Psychrophilic and psychrotrophic microorganisms

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Summary. Psychrophilic and psychrotrophic microorganisms have the ability to grow at 0 °C. Psychrotrophic microorganisms have a maximum temperature for growth above 20 °C and are widespread in natural environments and in foods. Psychrophilic microorganisms have a maximum temperature for growth at 20 °C or below and are restricted to permanently cold habitats. This ability to grow at low temperature may be correlated with a lower temperature characteristic than that of the mesophiles, an increasing proportion of unsaturated fatty acids in the lipid phase of the cell membrane, which makes it more fluid, and a protein conformation functional at low temperature. The relatively low maximum temperature of growth for these microorganisms is often considered to be due to the thermolability of one or more essential cellular components, particularly enzymes, while some degradative activities are enhanced, resulting in an exhaustion of cell energy, a leakage of intracellular substances or complete lysis. Psychrotrophic microorganisms are well-known for their degradative activities in foods. Some are pathogenic or toxinogenic for man, animals or plants. However in natural microbial ecosystems psychrotrophic and psychrophilic microorganisms can play a large role in the biodegradation of organic matter during cold seasons.
Key words. Psychrophily; psychrotrophy; microorganisms; temperature; physiology; activities.

The ability of microorganisms to grow at 0 °C was first reported by Forster in 1887[12]. He isolated bioluminescent bacteria from fish preserved by cold temperatures, and later other bacteria, from natural water, foods and fish, that were capable of growth at 0 °C. The term 'psychrophile' was proposed by Schmidt-Nielson in 1902[43] for microorganisms that had the ability to grow at 0 °C. Ingraham and Stokes[23] and Stokes[45] defined more precisely that psychrophiles are microorganisms which grow rapidly enough at 0 °C to become macroscopically visible in about one or two weeks and subdivided them into strict, or obligate, and facultative, depending on whether they grow most rapidly below or above 20 °C. Morita[33] debated the validity of this definition, since the facultative psychrophiles do not actually prefer a low temperature. He proposed that psychrophiles be redefined as 'organisms having an optimum temperature for growth at about 15 °C or lower, a maximum temperature for growth at 20 °C or below'. He used the term psychrotrophic for 'organisms previously referred to as facultative psychrophiles, the maximum temperatures of which being above 20 °C'. This last definition will be used in this paper. With the development of cold or freezing procedures for food preservation many pragmatic studies on psychrotrophic microorganisms have been made by food microbiologists. More fundamental investigations on psychrophilic microorganisms were carried out especially between 1958 and 1975[2,10,24,25,33]. However, for some years the fundamental interest in them has apparently decreased, while new fascinating 'extremophile' bacteria like thermophilic archaebacteria have attracted the attention of microbial ecologists and physiologists.

For eighty years, many investigators reported the isolation of 'psychrophilic' microorganisms from various environments: fish, meat, milk, vegetables, soils, continental and marine waters ..., but most of them were probably not true psychrophiles since the investigators usually had no refrigerated incubators, and were unaware of the thermolability of psychrophilic organisms at laboratory temperature. Furthermore the term 'psychrophile' had not the same meaning for all the investigators. It is difficult to list with certainty the genera and species of psychrophilic or psychrotrophic microorganisms which have been isolated. Some have not been identified. Table 1 gives examples of the most commonly isolated microorganisms. Psychrophilic or psychrotrophic microorganisms belong to various genera of gram-negative or gram-positive bacteria, cyanobacteria, fungi and eucaryotic algae. Psychrophilic bacteriophages have been isolated from psychrophilic bacteria[51].

Psychrotrophic microorganisms are widespread in natural environments and in foods. True psychrophilic microorganisms are restricted to permanently cold habitats: oceans (the temperature of more than 90 % of the volume of marine waters is below 5 °C), polar areas, alpine soils and lakes, snow and icefields, caves ... 0 °C is not per se an 'extreme' condition. Cold environments may be considered as extreme environments only if another factor creates hard conditions, like low water activity in arid antarctic soil, or low nutrient availability and high pressure in the deep sea. There are a number of reports on the microbiology of polar soils, especially in Antarctica,

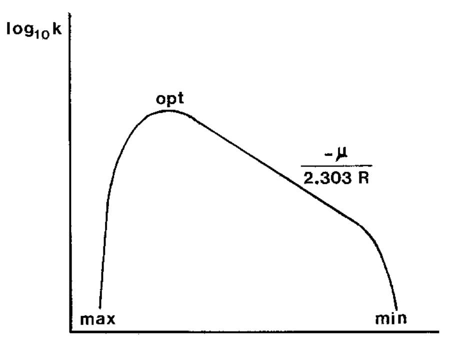
Table 1. Examples of psychrophilic and psychrotrophic microorganisms[2,4,5,15,21,23,41,45,51]. ^{p}, genera among which some psychrotrophic species are pathogenic for man or animals; ^{pp}, some are plant pathogens

Bacteria	Diatoms
Pseudomonas ^{pp}	*Fragillaria*
Vibrio ^{p}	
Acinetobacter/Alcaligenes	Green algae
Aeromonas ^{p}	*Chlamydomonas*
Chromobacterium	*Raphidonema*
Flavobacterium	*Chloromonas*
Serratia ^{p}	*Cylindrocystis*
Yersinia ^{p}	
Arthrobacter	Yeasts
Corynebacterium	*Candida*
Brevibacterium	
Cellulomonas	Filamentous fungi
Lactobacillus	*Typhula* ^{pp}
Brochothrix	*Leptomitus*
Streptococcus	*Mucor*
Micrococcus	*Rhizopus*
Listeria ^{p}	*Penicillium*
Bacillus	*Alternaria*
Clostridium ^{p}	*Cladosporium*
Cytophaga ^{p}	*Keratinomyces* ^{p}
Cyanobacteria	Virus
Nostoc	Bacteriophages of *Pseudomonas*

where dry valleys are extreme environments considered as models for testing the hypothesis of life on the planet Mars. The well-known phenomena of 'red snow' on snowfields in summer is the result of several species of 'snow algae' which do not grow at a temperature above 10 °C. The most common is *Chlamydomonas nivalis*. Its brilliant red spores are responsible for the red color. The alga probably grows within the snow as a green-pigmented vegetative cell and then sporulates; as the snow dissipates by melting, erosion and vaporization, the spores become concentrated on the surface[4].

1. *Physiology of psychrophilic and psychrotrophic microorganisms*

Microbial growth involves an interrelated sequence of chemical reactions, which are influenced by temperature. The basis for the relationship between temperature and growth rate is expressed by the Arrhenius equation:
$\log_{10}k = -\Delta E/2.303\ RT + C$
where k = reaction rate constant, that is, in the present case, growth rate; ΔE = energy of activation, which is referred to as the temperature characteristic (μ); T = absolute temperature; C = constant.
Consequently, if a plot of $\log_{10}k$ versus 1/T is made, then the slope of the linear portion of the curve equals $-\mu/2.303R$ and the temperature characteristic value can be calculated. The linear portion of the curve indicates the dependence of the growth rate on temperature on the Arrhenius principle regarding a chemical reaction (fig.). However, at temperatures below or above this range, a deviation from linearity occurs and growth finally terminates. A microorganism is characterized by its cardinal temperatures, which are the maximum, optimum and minimum growth temperature.



General form of an Arrhenius plot of bacterial growth; max, maximum; opt, optimum; min, minimum temperature for growth.

Table 2. Temperature characteristic of psychrophilic, psychrotropic and mesophilic *Arthrobacter* strains[7]

Psychrophile	*Arthrobacter glacialis*	12,200
Psychrotrophic strains		14,800
		15,100
		15,800
		18,000
Mesophiles	*Arthrobacter globiformis*	21,200
	Arthrobacter variabilis	21,800
	Arthrobacter ureafaciens	23,900

Table 3. Percentage of unsaturated fatty acids in total fatty acids related to growth temperature of three *Arthrobacter* strains, respectively mesophilic, psychrotropic and psychrophilic[8]

	Growth temperature (°C)				
	−2	+2	+10	+25	+32
A. globiformis (optimum growth temperature: 32°C)	–	23.1	12.8	2.6	0
A. sp. str. SI 55 (optimum growth temperature: 25°C)	37.5	32.3	16.5	2.6	–
A. glacialis (optimum growth temperature: 13°C)	32.3	31.2	17.8	–	–

The general form of the Arrhenius plot of growth rate is typical for all microorganisms studied, but the temperature characteristic and the cardinal temperatures vary widely among microorganisms. The cardinal temperatures are lower for psychrotrophic and psychrophilic microorganisms than for mesophilic microorganisms. It is difficult to establish the lower limits since growth below 0 °C is limited by the availability of liquid water or the ability to withstand the osmotic pressure required to maintain water as a liquid below the freezing point of pure water. Most bacteria grow over a range of approximately 40 °C; it is rarely greater but some bacteria grow only over a more limited range of growth temperature. For instance we found cave bacteria that grew at 10 °C and 20 °C but did not at 4 °C or 28 °C[14]. According to the classical definitions, they are neither mesophilic, nor psychrophilic or psychrotrophic. I suggest that the term 'stenotherm' be used for such bacteria.
The first point that has to be explained is how some microorganisms are capable of growth at low temperatures – or, in other terms, what prevents the growth of mesophilic microorganisms at temperatures above 0 °C. The second point is: what inhibits the growth of psychrophilic microorganisms at 20 °C?

2. *Growth at low temperature*

Temperature characteristic

One of the earlier hypotheses for growth at low temperature was suggested by Ingraham[22], using the comparison between Arrhenius curves of a psychrophilic *Pseudomonas* and of *Escherichia coli*: the temperature characteristic (μ) was lower in the psychrophilic bacteria. The same observation was made by several authors, for instance by Tai and Jackson[47] on *Micrococcus cryophilus* and its two mesophilic mutants (μ were respectively 10,000, 15,000 and 16,000 calories) and by Canillac[7] on psychrophilic, psychrotrophic and mesophilic strains of *Arthrobacter* (table 2). However, such a relationship has not been found for three species of *Vibrio*[18] and for yeasts[44].

Lipid composition and membrane fluidity at low temperature

Temperature effects on the composition of the lipids of poikilotherm organisms have been known for many years. In general, as growth temperature is decreased, the lipids are composed of an increasing proportion of unsaturated fatty acids which serve to maintain an optimal degree of fluidity in the lipid phase of the cell membrane. The same phenomenon applies to bacteria, for example *E. coli*[31]. Furthermore, experiments made with mutants of *E. coli* or *Salmonella* grown in a medium with unsaturated fatty acids showed a lower minimum growth temperature. In *E. coli*, temperature modulates the specificity of enzyme activity. In *Bacillus megaterium* three control mechanisms have been shown to regulate the level of the biosynthetic enzyme for unsaturated fatty acids: de novo enzyme synthesis is induced at low temperature, the enzyme is irreversibly inactivated at high temperature, and the ability to synthesize the enzyme is lost at high temperature[13]. Such a change in fatty acid unsaturation has been observed in a number of psychrophilic bacteria (*Bacillus, Listeria, Pseudomonas, Brevibacterium*) and yeasts[8]. Lipid composition of three *Arthrobacter* strains, respectively mesophilic, psychrotrophic and psychrophilic, were studied by Canillac et al.[8]. Incubation of three strains at temperatures below their optimum resulted in an increase of the ratio of unsaturated, of branched and of short-chain fatty acids (table 3). The mesophilic strain *A. globiformis* grew at 2°C but did not at 0°C. No unsaturated fatty acids were synthesized at 32°C which is its optimum growth temperature. After a shift from 32°C to 2°C during the mid-exponential phase, there was a lag phase until the proportion of unsaturated fatty acids reached 19–20°C. After a shift from 32°C to 0°C, the proportion of unsaturated fatty acids did not increase beyond 13% and no growth occurred. The inability to grow at 0°C may result from an inability to synthesize sufficient unsaturated fatty acids.

In *Micrococcus cryophilus* there are no growth-temperature-dependent changes in fatty acid unsaturation, but there are acyl-chain length changes[42].

Temperature effects on proteins

The study of cold-sensitive mutants has provided examples of loss of function at low temperature. O'Donovan and Ingraham[35] isolated mutants of *E. coli* which were cold-sensitive for the biosynthesis of histidine. The first enzymes of the histidine pathway were 100–1000-fold more sensitive to feedback inhibition by the end product of the pathway, that is histidine. Moreover, both wild type and mutant enzymes have 10-fold increased sensitivity for feedback inhibition at 20°C compared with 37°C. At low temperature the functioning of the enzyme was prevented by an intracellular concentration of histidine which was too low to allow protein synthesis. Mutations in genes encoding ribosomal proteins also frequently confer a cold-sensitive phenotype[17,48]. Such mutant strains are unstable to assemble ribosomal subunits at low temperatures.

So it seems probable that cold-sensitivity may result from mutations in genes encoding ribosomal proteins and from those in genes encoding regulatory enzymes, since both classes of proteins require a precise conformation to be functional. The weakening of hydrophobic bonds which occurs at low temperatures probably results in such conformational changes.

In contrast to the situation in mesophiles, all structural and metabolic proteins of psychrophiles have to be functional at low temperature.

Cold adapted mutants

In wild strains, it is generally assumed that a number of vital functions cease simultaneously at low temperature. Consequently a number of mutations, in genes encoding a variety of functions, must be required to extend the lower growth-temperature range. In spite of a great number of attempts, psychrotrophic mutants of *Escherichia coli* have not been isolated[26]. However, mutants with an extended low growth-temperature range have been found among Pseudomonads by Olsen and Metcalf[36]. A psychrotrophic mutant of *Pseudomonas aeruginosa* has been isolated, the growth-temperature range of which was 32°C to 0°C. *Pseudomonas aeruginosa* is almost unique among the various species of *Pseudomonas* in that it is not a psychrotrophe. The authors suggested that, since *P. aeruginosa* is closely related to psychrophiles, the number of mutational changes necessary to gain psychrophily might be expected to be small, and they demonstrated that psychrophily could be transduced from *P. fluorescens* to *P. aeroginosa*.

3. Determinants of low maximum temperature of growth of psychrophilic and psychrotrophic microorganisms

The molecular basis for the relatively low maximum temperature of growth for microorganisms which normally grow at low temperatures probably varies depending on the microbe. In general terms, the upper temperature limit for microorganisms is often considered to be due to the thermolability of one or more essential structural or functional cellular components; for example enzymes, regulation factors or membrane components.

Enzyme inactivation

The most susceptible cell components are proteins, particularly enzymes or other functional proteins, since a subtle change in their configuration may result in their inactivation. The susceptibility of enzymes to relatively moderate temperature variation may be responsible, partly or completely, for the low maximum growth temperature exhibited by psychrophilic microorganisms. A great variety of enzymes have been studied, particularly enzymes of oxidative and fermentative pathways, in psychrophilic bacteria, yeasts and molds, both with whole cells and with cell-free preparations[25]. Usually the enzymes of psychrophiles are more heat-sensitive than those of mesophiles, but a number of them remain active several degrees above the temperature which inhibits growth. Some are affected at the maximal growth temperature and their synthesis itself may be inhibited. It was found in studies of a variety of organisms that it was not always the same enzyme which was most thermolabile. The more essential a protein is for growth, the more drastic is the effect of its thermal inactivation. This is the case when the protein is involved in DNA, RNA or protein synthesis. In psychrophilic *Bacillus (B. psychrophilus, B. insolitus)*[3] and *Candida gelida*[34] protein synthesis is inhibited at the maximum growth temperature. One or more aminoacyl synthetases are often thermolabile. Glutamyl- and prolyl-tRNA synthetases of *Micrococcus cryophilus* were inactivated after 10 min at 30°C[30], and six aminoacyl-tRNA synthetases of *Candida gelida* were thermolabile after 30 min at 35°C[34].

In a psychrophilic *Pseudomonas*, Harder and Veldkamp[19] observed that cells grown in a chemostat showed the lowest RNA content at 14°C; at lower temperatures the cells compensated the temperature-induced decrease of reaction rates by increasing the concentration of RNA and respiratory enzymes. At temperatures above 14°C the cellular RNA content also increased, probably counteracting an impairment of protein synthesis. Above 18°C the RNA synthesis was inhibited, which resulted in a rapid decrease of protein synthesis, until between 19 and 20°C growth ceased entirely.

Enzyme activation

While some enzymatic activities are inhibited, others are less thermosensitive and may be enhanced at the maximum growth temperature, especially some degradative activities; this results in an exhaustion of cell reserves and loss of viability. If protein synthesis is reduced and intracellular proteolysis enhanced, protein degradation will be greater than new synthesis. Potier et al.[39] showed that protein breakdown in the psychrotrophic bacterium *Arthrobacter* sp. SI 55 was much greater than in mesophilic microorganisms such as *Escherichia coli,* and protein degradation increased when cells were grown at 32°C, which is the maximal growth temperature for this bacterium. Conceivably psychrophilic, psychrotrophic and mesophilic bacteria possess different mechanisms for the control of protein degradation, which determine their temperature range of growth. The very high degradation at 32°C and above may set an upper limit to growth in this organism.

On the other hand, extracellular lipases and proteases of psychrotrophic bacteria which develop in refrigerated raw milk are heat resistant; they are not inactivated by pasteurization or ultra high temperature treatment and their activity in dairy products results in bad odors or taste[27].

Cell structure modification

Relatively moderate temperature variation can alter the ultrastructure of the cell wall as shown by electron microscopy, as is observed with *Vibrio psychroerythrus*[9] and *Bacillus psychrophilus*[1], or they may be reflected in changes in overall cellular appearance. The psychrotrophic *Bacillus insolitus*[11] and *Arthrobacter* sp. SI 55[15,16] form filamentous cells at 30°C, but only normal-sized cells at 20°C. The psychrotrophilic *Arthrobacter glacialis* forms clumps of coccoid cells at 18°C and normal motile rods at or below 13°C[16]. Cell morphology of the psychrophilic fungus *Sclerotinia borealis* is altered at 25°C[49].

These alterations may affect both the cell wall and the cytoplasmic membrane, resulting in changes in cell permeability demonstrated by the leakage of intracellular substances or by actual complete lysis. This was observed in *Candida nivalis, Merulius lacrymans, Vibrio marinus, Bacillus psychrophilus*[25].

Lipids

Recently McGibbon and Russel[32] reported that a shift up from 0°C to 20°C of a culture of *Micrococcus cryophilus* resulted in a rapid turnover of phospholipids and the reciprocal shift down did not. They argued that a psychrophilic organism well adapted to growth at low temperatures is more stressed by a sudden increase of growth temperature than by a sudden decrease.

Thus there is no general and unique explanation for thermal sensitivity of psychrophilic microorganisms. It is probably the result of thermolability of one or more metabolic or structural components and activation of degradative reactions.

In conclusion, regarding the interaction of temperature with the cellular processes of psychrophiles, most investigations have concentrated on the effects of relatively high rather than low temperatures. This has resulted in the elucidation of various bases for the effects exerted by temperatures above the optimum for growth, but does not clarify the important problem of how they function at low temperatures, in spite of the fundamental interest of this question, and its important consequences and applications.

4. *Role of psychrotrophic and psychrophilic microorganisms in the biosphere*

Psychrophilic or, more exactly, psychrotrophic microorganisms are better known for their injurious activities rather than for their usefulness, especially in foods. They come from water, soil, air and sometimes from the food itself (marine fish, vegetables). The greatly increased use of frozen and especially chilled foods in recent years, and the increasingly longer periods of time between their production and consumption, have greatly increased the importance of psychrotrophic bacteria for the food industries.

The psychrotrophic population in dairy products is composed mainly of gram-negative rod-shaped bacteria: *Pseudomonas, Acinetobacter, Alcaligenes, Chromobacterium, Flavobacterium,* etc. Strains of *Pseudomonas* are the most troublesome psychrotrophes in the dairy industry because of their pronounced ability to produce undesirable flavors, odors and pigments. Furthermore, they produce thermally resistant enzymes (lipases and proteases) which are resistant to pasteurization and even to ultra high temperature treatment, and their activity does not stop after the bacteria have been killed[27]. For 20 years, milk cooling on the farm has been developed to decrease the cost of carriage. The mixture of 4–6 milkings is kept in refrigerated tanks and collected every 2 or 3 days. In the factory, milk is often stored in the cold for one or two days. Three to five days are sufficient for very large cell populations to arise and produce a significant amount of enzymes, since the generation time of the psychrotrophic *Pseudomonas* is about 8 h at 4°C[38].

In meat, in addition to psychrotrophic degradative bacteria or fungi, like *Lactobacillus viridescens, Brochothrix thermosphacta,* etc., pychrotrophic pathogenic or toxinogenic bacteria like *Listeria monocytogenes* or clostridia can develop at low temperature. Frozen meat is normally kept below −18°C, but the temperature may increase, resulting in meat thawing, in wholesale distribution channels, in stores or in home freezers. *Clostridum botulinum* type E is now the most common *C. botulinum* type in industrial countries where it replaces the previously dominant types A and B. In contrast to the toxins A and B which are synthesized near 30°C, the toxin E may be produced at +6°C and is rather more aggressive than that of the bacteria grown at the higher temperature[6].

Some transfusion accidents may be caused by psychrotrophic bacteria which develop in cooled blood. These are mainly strains of *Serratia, Pseudomonas* and less often *Yersinia* and *Proteus*[41].

Cytophaga psychrophila, the etiological agent of bacterial cold-water diseases of fish, is generally found in young silver salmon in early spring when water temperature is low, and abates when water temperature increases to 13 °C. In some hatcheries, up to 30% of the fry have been lost as a result of this disease[37].

The phytopathogenic *Pseudomonas syringae* which develops on buds. leaves and blossoms plays a significant role in the production of ice-nucleated particles at a relatively warm temperature of −2 °C[29]. It is responsible of frost damage to fruit trees and vines in spring[28]. Furthermore, frost is known to be a predisposing factor for infection. A biological control of this pest was suggested, which would consist of plant inoculation with non ice-nucleating mutants adapted to host colonization[28].

The snow mold is a fungus of the genus *Typhula* which is pathogenic on grass and winter cereals like wheat. It passes the winter in the vegetative state. It cannot grow at temperatures above 15 °C and remains dormant through the summer in a resting state as a *sclerotium*. When the plants become covered with snow, the *sclerotia* germinate and the fungus grows. Snow mold is much more common in areas where fairly deep snows occur because the thick layer of snow effectively insulates the plants from the winter cold and the temperature at the snow-plant interface remains around 0 °C during the whole winter[4].

The papermill fungus *Leptomitus lacteus* is a psychrophilic aquatic fungus which develops in winter as an abundant cotton-like mycelium in rivers receiving papermill effluents. It causes heavy pollution[21].

In natural microbial ecosystems, however, psychrophilic and psychrotrophic organisms can play a large role in the biodegradation of organic matter, not only in permanently cold areas, but also during late fall, winter and spring in habitats subjected to seasonal variations of temperature[2, 24]. Significant microbial activities have been demonstrated in soils at 2 °C during the winter; production of CO_2, cellulose and chitin degradation, ammonification and nitrogen fixation. Methane production has been demonstrated in peat at low temperature[46]. Photosynthesis by cyanobacteria and eucaryotic algae may be active in cold waters.

A thorough study of the microbial populations in northern and midarctic crude oils and their degradative activity has been carried out by Westlake et al.[50]. Under psychrophilic conditions the microbial populations that metabolize oils are not the same as those found under mesophilic conditions. Thus it may be useful to use microorganisms enriched under psychrophilic conditions for the breakdown of oil in a psychrophilic environment (e.g. in ocean oil spills).

Psychrotrophic microorganisms are more ubiquitous than psychrophilic microorganisms. However, Harder and Veldkamp[20] demonstrated that psychrophiles can successfully compete with psychrotrophes at low temperatures. They compared kinetics of growth of marine psychrophilic and psychrotrophic *Pseudomonas* at different temperatures and under different nutritional conditions in a chemostat. Both at low and high substrate concentration, obligate psychrophiles grew faster than psychrotrophes at 4 °C or below. At 10 °C the psychrophiles grew faster at high dilution rates. which reproduced the usual natural nutritional conditions of carbon- and energy-limitation. These results suggest that obligately psychrophilic chemoorganotrophes are responsible for mineralization processes in cold natural environments such as ocean waters and arctic or antarctic regions.

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Life without oxygen: what can and what cannot?

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Summary. The basic principles involved in the biotransformation of organic carbon compounds in the absence of molecular oxygen (dioxygen) are presented in this paper. The role of various electron acceptors during the breakdown of organic compounds is discussed and the metabolic end-products expected are summarized. The different biochemical possibilities and strategies for the anaerobic degradation of organic matter and the metabolic response of some organisms to anaerobiosis are elucidated. Positive and negative effects of anaerobiosis on environmentally relevant processes and their influence on man and on animals are reviewed. Finally, some examples of the biotechnological application of anaerobic processes are presented.
Key words. Anaerobic metabolism; anaerobic environment; biotechnological application of anaerobes; redox sequences; evolution of oxygen.

1. Introduction

Although our ideas about the sequence of events in the evolution of primitive life have changed in the last 40 years, it is now commonly agreed that the first living organisms are most likely to have resembled present-day fermentative bacteria[7]. They lived in the absence of molecular oxygen at the expense of abiotically synthesized organic compounds (anaerobic heterotrophy). When anaerobic life became more firmly established, anaerobic photosynthesizing bacteria emerged. These microorganisms possessed a light-harvesting and energy-transducing substance, chlorophyll, which enabled them to use energy from light to form organic matter from carbon