WATER MICROBIAL ECOLOGY – AN OVERVIEW

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Summary

Microorganisms are the smallest living organisms on Earth but at the same time they are the most abundant ones as they have occupied the entire biosphere. They are also the most diverse and in their majority unknown to scientists. They can be found in every macro or micro environment from the surface and the vast depths of oceans to the skin and digestive system of the humans and animals. The morphology (e.g. shape, size), taxonomy (classification) and biology (e.g. metabolism, reproduction) of microorganisms are studied by scientific disciplines as microbiology and molecular biology. But microbes been abundant and in extremely large numbers not only influenced but also influence their environment by participating or mediating important geochemical reactions which allow the composing of organic molecules from simple

elements (e.g. CO_2, N_2) and the decomposition of complex ones as the remains of dead organisms. Microbial ecology and particular the water microbial ecology studies the relationships among microorganisms and the way they influence water environments, by using principles and methods from microbiology, chemistry, physics, ecology, mathematics, and computer science. Despite the long history of the above disciplines - Anton van Leeuwenhoek back in 1670s was the fist who described microbes in rainwater samples – most of the progress in water microbial ecology occurred during the last 35 years. At present, further advances in molecular biology (e.g. genetic fingerptinting), microscopy (e.g. fluorescence, staining), culturing media and techniques as well as sampling capabilities increases our knowledge about the complex relations, nutrients and energy cycles occurred in water environments by the microorganisms. A brief description of the microbial communities occurring in various water environments, their adaptation and their role in nutrient and energy cycling are briefly described into the next chapters.

1. Introduction

A simple answer to the question “What is Water Microbial Ecology” is the study of the ecology of microbes in water or aquatic environments. ‘Microbes’ is the term referred to all organisms smaller than about 100 μm, which can be seen only with a microscope. Ecology refers to the interactions among microbes and the biogeochemical processes they mediate. Water microbial ecology according to a review article by Psenner, Alfreider and Schwarz (2008), aims at nothing less than explaining the world from “ecological scratch”. Therefore it develops theories, concepts and models about the small and invisible living world that is at the bottom of every macroscopic aquatic system.

Water microorganisms are capable of flourishing in all water habitants, from several kilometers below the seafloor to the first millimeter of a shallow trench created by rainwater and into acidic lakes. Three major domains compose water microorganisms- Eukaryota, Archaea and Bacteria – as well as viruses. Their omnipresence impacts the entire biosphere as they are the main producers of energy and carbon flow to other organisms. Some of the microbes decompose organic matter and thus they recycle nutrients in complex manner involved in various geochemical cycles as the nitrogen, the phosphorus and the carbon cycle.

Microbial ecology isn’t only the study of microorganisms *per se* but because of the multidisciplinary approach of the various interactions of the microorganisms, it produces knowledge in a wide manner giving answers and offering ways to explore the scientific fields of:

Evolution and creation: The study of the origin and evolution of microorganisms can provide critical evidence about missing links in the early stage of life on the planet.

Biodiversity: Understanding the natural processes led to the development of thousands of different species. This is a difficult task as we have study less than 1% of the microbial species.

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Ecology: There is an advantage using microbes to clarify the complex interactions between organisms and their environment. They are good laboratory material as they are small enough and reproduce rapidly. Another ecological aspect of bacteria is their use in biocontrol to help fighting pests.

Population interactions: Microbes are having the most complex interactions with other organisms such as the plants and animals as in the case of symbiosis, causing of diseases, creating biofilms or composing natural microfloras.

Recycling: Microbes characterized by unique recycling properties and they are used in solutions to many environmental problems as the waste treatment, composting, clean-up of toxic wastes (bioremediation) and transforming by-products and wastes to useful raw materials.

Biotechnology: Microbes are used in the production of various products from pharmaceuticals and chemicals to foods, fuels and other materials of higher added value.

Water covers seven tenths of the Earth's surface. Most of this water occurs between continents, where it is present as oceans (96.1% of global water). The remaining present within continental boundaries, occurs mainly as polar ice and ground water. These two main bodies (over 99 per cent by volume) of continental water – the polar ice-caps and exchangeable ground water – are extreme environments which have received relatively little microbiological attention until recent years. Life in the aquatic environment (freshwater and marine) has numerous potential advantages over terrestrial existence. These include physical support (buoyancy), accessibility of three-dimensional space, passive movement by water currents, dispersal of motile elements (gametes, genetic material) in a liquid medium, minimal loss of water (freshwater systems), lower extremes of temperature and solar radiation, and availability of soluble organic and inorganic nutrients (Sigee 2005).

2. Microbial Ecology of the Seas and Oceans

Although there is a long history of relation between man and the sea a great deal of what we know about the marine life and marine microbes in particular, has only been discovered since the mid-1970s. During the first half of the 20th century, scientists were aware that a variety of bacteria existed in the sea and they somehow involved in biogeochemical cycling. Important early studies were those of Waksman on the role of bacteria in organic matter decomposition (Waksman and Renn 1936), ZoBell on the microbial life adaptation in all parts of the ocean (McGraw 2004) and Morita with his work in the surviving of microbes to cold temperatures (Morita 1997). After the World War II and between 50s and 80s, marine microbial ecology received a great interest as researches start to comprehend the particular role of each microbial domain in the cycle of nutrients and energy flow (Pomeroy 1974). One of the major reasons that little is known about species composition and its variation is the difficulty of conventional identification which requires pure cultures (Gordon 1985). Another reason is that most water bacteria are non-detectable (CFU < 1%) or exist at very low levels. It is evident that there is the need for a rapid and simple technique that does not necessarily identify

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individual species but that can differentiate bacterial communities in terms of their constituents. In 80s and 90s, the boost to microbial ecology was given by the development of new techniques concerning mostly the direct count of microbes in seawater. The use of epifluorescence microscopy with DNA staining revealed that bacterial cells are by far abundant in each milliliter of water than the earlier methods of bacterial counting on agar plates had indicated. Advances in electronic microscopy gave a new perspective in the shapes and sizes of marine microorganisms. Other more sophisticated approaches included the use of radiolabeled amino acids and nucleotides and as they were quickly adopted, offered data on the bacterial productivity and substrate assimilation and utilization rates. The data gained by those, and other, studies were later incorporated into mathematical models trying to estimate and demonstrate the material flow through the various species (Ducklow et al. 2004). In the present, bibliography is possessed by studies evolving mostly molecular techniques as DNA cloning and DNA or RNA sequencing after the development of Polymerase Chain Reaction (PCR) technology in the mid 80s. This molecular biology revolution advanced the study on diversity and distribution not only of marine microorganisms but also of specific functional genes.

2.1. Functional Groups of Marine Microorganisms

2.1.1. Cyanobacteria

Cyanobacteria are found in most aquatic habitants and can range from 1μm in diameter to several 100 μm. Most of them are O_2 producing photosynthetic bacteria which utilize the CO_2 available in water and they are responsible for N_2 fixation. Their size gave the opportunity to scientists to study the taxonomy of these organisms more completely than that of the other bacteria. Additionally, the ecology of cyanobacteria is of concern as they often tend to increase in population (blooms) in eutrophic environments with a tremendous impact on seawater quality and aquatic life. The functional difference between terrestrial photosynthetic plants and phytoplankton is that the carbon fixation in marine systems in almost an exclusive privilege of the later which are free-floating microorganisms. Common elements are that both are autotrophs with CO_2 being their primary carbon source and they both fix CO_2 by the Calvin-Benson-Bassham cycle. But, unlike land plants most of phytoplankton and cyanobacteria are using carotenoids instead of chlorophyll as functional pigments. Large sized phytoplankton as diatoms have been studied for years as their size (up to 100 μm) and shape gives the opportunity for easier observation and identification. The skeletons of diatoms lived on the planet have accumulated in the ocean floor and gave birth to formations which define the morphology of our planet. Pico-sized phytoplankton has been studied less extended but its role is also quite important in marine ecosystems.

2.1.2. Bacteria

The bacteria are a ubiquitous class which colonizes any habitant of the planet having the grater active biomass than any other group of organisms. They are also the most metabolically diverse group that obtain energy from oxidizing organic carbon, parasitism, chemoautotrophy and photoautotrophy. In aquatic environments they play a crucial role in most biogeochemical fluxes as in nitrogen cycle, carbon cycle, oxygen and sulfur (Kirhman 2008). The bacteria may be pathogens to aquatic plants and

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animals and they are responsible for most of waterborne diseases in humans. The methods used to determine groups of bacteria are based on their morphology or various metabolic characteristics (Holt et al., 1994). Differentiation among bacteria species or strains is based on reaction to staining compounds as in the case of Gram's stain, morphology, mobility, utilization of various compounds (sugars), temperature, oxygen or pH tolerance. Besides the various methods employed for their cultivation, isolation and identification including molecular techniques less than 1% of bacterial species have been studied. The main reason for this is the difficulty to simulate their different environments in the laboratory and also the difficulty to collect and preserve samples from such diverse habitats.

In the oceans, various types of bacteria have been identified including cyanobacteria, ptoteorhodopsin bearing bacteria and aerobic anoxygenic bacteria. Except cyanobacteria, all other groups are not autotrophs and do not contribute to primary production (Kirhman, 2008) but instead they contribute in dissolved organic matter assimilation in the oceans. The main reason for this domination is their large surface to volume ratio and thus their ability to compete other microbes for dissolved compounds. In general, this ratio is greatly correlated to the abundance not only in heterotrophs but in all groups of microorganisms in marine environment with viruses being the most abundant of all.

2.1.3. Viruses

Viruses and particularly bacteriophages despite their sizes are probably the most abundant group of microorganisms in the marine environments. Wommack and Colwell (2000) estimated that there are approximately 10^7 viruses per milliliter of seawater and even more per gram of sediments. Global marine viral biomass is estimated to be 200 Mt of carbon which makes viruses the ocean's second largest biomass.

Viruses, or virus-like particles (VLPs), have been observed in numerous phytoplankton species estimated that a specific group of marine viruses, the phages, may lyses 2 to 24% of the bacterial population per hour and thus be a major cause of bacterial mortality in aquatic ecosystems having a significant impact on the carbon and nutrient flow in aquatic environments. Other reports suggest that viruses are also significant agents sustaining the intra-species diversity of microalgae. Thus, viruses are regarded as an important member of the aquatic microbial community affecting the nutrient cycle in aquatic environments.

2.1.4. Archaea

The Archaea are prominent in extreme environments including anaerobic waters, hot springs, deep-sea vents, mine wastes and hypersaline environments such as salt lakes. Some groups such as the methanogens have global biogeochemical importance, especially those populations found in wetlands. Others tend to defy known biochemical pathways by utilizing toxic elements as arsenic for their energy sources (Wolfe-Simon et al, 2011). The identification of species or strains of archaebacteria is based in molecular analysis or on various metabolic characteristics (Holt et al., 1994). Although these organisms have a close morphology to the Bacteria, analysis of ribosomal RNA sequences, classifies them separately from the later. Distinct difererences are the lack of

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peptidoglycan found in bacterial cell walls and the presence of unique lipids. In marine environments, archaea are everywhere, but they are particular abundant in deep water. Recent micro autoradiographic studies suggested that over 80 percent of archaeal carbon is from CO_2 assimilated via chemoautotrophy driven from ammonium oxidization (Ingalls et al. 2006).

2.1.5. Fungi

Yeasts and fungi are heterotrophs found in the oceans but their densities seem to be lesser than those of other groups of microorganisms. They are competing heteroptrophic bacteria and archaea for the organic matter but their low abundance is probably an indicator of being incapable to compete efficiently the other groups of microorganisms. Fungi and yeasts are most abundant in decomposed plants masses floating on water surface (Newell 2003).

2.2. Important Biogeochemical Cycles In Marine Environments.

The main types of carbon in marine environments are the in the dissolve or in particulate form called Dissolved Organic Matter or Carbon (DOM/DOC) and Particulate Organic Matter or Carbon (POM/POC) respectively. These forms compose a vast reservoir of carbon being about 60 times greater than the atmospheric. Carbon enters the marine environments from atmosphere, rivers, man-made sources, runoffs from the natural decay of vegetation or up wells from sinks at the bottom. The simplest form of carbon, CO_2, dissolves in seawater and can remain as is or converted to carbonate, bicarbonate or carbonic acid (pH depended). In the photosynthetic zone, microorganisms (primary producers) utilize CO_2 and their metabolites or decayed bodies settle into the deeper water layers as Particulate Organic Matter were may be consumed by filter or deposit feeding organisms or buried in the sediment and enriches those zones with Dissolved Inorganic Carbon and other dissolved nutrients. As a consequence of this remineralization of carbon, mostly by bacterial respiration there is much more inorganic carbon in deep waters than in the surface. Various studies suggest that different groups of microorganisms play specialized roles in their utilization of various substrates and nutrients. Thus understanding substrate and resource flux also requires knowledge of those specific groups.

Nitrogen is an essential but non-available micronutrient in suitable form or concentration to support biological production. Only few microorganisms have the ability to utilize N_2 by fixation (fixing cyanobacteria) thus converting it to utilizable forms as ammonia or ammonium. Nitrifiers then oxidizes further this forms to nitrites and nitrates and denitrifiers converts them back to N_2 or (by assimilation) to ammonia thought the action of grazers and zooplankton. In this cycle, archaea plays an important role of ammonium oxidation in deep ocean. Various other nutrients such as phosphorus and iron seem to play an important role as their availability controls utilization of C and N (Madigan and Martinko 2006).

3. Microbial Ecology of Freshwater.

Freshwater environments can be grouped into standing waters and flowing waters. Standing water includes lentic systems – ponds, lakes, marshes and other enclosed

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water bodies while flowing waters includes lotic systems as rivers, estuaries and canals. The distinction between lentic and lotic systems is not absolute, and almost all water bodies have some element of through-flow. There are key differences between lentic and lotic systems particularly in terms of carbon availability and food webs which defines or alters their microbial ecology.

3.1. Biodiversity of Microorganisms in Freshwater Environments.

Three major domains constitute the most fundamental element of taxonomic diversity within the freshwater environment. With the exception of viruses (as non-freeliving organisms) these domains are the Bacteria, Archaea, and Eukarya. Organisms within these domains can be distinguished in terms of a number of physiological, structural and biochemical characteristics.

Bacteria are represented in all freshwater environments and contain a single kingdom, the Eubacteria, which includes bacteria, actinomycetes and blue-green algae. Members of the domain Archaea tend to be restricted within extreme freshwater environments. In the case of planktonic organisms, the maximum linear dimension ranges from below 0.2 mm and up to 200 mm, separated into five size categories, from femtoplankton to macroplankton.

Microorganisms in freshwater environments may also be divided according to their feeding habits into two major groups: The autotrophs which synthesize their complex carbon compounds from external CO_2. They obtain their supplies of nutrients as nitrogen and phosphorus from inorganic compounds. These microorganisms include microalgae and photosynthetic bacteria, and they are considered the primary producers in freshwater ecosystems. The other group, the heterotrophs are using organic compounds as a source of carbon. Most of freshwater microorganisms, bacteria, protozoa and fungi are heterotrophic. Heterotrophs nutrition involves saprotrophy, predation and in association with living organisms. Saprotrophic organisms obtain their nutrients from non-living material either by direct uptake of soluble compounds or indirect uptake by secretion of external enzymes followed by absorption of the hydrolytic products (bacteria and fungi) and ingestion of particulate matter by phagocytosis as protozoa.

The third aspect of heterotrophy involves associations with living organisms like parasitism and symbiosis. In parasitism there is a strict dependence of the parasite to the host organism. The general scheme of food web in freshwater environments isn’t much different from the oceanic as these also include the uptake of DOC by planktonic bacteria, the breakdown of detritus by benthic organisms as bacteria, fungi, and protozoa and limitation of phytoplankton populations by parasitism with viruses and fungi.

One special application of the water monitoring is the analysis of spore and vegetative forms of *C. perfringens* and their distribution. It is known that *C. perfringens* survives the longest in polluted waters (Bezirtzoglou et al. 1996, Bezirtzoglou et al. 1997, Maipa et al. 2001) and is therefore, an excellent indicator of fecal contamination (Bezirtzoglou et al. 1994, Savvaidis et al. 2001). In various studies it was observed that the proportion

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of bacterial spore forms in rivers is statistically more important than the vegetative forms, and this formulation should be explained by considering the following points. The extended length of rivers, the heterogeneicity of sampling locations (e.g. source, downstream) and the influence of stress and environmental factors does not allow for easy survival of vegetative forms in their upstream flow (Bezirtzoglou et al. 1996). Spore forms being more tolerant to these effects are generally found in rivers (Bezirtzoglou et al. 1996). *C. perfringens* is used in many countries to detect faecal pollution of remote origin (Bezirtzoglou et al. 1996, Hirn et al. 1980, Pinfold et al. 1990), but since the organism is excreted by both humans and animals, it cannot be used as a specific indicator organism to distinguish between human and animal faecal contamination (Oragui et al. 1983). In order to fulfil epidemiological investigations and trace the source of water contamination (Sorensen et al. 1989), *R. coprophilus* was used as a specific indicator of animal faecal pollution (Oragui et al. 1983).

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Biographical Sketches

Athanasios Alexopoulos, Assistant Professor in Food Hygiene with special interest and 15 years experience in drinking water quality.

Stavros Plessas, Lecturer in Food Quality with 10 years experience in food and water biotechnology.

Eugenia Bezirtzoglou, Professor in Microbial Ecology with 25 years experience in the field of microbial ecology of intestinal, food and water ecosystems. Professor Eugenia Bezirtzoglou has specific expertise in:

-gastrointestinal microflora
-bacterial biofilms
-anaerobic bacteria
-food microbiology and hygiene
-water microbiology and hygiene
-microbial ecology methods and techniques at cultural and molecular level
-developing methods for sampling and culturing bacteria
-designing experimental protocols to investigate the gastrointestinal ecosystem and factors influencing food microflora in health and disease. In addition, she has been involved in many European (ECDC, EFSA) and National bodies (Ministries, Chemical State Laboratory) to offer her laboratory and teaching expertise on the above scientific fields.

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