whitting et al. 1976).

In addition to the adaptations mentioned, organisms have developed mechanisms to avoid low nutrient environments, or to "escape" from predation. Such adaptations are beyond the scope of the present paper. For a detailed discussion the reader is referred to Dow and Whittenbury (1980).

Maintenance, Survival, and the Minimum Growth Rate

Organisms, even when not growing, require a certain amount of energy per unit time and biomass. This is required first of all for the maintenance of a proton motive force (p.m.f.) over the membrane (Konings and Veldkamp 1980) which is needed for the performance of such life-sustaining processes as osmotic regulation, control of internal pH, synthesis of ATP, and turnover of essential macromolecules (Pirt 1975; Tempest 1970; Dawes 1976). The energy required for all processes not directly related to growth is collectively called the energy of maintenance. As a consequence, the curve describing the relation between the specific rate of growth and the concentration of the limiting source of energy does not pass through the origin. In such a plot, the ordinate intercept of the extrapolated curve has been described as the specific maintenance rate, μ_e (Powell 1967). The same principle applies to the growth rate dependency of phototrophic bacteria on the intensity of light. A high μ_e value of organism A compared to organism B then indicates that organism A requires a higher irradiance to maintain its cellular integrity.

In the continuous cultivation of microorganisms, the specific rate of growth, μ, is usually considered identical to the dilution rate, D, once a steady state is established. Also, the magnitude of the energy of maintenance is considered small compared to the energy required for growth processes. Actually, however, under steady-state conditions μ is always somewhat higher than D because the viability is somewhat lower than 100% (Sinclair and Topiwala 1970; Veldkamp 1976). Also, the steady-state cell densities are somewhat lower due to the fact that some of the (limiting) energy source is used for maintenance purposes. In most studies the dilution rates employed are such that neglecting the maintenance rate, μ_e, and the death rate, γ, can be justified. However, with decreasing dilution rate these parameters become increasingly important. It can be argued, therefore, that it is not justified to apply the original Monod equation to growth under oligotrophic conditions.

The increasing importance of the energy for maintenance at decreasing dilution rates becomes noticeable in cell densities which are lower than expected from the yields at higher growth rates. This phenomenon has been observed in a number of bacteria and yeasts, and can be assumed to hold for all organisms whose growth rate is limited by the concentration of the energy source. In other words, under conditions which only allow growth at a very low specific rate, the size of the standing crop is low compared to the supply of nutrients. Therefore, under oligotrophic conditions, a substantial amount of the limiting energy source must be expected to be utilized for maintenance purposes.

Conceivably, at very low dilution rates the energy source could eventually be utilized exclusively for maintaining the cell integrity, thus resulting in a specific rate of growth of zero. However, there is evidence that μ cannot be any given value between μ_{max} and zero, but has a certain minimal value, μ_{min}. Progressively decreasing viabilities were observed by Tempest et al. (1967) upon a decrease in the dilution rate of carbon- and energy- (glycerol) limited cultures of *Aerobacter aerogenes*. At a D of 0.04 h^{-1} ($\mu_{max} = 1.0$ h^{-1}), the viability was still 90%, but decreased to less than 40% at a dilution rate of 0.004 h^{-1}. As a consequence of the fact that steady states could be established, the specific rate of growth of the viable population must have been higher than the dilution rate (compare Fig. 5). It was estimated that the minimum value of μ was 0.009 h^{-1}, which is approximately 1% of μ_{max}. Considering the nature of the growth limitation, this might be attributed to a shortage of energy. However, the comparison with ammonia-limited cultures receiving the energy source in excess throughout, revealed that energy is not the (only) decisive factor. The minimum rate of growth of the viable population under ammonia-limitation was found to be about 0.007 h^{-1}.

It is to be expected that organisms with lower μ_{max} values will have correspondingly lower μ_{min} values. As mentioned earlier, competition studies between the specialist *Thiobacillus neapolitanus* and the versatile *Thiobacillus* A2, performed under thiosulfate limitation at dilution rates of 0.025 h^{-1}, 0.050 h^{-1}, and 0.075 h^{-1}, resulted in a dominant population of *T. neapolitanus*. The sustained presence of *T.* A2 (5-10%) was attributed to the utilization of glycollate excreted by the specialist. However, similar experiments at a D of 0.004 h^{-1} resulted in increased *T.* A2 numbers (up to 70% after five volume changes), concomitant with more drastically reduced viabilities of *T. neapolitanus* (40% viable) compared to *T.* A2 (85% viable). A dilution rate of 0.004 h^{-1} is only 1.1% of the μ_{max} of *T. neapolitanus*, but is still 4% of the μ_{max} of *T.* A2.

From the considerations mentioned above, it may be expected that organisms which are adapted in such a way that growth under oligotrophic conditions is possible are slow growing organisms even at higher substrate concentrations (low μ_{max}). The fact that cells

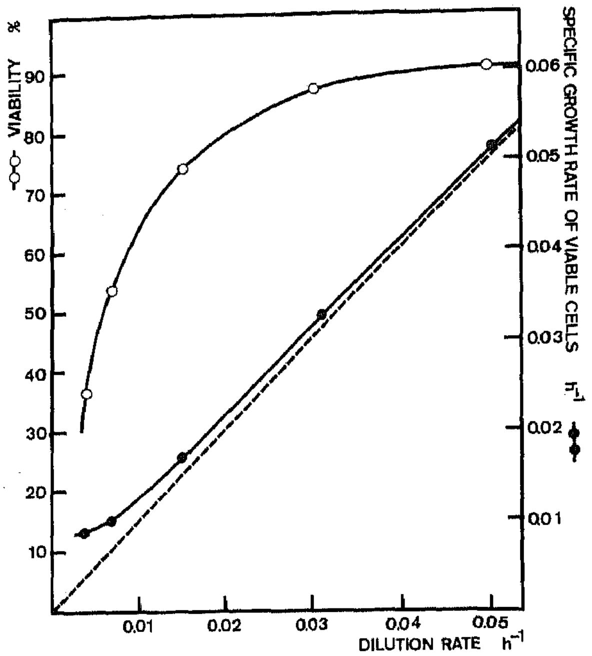

Figure 5. Steady-state viability (● — ●) and specific growth rate of the viable cells (■ — ■) of glycerol-limited Aerobacter aerogenes. Dashed line represents the specific growth rate assuming a 100% viability. Redrawn from Tempest et al. (1967).

geared to an extremely accurate control over biosynthesis at very low rates have low maximum specific growth rates, may well be related to the properties of enzymes (see S/V ratio). Again the principle of the "limited elasticity" is applicable.

It is obvious that organisms thriving in oligotrophic habitats, conceivably often facing starvation conditions, will not have high endogenous rates of metabolism. Such a "strategy" would result in even less energy available for growth. Genuine oligotrophs such as Arthrobacter, Caulobacter, and Hyphomicrobium may be expected to show low endogenous growth rates (Poindexter 1981; Boylen and Ensign 1970a, b). An interesting observation on this respect was made by Meiberg and Harder (unpublished) in starving Hyphomicrobium cells. After a week the O_2-uptake rate became undetectably low but all cells remained viable; even after 3 months of starvation methanol was respired immediately.

In most heterotrophic organisms the energy source also serves as the source of carbon; thus under oligotrophic conditions many organisms can be expected to be both carbon- and energy-limited. However, in phototrophic bacteria the effect on survival of a low rate of energy supply (low irradiance) can be separated from the effect of low fluxes of carbon. Such studies have been performed with a Chromatium strain in the presence of both an organic (acetate) and an inorganic (CO_2) carbon source (van Gemerden 1980). In the studies the relation between the specific growth rate and the light intensity was estimated, and by extrapolation of the data to zero growth rates, the light intensity required for maintenance purposes was deduced. Indeed, at this light intensity Chromatium maintained a 100% viability for 10 days without visible growth. In the corresponding dark cultures which also contained the carbon sources, the viability decreased rapidly. This was confirmed with ATP data. It is to be expected that similar observations can be made for green algae, cyanobacteria (Gons and Mur 1975), and green bacteria. In this respect it is of interest to note that green sulfur bacteria appear to require less light for the maintenance purposes than purple bacteria (Biebl and Pfennig 1978; van Gemerden 1980). This can be ecologically significant. In layered ecosystems green sulfur bacteria usually are found below purple sulfur bacteria; thus they will receive less light. The ability of the purple sulfur bacteria to outcompete the green sulfur bacteria at levels closer to the surface may be related to the fact that growth of the green sulfur bacteria is completely inhibited by traces of oxygen. This is not the case for the purple sulfur bacteria who can grow chemolithotrophically under microaerophilic conditions (Kämpf and Pfennig 1980). Although habitats which support the exuberant growth of phototrophic bacteria are usually characterized as being (highly) eutrophic, this is to be considered a misconception with respect to light.

The differences just described with respect to the intensity of the light required for the maintenance between the purple and the green sulfur bacteria are similar to those found between the green algae and cyanobacteria (Gons and Mur 1975; van Liere 1979). It was suggested by Raven and Baerdall (1981) that the lower μ_e value observed in the green sulfur bacteria and the cyanobacteria is related not only to the quality and quantity of the pigment of these organisms, but also to the spatial distribution of the (bacterio) chlorophylls within the cells. For instance, the Chlorobium vesicles would prevent an excessive leakage of protons to the external medium. Without the vesicles, an additional amount of energy would be required to compensate for this leakage; this would raise the maintenance requirements. In addition, the high pigment content of these organisms enables them to scavenge the available photons more effectively. Such organisms thus can best be described as well adapted to oligophototrophic conditions. In the green sulfur bacteria such a strategy may well have been developed during evolution as a result of the obligate anaerobic growth requirements which forced them to inhabit the deeper layers to avoid the toxic oxygen produced at the surface layers. At the same time, their most common electron donor, sulfide, usually is produced in the underlying sediments.

THE "MODEL" OLIGOTROPH

Thus far we have described differences between organisms. From these facts we have speculated about how the properties we observe allowed the organisms to survive.

Another way to proceed is to first try to design an ideally equipped organism for a certain job and then by looking at examples found in nature see how well evolution has been able to design such an organism. In the present context we would like to discuss the profile of the "model" oligotroph on the basis of the stimulating thoughts and ideas put forward by Poindexter in Hirsch et al. (1979).

Oligotrophs are defined as those organisms known to be able not only to survive but particularly to multiply under conditions of extremely low and often discontinuous supply of nutrients. In other words, organisms adapted to low and irregular fluxes of substrates. One might wonder what properties such organisms should have to survive optimally and what the consequences would be. Table 8 (Hirsch et al. 1979) summarizes the characteristics of such an organism with respect to the uptake of nutrients, and Table 9 (Hirsch et al. 1979) does the same with respect to the utilization of substrates.

a. A high surface/volume ratio would enable such organisms to scavenge substrates, due to the relatively high proportion of uptake sites. In addition, the internal diffusion pathway would be reduced. The "model" oligotroph thus can be expected to be small, elongated, and may have cellular appendages.

Studies with bacteria isolated from the same habitat generally show that bacteria with comparatively low surface-volume ratios are outcompeted at low substrate concentrations. Examples obeying this statement are summarized in Table 2.

The occurrence under nutrient limitation of fully expressed prosthecae in *Ancalomicrobium* and the long stalks in *Hyphomicrobium* also are in acccordance with these expectations.

b. The preferential utilization of energy for the uptake of nutrients can be expected to be of advantage under conditions of low nutrient supply. The continued uptake of substrates at concentrations which are either too low to permit growth at $\mu > \mu_{min}$ or are unbalanced, would lead to the synthesis of reserve materials. These substances could later, when conditions have changed, be used to replenish the nutrients which are available at that time and in this way allow growth. Storage material could even be synthesized under conditions enabling growth. At first glance, it seems unlikely that reserve materials are synthesized under energy limitation, nevertheless it has been reported to occur. Matin and Veldkamp (1978) observed increased amounts of PHB with decreasing dilution rate in

Table 8. Characteristics of nutrient uptake and their consequences for model oligotrophs (from Hirsch et al. 1979).

Characteristic	Consequence
High surface/volume ratio	a) cells small and/or elongated b) morphology possibly unusual c) relatively high proportion of uptake sites among surface components d) decreased diffusion path from surface to internal sites of utilization
Metabolic energy used preferentially for nutrient uptake, especially when not actively growing and reproducing. Energy not dissipated in irrelevant activities.	a) relatively high proportion of endogenous metabolic energy would be consumed for uptake b) possession of specific electron donors that could be quickly tapped for the generation of the proton gradient allowing transport. The proton gradient could be coupled to inhibition mechanisms for other gradient-consuming processes.
Constantly capable of uptake	Could not afford to produce resting stage cells impermeable to nutrients or unable to perform transport (except possibly for dispersal).
Uptake systems of high affinity; possibility for simultaneous uptake of mixed substrates	a) highly sensitive to toxic solutes of biotic and abiotic origin b) during nutrient enrichment, a relative excess of one nutrient might interfere with uptake of others c) A nutrient present in relative excess will be stored; the ability to store a variety of reserve materials would be needed
Uptake would lead to reserve accumulation, particularly when the rate of uptake was submaximal and metabolic pools were not saturated due to extremely low substrate concentrations, but possibly also when substrates were adequate for balanced growth.	Relatively low maximum growth rates. In laboratory cultures, this could result in burdensome accumulation of reserve materials.

Table 9. Characteristics of nutrient utilization and their consequences for model oligotrophs (modified from Hirsch et al. 1979).

Characteristic	Consequence
Low endogenous metabolic rate with energy used principally to support uptake constituents	a) slow degeneration of reserve materials and cellular b) prolonged viability in absence of exogenous nutrient supply
Increase in metabolic rate to a level supportive of net biosynthesis would occur only when both the uptake systems and the metabolic pools were saturated	Slow response to nutrient enrichment (as measured by net biosynthesis or overall multiplication); the oligotroph would, therefore, not be among the first flush of microbial multiplication following nutrient enrichment
Biosynthetic rate would drop rapidly in response to decline decline of uptake rate due to exhaustion of substrates below a concentration that allowed maximal rate of transport	Growth rate would not be maintained during depletion of exogenous nutrients, although uptake rate would be maintained and nutrient accumulation would continue
Catabolically efficient and versatile	a) would posess at least average genetic complexity b) would by oxybiontic
Large proportion of catabolic enzymes would be inducible, while carriers would be constitutive	a) the usual economy of specific protein synthesis b) reduction in variety of enzymes available for endogenous metabolism c) slow response to nutrient enrichment d) even with constitutive carriers, low specificity of uptake might cause interference by a relatively excessive nutrient with uptake of others
Minimal level of catabolite repression	Ability to utilize several carbon- and energy-sources simultaneously
Ability to store a variety of reserve materials	a) relatively large proportion of cell volume could become committed to storage, reducing capacity for other activities b) growth (as net, balanced biosynthesis) could result from unbalanced, single-nutrient enrichment in the environment of the well-stocked cell

lactate (carbon and energy!) limited continuous cultures of a Spirillum sp. A similar phenomenon has been observed in Hyphomicrobium (Meiberg and Harder, unpublished).

c. Low endogenous rates of metabolism would result in a slow degradation of the reserve materials or essential macromolecules. It would enable an organism to grow faster under supporting conditions, or to remain viable for longer periods of time under starvation conditions.

Low endogenous rates have indeed been reported to occur in such genuine oligotrophs as Arthrobacter, Caulobacter and Hyphomicrobium.

d. The possession of inducible carriers under conditions of low and discontinuous supply of nutrients can be expected to be fatal. Similarly, the production of resting cells impermeable to nutrients can be concluded to be highly disadvantageous. Thus, the "model" oligotroph does not form spores and has constitutive uptake carriers. The possession of non-specific carriers would be most favorable.

In addition, the uptake systems must have a high affinity towards the substrate(s). In Caulobacter crescentus a high affinity uptake system for NH_4^+ has indeed been reported to be present on a constitutive level (Ely et al. 1978). According to Hirsch et al. (1979; see Table 8), the "model" oligotroph should have the possibility to utilize a number of substrates simultaneously. This is correct, but seems not be a special feature of oligotrophs; all organisms studied so far in this respect can simultaneously utilize different substrates, provided the concentration of the nutrients is low.

e. Under conditions where not all requirements for growth are met, uptake should continue and result in the accumulation of reutilizable storage materials. Balanced net biosynthesis (growth) should not occur unless both the uptake systems and the internal metabolic pools were saturated. As mentioned earlier, the synthesis of storage materials has been observed even when conditions are such that balanced growth is possible.

As a consequence, the "model" oligotroph can be expected to have a relatively low μ_{max}, and in addition shows a slow growth response upon a sudden increase in the concentration of nutrients.

f. A continuation of growth under conditions where uptake rates decline (as the result of decreasing substrate concentrations), would result in the exhaustion of intracellular reserves. The "model" oligotroph may therefore be expected to show a rapid decline in growth rate in response to a decreased uptake rate. Consequently, an internal nutrient enrichment is to be expected upon the continued uptake even if the rate of uptake would be somewhat reduced as a result of the decreased concentrations.

g. Being a specialist, and handling available energy sources inefficiently, cannot be regarded a "wise" strategy under conditions of low and irregular nutrient availability. Thus, the utilization of nutrients by "model" oligotrophs can be expected to be catabolically versatile and with a high efficiency of energy conversion.

In general this is indeed found in most of the cases. Caulobacter sp. and Arthrobacter sp. are best described as versatile organisms. On the other hand, Hyphomicrobium species are mainly restricted to growth on C_1-compounds. One might wonder why the latter organism, even under oligotrophic conditions, has been able to specialize on so few substrates. It may mean that in many environments a continuous supply of C_1-compounds is available. For example, air invariably contains volatile C_1-compounds other than CO_2, and the same is true for environments where CH_4 is formed.

h. Under oligotrophic conditions, a delay in the uptake can be a disadvantage of capital importance in the presence of other organisms. Therefore, the "model" oligotroph does contain its uptake carriers on a constitutive level. On the other hand, having a wide variety of catabolic enzymes constitutively at high levels is meaningless under conditions of low and irregular substrate availability. Thus, most catabolic enzymes can be expected to be inducible, or possibly constitutive but then at low levels. Therefore, upon nutrient enrichment, the "model" oligotroph would show a fast response with respect to uptake, but a slow response with respect to growth.

i. From the foregoing it is obvious that under conditions where the rates of uptake and growth are not comparable, the capability to synthesize reserve materials becomes essential. The ability to store a variety of reserve materials enhances the chances for a full and complete utilization of the uptake potential under the conditions encountered.

In conclusion, it can be stated that known oligotrophs to some extent fulfill the expectations. In some other cases at least the biochemical mechanisms for the "model" oligotroph are available.

However, some of the properties mentioned are observed in organisms which cannot be regarded as oligotrophs at all. The secret of the sustained life under oligotrophic conditions would then lie in the combination of as many of the properties listed as possible. Thus, not necessarily all properties would be encountered in all oligotrophic organisms. It may very well be that there does not exist a single organism possessing all these properties, but rather that a whole spectrum of oligotrophs exist which possess one or more of the properties to a greater or lesser extent. The educated guess of Poindexter (Hirsch et al. 1979) may help us to look specifically for those characteristics which can be expected to be of particular relevance for the strategy of the oligotrophic mode of life.

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