

# The importance of diffusion in the microbial world

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## Abstract

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One outstanding property of the prokaryotic way of life is its wide occurrence in the current world. Microorganisms appeared on Earth in advance to other biological systems. Likely, they will remain here after the sunset of other living forms. This suggests that their basic endurance must rely upon relatively simple and stable physicochemical laws. In this short review we explore some theoretical considerations related to the characteristic small size of the representatives of the microbial world. We emphasize that diffusion, in particular, is an important driving force at microscale level on which prokaryotes have based great part of their biological success. Because of their dependence on diffusion, this feature imposes necessarily restricted limits to the size of prokaryotes without apparently affecting their efficient performance.

**Key words:** Microbial size, Biological evolution, Diffusion, Prokaryotic adaptation

## Resumen

*La importancia de la difusión en el mundo microbiano.*

Una característica destacable de los procariotas es su amplia distribución actual. Los microorganismos aparecieron en la Tierra antes que otros sistemas biológicos, y es posible su continuidad tras la desaparición de otras formas vivientes. Este hecho sugiere que su persistencia descansa en leyes fisicoquímicas simples que deben ser muy estables. El presente trabajo plantea algunas consideraciones teóricas relacionadas con el pequeño tamaño de los microorganismos para destacar que la difusión constituye a microescala un parámetro importante al que el mundo microbiano debe gran parte de su éxito. Debido a su dependencia de la difusión, los procariotas deben presentar necesariamente un pequeño tamaño que no limita, sin embargo, su eficacia metabólica.

**Palabras clave:** Tamaño celular, Evolución biológica, Difusión, Adaptación

## Introduction

Although microorganisms have a leading role in our daily life, this is not apparently quite obvious. Because of their small size, they may well be ignored or just considered as mere bystanders of our existence. However, the physical and biochemical processes they

achieve are of fundamental importance to life on Earth. No less than 80% of the Earth's atmosphere is the product of bacterial denitrification, in whose absence land masses would be deprived of fixed nitrogen, which would then accumulate in the oceans. Even the environment on which our society relies could not exist without their constant recycling of natural and

synthetic materials. These facts were wisely summarized in the words of the late microbiologist H.W. Jannasch, which expressed that all higher forms of life on our planet depend on microbes. Considering the elegance of microbial physiology and biochemistry, small is not only beautiful but also intrinsically powerful. Highly dispersible through their smallness and enduring extreme conditions, microbes are ubiquitous in the biosphere and they define its limits. With their enormous metabolic and genetic diversity, microbes controlled the chemical balance of the global environment for more than two thousand millions years before other organisms appeared on stage. They made, and are still making, the planet livable for these more complex and fragile forms of life (Jannasch 1997).

Occasionally, the microorganisms can be also detrimental agents that influence our lives by causing diseases in ourselves, animals and plants, by spoiling our food or by impacting our activities in many other ways. But, above all, the most impressive feature of these living beings is that they represent the primeval framework found in the origin of life. However, instead of disappearing by the selective forces of evolution, they are here to stay. This fact represents a big paradox for classical biology, a puzzle that can only be solved by considering that their continuity encloses the clues for a remarkable fitness to the conditions that make life possible. Thus, these forms of life appear to be not only the seed of evolution but also the guarantee of life itself. No other biological group as a whole has achieved an endurance and lifespan similar to microorganisms.

Modern interpretations of darwinian evolution envisage that the ultimate function of the living systems is the survival of the genomes, and that all the resources of the natural world, including selective forces, tend to maintain only what is effective in terms of replicative efficiency (Dawkins 1989, Ridley 1993). If this is indeed the hidden «pathos» of life and the real sense of evolution, then the hystorical maintenance of the microbial world indicates that microorganisms are themselves the very point of the arrow in evolution rather than relics of scarcely evolved systems. In any case, the microbial world offers some physical perspectives of which we are not usually well aware. We shall attempt to underline this further in the lines below.

### **Size is important...but the smaller the better**

As pointed out above, many textbooks on biology often reflect the dubious axiom that biological

evolution moves toward an ever increasing complexity in the living systems. This common view is supported by the interpretation that complexity endows a fine adaptation to the dynamic environmental conditions in which organisms evolve. An usual corollary derived from such grounds is to consider that, by definition, the complex living beings are also much more evolved than the simpler ones, i.e., that their comparative tuning with the surrounding medium is much more efficient than that achieved by previous, less elaborated forms of life. Nevertheless, although this may be historically true, the trends of life dismiss this general assumption. Evolution has to be understood not as a pattern of morphological complication but in terms of physiological and genetic efficiency. In this sense, the trend of biological progress does not always correlate with size or complexity.

Biological systems appeared early on pre-cambrian Earth as small drops of life in the form of quite minute prokaryotic microorganisms (Bengston 1994). If these biological systems were to represent only a triggering stage for evolution, one should expect their disappearance in parallel to the emergence of a progressive complexity. Surprisingly, however, the initial strategy of life persists among us nowadays. In other words, the prokaryotic way of life was not only maintained for miriads of years throughout the ensuing evolution but, in addition to precede all other living forms, coexists now with them, and most importantly, it will likely continue to exist in the future, even after the extinction of so-called more evolved forms of life. As an example, *Micrococcus radiodurans* is able to resist an atomic radiation of 6,5 millions roetgens, which is a dosage 10.000-fold more lethal than the survival limit for human beings (Matthews 1996).

These observations raise questions as to what are the clues for the success of microorganisms in the biosphere, why are they so essential and perpetual, and where does reside their tremendous efficiency as biological systems. Undoubtly, the answer to all these complex queries is a difficult matter whose approach would require to consider many different aspects. But let us just to focus in one based in their physical nature. One of the most relevant features of microorganisms is, precisely, their small size.

### **Dwarfs and giants within the prokariotic world**

The scale of living organisms has enlarged considerably due to the discovery of both big and small bacteria during the last decades. These new

descriptions help to know the lower limits of cellular life and enlighten that size diversity among prokaryotes is much larger than previously suspected. The classical size-based working definition for microorganisms, that included all those organisms not surpassing 0.1 mm during their life cycle (Stanier et al. 1986), can not be maintained any longer in view of the relatively giant size of some new bacteria, some of which can be perceived almost with the naked eye.

Among the megabacteria is *Thiomargarita namibiensis*, a colorless sulphur spherical bacteria, 750  $\mu\text{m}$  in diameter, recently discovered in sea sediments (Schulz et al. 1999). Similarly, a giant within the prokaryotic world is the rod-shaped heterotrophic bacterium *Epulopiscium fishelsoni*, which lives in fish gouts and reaches a size of 80 x 600  $\mu\text{m}$  (Clements & Bullivant 1991). Big bacteria also include some *Beggiatoa* spp. (Nelson et al. 1989) as well as the gigantobacterium isolated from the Ebro Delta microbial mats which has received the suggestive name of *Titanospirillum velox* (Guerrero et al. 1999). On the contrary, among the smallest prokaryotes known are the eubacterium *Mycoplasma* spp. and the archaea *Thermoplasma* spp., with a diameter down to 0.2  $\mu\text{m}$  (Heimmelreich 1996, Stetter 1999). Nanobacteria or ultramicrobacteria is the collective designation for even smaller cellular forms (Vainshtein & Kudryashova 2000). As a comparison, let us to recall that a typical bacteria, like the well known *Escherichia coli*, shows a size of about 1 x 3  $\mu\text{m}$  (Madigan et al. 2003). When we elevate the linear dimensions of these cells to the third power, to estimate as a whole their approximate global volumes, we observe that the biovolumes of prokaryotic cells may cover an extensive range within more than 10 orders of magnitude, i. e., from <0,01  $\mu\text{m}^3$  for the smaller to 200.000.000  $\mu\text{m}^3$  for the largest prokaryotic cells.

Taking into account all living organisms in this picture, with the smallest bacteria in one extreme and the big eukaryotes such as the blue whales on the other, the scale of life is so vast that is difficult to comprehend. The smallest prokaryotes are at the resolution limit of the light microscope (0.2  $\mu\text{m}$ ), whereas the blue whales reaching 30 m in length are at the other end of the spectrum (8 orders of magnitude larger than the nanobacteria). The span in biomass between bacteria and whales is thus about the third power of their difference in size (somewhat less, because whales are not spherical), i.e.,  $10^{22-23}$ . Interestingly, this is a value quite close to the volume ratio between the Earth and humans. In other words, within the current living world, the smaller organisms «see» the biggest ones just as man sees the whole

planet. Gross calculations allow us to further consider another unexpected fact. By a chance of Nature, this same round figure approaches the Avogadro's constant ( $2,03 \times 10^{23}$ ), i.e., the number of molecules contained in a mole of a given chemical substance. Hence, it is again somewhat surprising that, conceptual rigourness aside, a blue whale is something like about a mole of nanobacteria in the living world.

As others have recently noted (Schulz & Jorgensen 2001), it is no wonder that the world at the bacteria microscale is quite different from the world that we humans perceive. When we go down in scale into the aqueous microenvironments of bacteria, many physical laws of Nature appear to change in significance. In the living microworld, for instance, viscosity becomes the strongest force affecting motion, and molecular diffusion is the fastest transport. Let us analyse this later aspect in more detail.

### The meaning of microbial life

The sphere is the geometrical form which exhibits more surface relative to the volume that it encloses. Therefore, this shape is important because offers the possibility of more transport in and out the cell membrane per unit of volume. It is no surprising to find that spherical, cuasi-spherical or spherical-derived shapes are quite common among prokaryotes. Furthermore, another characteristic connected not to shape but to size, reinforces the potential efficiency of minute forms for the most primitive ways of transport. A simple surface/volume calculation reveals that, within spherical forms, this ratio is inversely proportional to the radius of the sphere ( $S/V = 4\pi r^2 / 4/3\pi r^3 = 3/r$ ), which means that the smaller the sphere is, the higher is the relative ratio of surface to volume. Hence, per a given unit of volume, to be small has the advantage of a better performance respect to interface mechanisms of exchange with the external medium (Madigan et al. 2003).

Molecules move through water in random directions by molecular diffusion. Recently, much attention has been drawn on diffusion as a key factor explaining the reduced size in prokaryotes. Diffusion may be an efficient and simple mechanism to incorporate nutrient molecules from the medium as well as to eliminate undesired molecules outside the cells. The diffusion force determines the basic flux of solutes to within and from bacterial cells and, as we will see below, may impose limits to the maximum size of biological systems where it is important. A fundamental equation governing diffusion is:

$$t = \pi L^2 / 4D \quad [\text{Equation 1}]$$

which is equivalent to the basic expression:

$$L = (4Dt/\pi)^{1/2}$$

where L is the distance that a given molecule travels by diffusion during the time t, whereas D is the diffusion coefficient for such a molecule at a given temperature (Schulz & Jorgensen, 2001). We find here something shocking, since the distance that molecules are likely to travel by diffusion increases with the square root of time, not with time itself, as it happens in the locomotion of objects and fluids that we generally know from our macroworld. Our intuition may fail due to this unusual feature, because the equation also shows that the velocity of the movement of the molecules (not only the distance travelled) depends on the time over which observe the movement:

$$L/t = \text{velocity} = (4Dt/\pi)^{1/2} / (t^2)^{1/2} = (4Dt/\pi t^2)^{1/2} = (4D/\pi t)^{1/2}$$

All this has far reaching consequences. As an example, oxygen molecules, which typically diffuse 1 mm in an hour, will take a day to travel 2 cm by mere diffusion, and about 1000 years to reach 10 meters. However, over the scale of a normal 1  $\mu\text{m}$  long prokaryote, they will take only about  $10^{-3}$  seconds, that is, a millisecond (Schulz & Jorgensen, 2001). It is difficult to imagine a transport mechanism more rapid than this one. This argument, inserting the corresponding D value, can be applied as well to many other soluble substances in aquatic media.

### Diffusion limits the size in prokaryotes

In contrast to larger eukaryotic cells, prokaryotic microorganisms lack systems for internal transport and are devoid of cytoskeleton, actin filaments or microtubules which causes hydrodynamic flows. Apparently they do not need such mechanisms because the scale of their size makes diffusion an important key which is sufficient enough either to support intake (for permeable substances) or to distribute the internal substances within the cell eliminating intracellular solute gradients. This can be clarified by considering again the Equation 1 above, and by taking now the value of L as the linear size of the cell. For a bacterium of 1  $\mu\text{m}$  in size, the time taken before a molecule observed at some point can be seen

anywhere else within the cell volume will be proportional to  $L^2/D$ . Considering a typical diffusion coefficient (D) for small molecules, the time is of the order of milliseconds. Because the turnover rate for most enzymatic reactions is a few hundreds per second, substrate and product molecules can thus move through the entire cell volume many times within the timing of a single round of enzyme action. In other words, in bacteria, the rapid communication due to diffusion makes possible that different enzymes can work as a dynamic system in a coupled fashion, without the need of internal compartments or additional transport mechanisms. The rapid mixing has the additional consequence that the theoretical time for two molecules to meet each other within a cell is extremely short (Mikhailov & Hess 1995). In statistical terms, any substrate molecule in the bacterial cell can meet any enzyme molecule in every second.

The cells can take up substrate from the surrounding more efficiently when they are small (due to their S/V ratio and their minute dimensions) but the advantages of having a small size have, nevertheless, a limit. A larger size breaks the thermodynamic success that diffusion exhibits on microscale. The scale for the time of diffusion inside a bacterial cell is critical and imposes constraints on its structural and functional organization. In big cells the mixing time by diffusion ( $L^2/D$ ) may be of the order of minutes and the traffic time ( $L^3/D$ ), which increases proportionally to the cell volume, may take several hours. This allows in the larger cells the establishment of internal microenvironments within the cytoplasm, creating regimes completely different to those in small cells. Hence, it is tempting to speculate that, in larger cells, the escape from diffusion might represent the origin of the membrane bounded compartmentalization. Since big cells can not further rely on diffusion as the main intracellular transport system, we arrive to the obvious conclusion that molecular diffusion sets limits to the size of the cells. Consequently, outside of the microworld, other strategies have evolved to cope with this problem.

### Adaptations to diffusion

In the microbial world, even the giants show adaptive patterns to exploit diffusion efficiently. A common property of the largest bacteria (megabacteria) is that they have unique intracellular structures not shown in smaller cells. The giant cells of *Epulopiscium* contain a cortex along the periphery that consists of vesicles and tubules. The analysis of this complex structure suggests that it has an excretory function in the removal of waste products, leading to the

interpretation that the constraints imposed by diffusion limitation due to the large cell size may be overcome by an intracellular system of transport organelles (Robinow & Angert 1998). Apart from this symbiotic bacterium, which is presumably related to the spore-forming clostridia (Clements et al. 1991, Angert et al. 1993), many of the particularly large prokaryotes are either cyanobacteria or sulfide oxidizers which show prominent non soluble internal inclusions. For instance, *Thiomargarita* cells are mostly comprised of a large vacuole and sulfur inclusions, with only 2% of the biovolume representing an active protoplasm (Schulz et al. 1999). Thus, in spite of its apparent big volume, the reduced portion representing the «alive» cellular volume may render the cell still manageable in terms of rapid thermodynamic equilibration due to diffusion. Also, many other large bacteria harbor massive cell deposits that reduce the metabolically active volume and, likely, the diffusion limitation. As other example, the large cells of *Achromatium oxaliferum* are unique among the big prokaryotes in that they store a high amount of calcium carbonate (calcite) inside the cells. These inclusions, together with internally stored sulfur globules, take up a large portion of the total cell volume (Head et al. 2000). On the other hand, the microscopic examination of the filamentous *Beggiatoa* reveals that the cells are composed by a highly hollowed protoplasm (Larkin & Henk 1996). Taken together, these evidences clearly point to the common trend that, unless cell size (or its equivalent «metabolic size») results limited, diffusion can not afford for its usual role. In larger cells, where diffusion is no longer the solution for efficient internal transport, other alternatives have been assayed in evolution giving rise to the elaborated transport systems shown by eukaryotes.

In biological terms, microorganisms should not be just regarded as a primitive vestige of the past. This may be true only on a time scale but, from an operative point of view, they are an example of continuous adaptation to a simple law of Nature which will favor their existence provided that the basic principles of thermodynamics remain valid. They exploit to the limit a fundamental physical force, diffusion, and by so doing they assure their endurance to reach much more ecological niches than other more sophisticated forms of life which depend upon less stable principles.

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