The starvation-survival state of microorganisms in nature and its relationship to the bioavailable energy *

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Summary. Although one can measure the organic matter in various ecosystems in terms of organic carbon, this measurement does not indicate what portion of the organic carbon is bioavailable to the microorganisms. Most organic matter is recalcitrant and, therefore, most microorganisms do not have sufficient energy to carry on their metabolism for growth and reproduction. As a result, many species of bacteria will form ultramicrocells and enter a physiological state known as starvation-survival. This physiological state results in metabolic arrest which permits the organisms to survive for long periods of time without sufficient energy for growth and reproduction.

Key words. Starvation-survival; energy; ultramicrocells.

Introduction

Microbiologists are concerned with growth in the laboratory under optimal conditions (excellent medium, optimal pH, temperature, and Eh, etc.) but conditions in nature, except in rare instances, do not offer the microbes such a luxurious life-style so that they can grow and multiply. Like all other biological forms, they have to seek an energy source so that growth and reproduction can occur. Because laboratory conditions are so favorable, people who look through a microscope at cultured bacteria are generally looking at giant cells, and Bergey's Manual of Determinative Bacteriology (all editions) gives a much larger size for most bacteria than would be found in nature. Because all organisms, especially the bacteria, seek energy, they drastically reduce the supply of energy in the environment, so that most ecosystems are grossly oligotrophic. Thus, we must consider the physiological state where sufficient energy is not present. Because of this situation, I have coined the term 'starvation-survival', which ensures the survival of the species. Starvation-survival has been defined as a physiological state resulting from the amount of nutrients available for growth and reproduction being insufficient. Although the starvation-survival state may be termed a dormant state of the microorganisms, it does not necessarily signify that the cell will remain in a dormant state. All that is necessary is that the appropriate nutrient (including energy) source becomes available in the environment so that the cell can metabolize, and, if the amount of energy or nutrients is sufficient and environmental factors are satisfied, then growth and multiplication can be initiated. The breaking of this type of dormancy is brought about by the availability of nutrients, especially energy. It is recognized that growth in most environmental systems is sporadic. The flow of energy in nature can also be sporadic; it may be present at all times, but in the latter situation, the amount may be very small. Thus, in nature, when small amounts of energy-providing nutrients become available, they can be metabolized, but may not be sufficient for growth and/or reproduction. Thus, the cell is just waiting until energy becomes available for its use. Within the microbial population, there are many types of organisms utilizing various types of energy sources as well as physiological differences in the population of the same species in nature. Another factor we must take into consideration is the time factor by which we measure metabolic reactions. Conditions in nature are not like those in enzymology, where most reactions are run at substrate saturation, optimum pH, optimum temperature, and Eh. Therefore, degradation of the more recalcitrant energy sources may not be measurable in laboratory time but in terms of days, weeks, months, or even years. It is commonly recognized that bacteria were the first type of organisms on earth, and the microorganisms have been on earth much longer than the eukaryotes, especially the green plant eukaryotes. Thus, mechanisms for the survival of heterotrophic, non spore-forming bacteria despite a lack of energy must have evolved, since this would have been essential for the survival of the species. All that is necessary is that one organism survives in a given environment, and when conditions become favorable again it will express itself[30]. For more information concerning this subject the reviews by Morita[30, 32], Roszak and Colwell[43], and Kjelleberg et al.[20] should be consulted.

Bioavailability of energy in nature

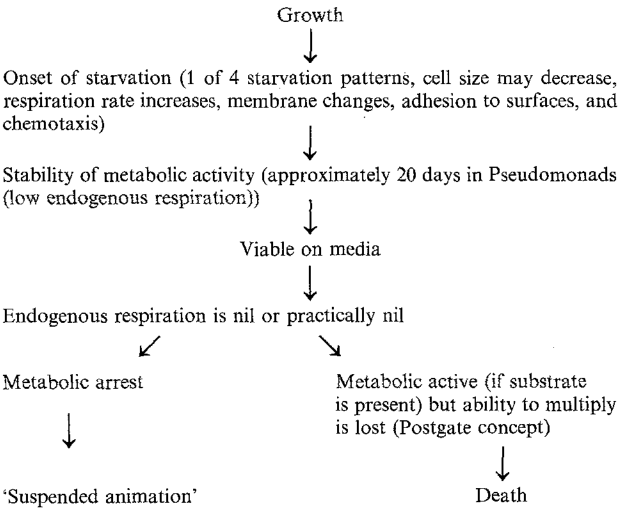
Energy in various ecosystems is measured by many ecologists as the amount of organic matter present. The organic matter content of given ecosystems does not provide an assessment of how much can be utilized as an energy source by the organism in question. For instance, the amount of available carbon in sediments varied from 0.5 to 1.0 % of the total organic carbon at levels from 0 to 1 cm, and was less than 0.2 % at depths less than 10 cm[36]. Unfortunately, no chemical analysis of the organic matter provides data as to how much is bioavailable for organisms. Although a chemist can measure certain readily utilizable compounds, it does not mean that all that is measured is available to the cell. In a nearshore sediment, the ratio of biologically available to biologically unavailable alanine was 1:80[7]. Even in soil the amount of available energy would only permit the microbial biomass C to turn over from 0.4 to 5.5 times per year[10, 26], or once every 912.5 to 66.36 days. According to Anderson and Domsch[5, 6] the energy theoretically required for maintenance of microbes in soil would consume far more energy than is actually available in the soil. The organic content of soil is much greater than that of aquatic systems. In addition, when macroorganisms are starved, their microflora also undergo starvation[10]. The lack of energy in various ecosystems is discussed in more detail by Morita[33].

The starvation-survival process

All degrees of starvation may be found among organisms, and how long the starvation period for a microorganism persists depends on the ecosystem in which it exists. This period of nutrient deprivation can last for long periods of time; viable microorganisms have been found in water droplets in salt crystals in salt mines[42], in deep ocean water where the organisms may have resided for over a thousand years[44], in estuaries[15], in ancient rocks[23], in deep strata of open ocean sediments[34], etc. Accidental intrusions of energy into the ecosystem, such as pollution, may add the energy necessary for the cells to break out of the starvation mode. In the laboratory, marine bacteria have been starved for long periods of time; the longest period recorded was 7 years[37]. Nonmarine bacteria are known to persist without energy for long periods of time. However, it is not known how long starved cells will survive in the laboratory.

In laboratory studies four patterns of starvation-survival process have been observed[1, 32]. These are: 1) cells decline rapidly until a few viable cells remain, 2) cells increase in number (with no added energy) before a rapid decline occurs, leaving a small percentage of viable cells, 3) cells increase in number but never decline in numbers, and 4) cells neither decrease nor increase in numbers. This last type has been studied mainly with the chemoautotroph, *Nitrosomonas cryotolerans*[19]. Pattern 2 has been observed the most. Ultramicrocells are formed during starvation[39], but not in all species. Most microbes in the marine environment[27] and in soil[7] are ultramicrocells, but it is not known what percentage of these cells are in this form as a result of starvation. Studies on marine waters by the microcultural slide method indicate that many of the ultramicrocells do have the ability to grow to a size that is typical of those which most microbiologist are used to observing, but some ultramicrocells remain small[45]. It is known that some ultramicrocells will even pass through a 0.2-µm filter[22].

Degrees of starvation-survival



The figure illustrates the possible changes that cells may undergo during starvation. With the onset of starvation conditions, one of the four patterns of starvation takes place. With increasing periods of starvation, various events could take place. At the beginning of the starvation process, the cell utilizes its endogenous energy reserves. Depending on the species of microorganism, the endogenous metabolism may degrade many of the cell's enzymes, but not those associated with its ability to utilize energy. Thus, if energy again becomes sufficient in the ecosystem, metabolic processes can start again, and the cell can re-synthesize its entire complement of enzymes, since the DNA is still present. The precise point at which metabolic arrest takes place with respect to the utilization of cellular components, probably depends on the species. The state of metabolic arrest brought about by the lack of energy is probably the mechanism by which cells can survive for long periods of time without an energy source. However, we also recognize that many cells can metabolize, even though they cannot replicate themselves[16, 41]. In fact, Hoppe[16] attributes the breakdown of organic matter in the sea to the small heterotrophic bacteria that cannot be cultured. These cells eventually die. However, the starvation process can be interrupted at any point in the figure, and again, if the quantity of nutrients is sufficient, growth and reproduction will again occur.

Most studies on starvation-survival have been conducted with cells grown in rich medium and generally from the log growth phase. However, it is now known that if cells are grown in a chemostat (D = 0.015/h) at a slow growth rate the cells always remain small – much smaller than cells grown in rich medium and then starved[35]. For instance, batch culture Ant-300 cells (5.94 ± 0.465 µm^3) are 0.245 ± 0.053 µm^3 when starved. Chemostat-grown cells (D = 0.015/h) are 0.478 ± µm^3 when not starved and 0.046 ± µm^3 when starved. Since most ecosystems are oligotrophic, cells grown at a slow growth rate more truly represent cells that one would find in the environment. Thus, future studies should be conducted with cells, produced at a slow growth-rate.

For a *Pseudomonas* sp. it took approximately 18–25 days for the cells to stabilize in the starvation-survival process[21]. For a *Vibrio,* Ant-300, Moyer and Morita[35] found that there were three stages in the starvation-survival process, the third stage being the stabilized state. The first stage lasted approximately 14 days and the second approximately 70 days; starved cells were in what might be called the 'metabolic arrest' stage in the period beyond 70 days. When cells are placed in a starvation menstruum, there is a large fluctuation in the viable counts with a moderate decrease during the first stage, followed by a 99.7 % decrease between days 14 and 70, before the viable counts stabilize during the third stage. The total direct counts still remain high during this period.

In another marine isolate, S14, it was shown that an increased endogenous respiration occurs with DNA synthesis when cells are placed in a starvation menstruum[28,29]. During the initial starvation state, *Vibrio cholerae* utilizes its entire reserves of poly-β-hydroxybutyrate in seven days[14]. This also occurs in *Vibrio* S-14[25]. In the initial stages of starvation, various endogenous materials are utilized by the cell for energy purposes. Changes in the fatty acid profile in *V. cholerae* have also been observed[13], as well as in carbohydrates[14]; both decrease with starvation time. The total lipid for nonstarved cells of *Vibrio cholerae* was 3679.1 nmol/10^{10} cells; for cells starved for 7 and 30 days the values were 7.75 and 2.43 nmol/10^{10} cells respectively[14]. The phospholipid content was also drastically reduced. Considering that phospholipids are an integral part of the membranes of bacteria, a drastic change must also occur in the membranes of starved cells. Because *cis*-monoenoic fatty acid can be used during starvation, and the *trans*-monoenoic fatty acids are not easily metabolized and/or synthesized, the *trans/cis* ratio increases. If this ratio is greater than 1.0, it could be used as a lipid index to indicate stravation[14].

Macromolecular changes during starvation

During the starvation process some cellular proteins disappear and others remain, while new proteins are synthesized. Starvation proteins were first demonstrated by Amy and Morita[2] in Ant-300, and later demonstrated in other *Vibrio*[17], as well as in *E. coli*[12]. The exact role that these proteins play in the ability of cells to remain viable for a long period of time is not known.

In regular medium, during the log growth phase, batch grown cells show up to four Feulgen bodies per cell[39]. When cells are placed in a starvation menstruum DNA is synthesized[29]. During the first stage of starvation there is a fluctuation in the levels of DNA, RNA and protein (Moyer, M. S. Thesis, Oregon State Univ., Corvallis, OR, 1988) and in all probability there are cellular adjustments in the amount of these macromolecules in stage 1. However, no fluctuations are observed in the second stage, and during the third stage these macromolecules have stabilized. Nevertheless, the amount of DNA is much smaller in starved cells. This can be shown by chemical estimation as well as in electron micrographs. Cells from $D = 0.015/h$ had estimated nucleoid and cell volumes of 0.018 and 0.05 μm^3 respectively, giving a nucleoid volume to cell volume ratio of 0.4. The nucleoid and cell volumes of batch-grown starved and unstarved cells were 0.017, 0.28, and 0.33, 5.94 μm^3 respectively.

Processes in preparation for recovery

Are there any steps taken by the cells during the starvation process that permit them to recover when nutrients become available? This question has not been investigated to any great extent. It is known that starved cells retain their ability to take up energy-producing substrates when these are present[39], and that starved cells can 'see' a concentration of glutamic acid as low as 10^{-12} M[31]. In this concentration of glutamic acid, the production of a high-affinity uptake system[11] takes place. During the first stage of starvation-survival, chemotaxis towards glutamic acid is induced after 48 h of starvation in Ant-300[46], whereas when the same organism is growing in batch culture the chemotaxis process is minimal. Three patterns of chemotaxis during starvation-survival occur in different organisms. These are as follows: 1) rapid decline in chemotaxis (as in *E. coli*), 2) chemotaxis only after starvation and 3) an intermediate pattern[46].

Recovery process of starved cells

The longer the cells are starved, the longer is the lag phase[3]. In nutrient-deprived *E. coli,* the cell envelope microviscosity is altered so that it becomes more fluid; thus permeability to substrates increases[24]. This may be the result of the utilization of lipid material, as mentioned previously. On the other hand, when starved Ant-300 cells are much harder to disrupt by sonication than cells that have not been starved (J. M. Upadhyay, personal communication). As mentioned before, it has been demonstrated in the laboratory that upon exposure to an energy source, starved cells will immediately take up the substrate[39]. Even if some biosynthetic enzymes had been utilized for energy during the starvation process, the DNA is still intact, so that once energy is supplied it will permit the DNA to express itself fully so that growth and reproduction can again occur.

Genetically engineered microorganisms and starvation-survival state

Today, we must also consider genetically engineered microorganisms (GEMs) placed in the environment, especially for agricultural use. It should be realized that nearly all ecosystems are oligotrophic, which means that any microorganism, indigenous or introduced, must face a shortage of energy. The main source of added energy in a natural situation is plant debris, and most indigenous microorganisms rely on syntrophy for their energy source. Artificially introduced organisms are generally not able to compete for the limited available energy source, and will not be able to grow successfully unless energy is added by accident, for example by sewage pollution.

We have starved various species of GEMs, with one plasmid engineered into the various species, in sterilized well water for long periods of time up to 624 days[9]. Three patterns of host plasmid expression were noted which were 1) loss of plasmid expression, 2) loss of plasmid expression on initial recovery with subsequent expression upon resuscitation, and 3) loss of capability to produce functional plasmid resistance. Thus it appears that the plasmid expression depends upon the species as well as the plasmid genetically inserted into the host cell.

Microorganisms containing plasmids have been isolated from well water and aquifers by numerous investigators. Since these environments contain little or no energy-producing substrates, metabolic arrest has taken place so that plasmids are retained. Metabolic arrest is also the reason why Ant-300 can survive the period from when the cells sink in the Antarctic Convergence to the time when they are upwelled[40], or why microorganisms are found in ancient rock[22], deep ocean[44], water droplets in salt crystals[42], etc.

Conclusion

The starvation state of microorganisms in ecosystems is real, and should be considered as the normal state of most microorganisms in nature. Unfortunately, microbiologists think in terms of cells grown in rich medium where ideal conditions exist for growth and reproduction. This idealized situation generally is not the real world, thus future research in ecological situations must reflect the starved physiological state. Certain pathogens also undergo the starvation-survival process and, again, the starvation state must be taken into consideration.

However, we still do not know why certain cells have the ability to survive for long periods without an energy source while others do not. Whether the determinant is genetic or physiological still remains to be seen.

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Addendum. The data reported, dealing with cell volume and nucleoid volume of Ant-300 have been published by Moyer and Morita (Appl. envir. Microbiol. *55* (1989) 2710–2716).

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Energetics of bacterial adhesion

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Summary. For the description of bacterial adhesion phenomena two different physico-chemical approaches are available. The first one, based on a surface Gibbs energy balance, assumes intimate contact between the interacting surfaces. The second approach, based on colloid chemical theories (DLVO theory), allows for two types of adhesion: 1) secondary minimum adhesion, which is often weak and reversible, and 2) irreversible primary minimum adhesion. In the secondary minimum adhesion a thin water film remains present between the interacting surface. The merits of both approaches are discussed in this paper. In addition, the methods available to measure the physico-chemical surface characteristics of bacteria and the influence of adsorbing (in)organic compounds, extracellular polymers and cell surface appendages on adhesion are summarized.

Key words. Bacterial adhesion; long-range forces; short-range forces; electrostatic interaction; DLVO-theory; hydrophobicity; surface Gibbs energy.

Introduction

Surfaces are abound in nature and bacteria eventually colonize them. To prevent removal from a surface, bacteria have to attach to it. Attachment can roughly be divided into two steps. First, the organisms adhere. This initial adhesion is governed by pure physico-chemical surface properties of the bacteria and the solid, and the type of solute[34]. Second, organisms may eventually anchor themselves to a surface using specific appendages or cell surface structures. This process strongly depends on the type of bacterium/surface combination.

Initial adhesion can further be divided into two separate stages, namely reversible and irreversible adhesion. Reversible adhesion may be defined as deposition of bacteria on a surface in such a manner that the bacteria continue to exhibit a two-dimensional Brownian motion, and can be removed from the surface by e.g. the bacterium's own mobility. Irreversibly adhering bacteria no longer exhibit Brownian motion and cannot be removed by a moderate shear force.

In the following we will discuss the physico-chemical models which can describe reversible and irreversible adhesion. In addition, methods to determine the individual