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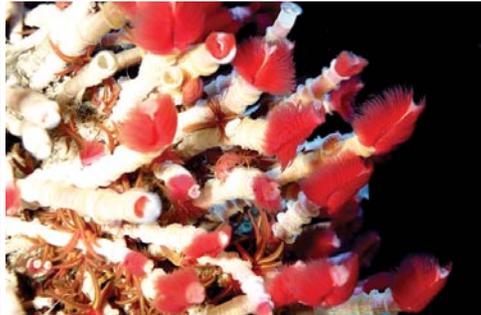
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MICROBES – FRIEND OR FOE?

Microbes or microorganisms, as the name suggests, are single celled and the smallest living organisms which are too small to be visible to naked eye. Even before its first observation by Antony van Leeuwenhoek (who invented microscope) in 1683, it was being used by man for fermenting beverages and for making bread, cheese etc. The discovery of microbes was a breakthrough in the field of science and people began to study them in detail which gave way to the development of a new branch of science, the microbiology. The different classes of microbes are algae, bacteria, fungi, protozoa, virus etc.

Microbes, though invisible, are abundant in earth and are found virtually in every habitat, including hostile environments such as poles, deserts, rocks, geysers and deep sea. Though many of these are harmless and helpful some can cause diseases. Microbes are the cause of many deadly disease outbreaks worldwide, viz. Tuberculosis, Malaria, Chickenpox, Smallpox, Plague, Polio and the like. They can also cause spoilage in canned food ('Botulism' caused by *Clostridium botulinum*). *Anthrax* is another threat from the world of microbes. It is common in the domestic animals but now found to occur in human beings also, if exposed to infected animals.

Many microbes have found their way to the industry in the production of fermented foods. Besides, microbes play a major role in the formation of fossil fuels and also the future fuels like hydrogen and ethanol. Bacterial cultures have found use in aquaculture. A new field, Probiotics- uses of bacterial culture (*Lactobacillus sp.*) as dietary supplements and as a therapeutic, has also been developed. In nature, bacteria play a role in nitrogen fixation thus improving the productivity of the soil which is a boon to agriculture. Microbes can sometimes help fighting diseases too. Many vaccines and antibiotics have been developed from microorganisms for use against disease.

Technological advances have made many leaping steps in the study of microbes, increasing their utility day by day in the industry. In the same time man has also gained the knowledge to use it in biological warfare. So it is essential that proper measures are taken by all countries to restrict their misuse. Quarantine measures are also to be adopted to assure that no new/harmful microbes are transmitted while importing microbial products (probiotics etc.) or new animal breeds. Thus the microbes are beneficial as well as harmful to mankind. It is now in the hands of man to decide – whether to use it as a friend or foe.

30 September 2006

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Editor

ARTICLES

MICROBES

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Microbes are everywhere - a largely unseen world of activities that helped to create the biosphere and that continue to support the life processes on earth. "Microbes were the first inhabitants of the Space Station," said Monsi Roman, chief microbiologist for the Environmental Control and Life Support Systems (ECLSS) project at NASA's Marshall Space Flight Center. "Just stand and breathe, and you're releasing microbes," Roman said. "You can wash and scrub and use antiseptic soap, and you'll still have microbes on your skin. You have them everywhere: in your clothes, on your skin, in your hair, in your body -- everywhere you could think of." Microbes constitute the vast majority of marine biomass and are the primary engines of the Earth's biosphere. They can be found almost anywhere in the taxonomic organization of life on the planet. They are unicellular organisms (or are at least capable of existence as single cells), too small to be seen with the naked eye. Among all forms of life on the earth, microorganisms predominate in numbers of species and in biomass, but their occurrence is generally unappreciated because of their small size and the need for a microscope to see individual cells.

A microorganism or microbe is an organism that is microscopic (too small to be visible to the naked eye). Microorganisms are often described as single-celled or unicellular organisms; however, some unicellular protists are visible to the naked eye, and some multicellular species are microscopic. Although most microorganisms are unicellular and do not differentiate or

develop into multicellular forms composed of different types of cells, there are many exceptions, so that this criterion cannot be used alone to differentiate a microorganism from a macroorganism.

Habitats and Ecology

Can you think of any places that microbes might not live? Microbiologists have found microbes living just about everywhere: in the soil, water and air; in animals, plants, rocks and even us! Microorganisms are found in virtually every habitat present in nature. Even in hostile environments such as the poles, deserts, geysers, rocks, and the deep sea, some types of microorganisms have adapted to the extreme conditions and sustained colonies; these organisms are known as extremophiles. Some extremophiles have been known to survive for a prolonged time in a vacuum, and some are unusually resistant to radiation. Many types of microorganisms have intimate symbiotic relationships with other larger organisms; some of which are mutually beneficial (mutualism), while others can be damaging to the host organism (parasitism). If microorganisms can cause disease in a host they are known as pathogens. Other interesting features of marine microorganisms are their ability to survive at very low temperature and at high salinity. The groups exhibiting the above characteristics are referred to as psychrophiles and halophiles respectively. In cold environments, psychrophilic bacteria cope with the conditions by maintaining flexible membranes and by circulating natural antifreeze compounds throughout the cell. In areas of high salt concentrations, many halophiles have adapted by altering their proteins, making them acidic, hence resistant to the protein-scattering effects

of high salt concentrations. In extremely hot areas of the oceans, hyperthermophilic microbes use subtle changes in their proteins to maximize the number of stabilizing salt bridges and ion pairings that keep enzymes together in the heat. Reverse gyrase, which allows DNA to maintain its structure during replication at high temperatures, is another unique enzyme used by hyper-thermophiles. Scientists have discovered microbes living in the boiling waters of hot springs in Yellowstone National Park. These microbes "eat" hydrogen gas and sulfur and "breathe" hydrogen sulfide. Other heat-loving microbes live in volcanic cracks miles under the ocean surface where there is no light and the water is a brew of poisonous arsenic, sulfur and other nasty chemicals. Other microbes live in the permanently frozen ice of Antarctica. Some scientists even believe there is the possibility bacteria may have once lived on Mars. Microbes are very diverse and represent all the great kingdoms of life. In fact, in terms of numbers, most of the diversity of life on Earth is represented by microbes.

Types of microbes

Microbes can be divided into six main types: Archaea, Bacteria, Fungi, Protista, Viruses, and Microbial mergers.

Archaea: Life's extremists...

Achaean are among the earliest forms of life that appeared on Earth billions of years ago. It's now generally believed that the archaea and bacteria developed separately from a common ancestor nearly 4 billion years ago. Millions of years later, the ancestors of today's eukaryotes split off from the archaea. So historically, archaeans are more closely related to us than they are to bacteria. Archaea are tiny, usually less than one micron long. Archaea are a

group of unicellular prokaryotic cells that sometimes produce methane (CH₄) during their metabolism, and which often live in extreme environments such as high temperature, low pH or high salt concentrations. They are specifically adapted to these conditions by means of special types of membranes and metabolism. The most striking chemical differences between Archaea and other living things lie in their cell membrane. There are four fundamental differences between the archaeal membrane and those of all other cells: (1) chirality of glycerol, (2) ether linkage, (3) isoprenoid chains, and (4) branching of side chains. Archaea are single-celled organisms lacking nuclei and are therefore prokaryotes classified as Monera in the five-kingdom taxonomy. Archaea are usually placed into three groups based on preferred habitat. These are the halophiles, methanogens, and thermophiles. Halophiles live in extremely saline environments. Methanogens live in anaerobic environments and produce methane. These can be found in sediments or in the intestines of animals. Thermophiles live in places that have high temperatures, such as hot springs.

Bacteria: Masters of every environment

Bacteria are the most abundant of all organisms. They are ubiquitous in soil, water, and as symbionts of other organisms. Many pathogens are bacteria. Most are minute, usually only 0.5-5.0 µm in their longest dimension, although giant bacteria like *Thiomargarita namibiensis* and *Epulopiscium fishelsoni* may grow past 0.5 mm in size. They are an amazingly complex and fascinating group of creatures. Bacteria have been found that can live in temperatures above the boiling point and in cold that would freeze your blood. They "eat" everything from sugar and starch to sunlight,

sulfur and iron. There's even a species of bacteria—*Deinococcus radiodurans*—that can withstand blasts of radiation 1,000 times greater than would kill a human being. A typical bacterium has a rigid cell wall made out of peptidoglycan and a thin, rubbery cell membrane surrounding the fluid, or cytoplasm, inside the cell. A bacterium contains all of the genetic information needed to make copies of itself—its DNA—in a structure called a chromosome. In addition, it may have extra loose bits of DNA called plasmids floating in the cytoplasm. Bacteria also have ribosomes, tools necessary for copying DNA, so bacteria can reproduce. Many move around using flagella, which are different in structure from the flagella of other groups.

Cyanobacteria fossils date back more than 3 billion years. These photosynthetic bacteria paved the way for today's algae and plants. Cyanobacteria grow in the water, where they produce much of the oxygen that we breathe. Once considered a form of algae, they are also called blue green algae. Bacteria are among the earliest forms of life that appeared on Earth billions of years ago. Scientists think that they helped shape and change the young planet's environment, eventually creating atmospheric oxygen that enabled other, more complex life forms to develop. Many believe that more complex cells developed as once free-living bacteria took up residence in other cells, eventually becoming the organelles in modern complex cells. The mitochondria that make energy for your body cells is one example of such an organelle.

Fungi: Of athlete's foot, champagne, and beer...

The organisms of the fungal lineage include mushrooms, rusts, smuts,

puffballs, truffles, morels, molds, and yeasts etc. A fungus is a eukaryotic organism that digests its food externally and absorbs the nutrient molecules into its cells. They range in size from the single-celled organism we know as yeast to the largest known living organism on Earth — a 3.5-mile-wide mushroom. Fungi come in a variety of shapes and sizes and different types. They can range from individual cells to enormous chains of cells that can stretch for miles. The branch of biology involving the study of fungi is known as mycology. A fungus of the species, *Armillaria ostoyae* may be the largest organism on the planet. It was discovered in the Malheur National Forest in Oregon, and its underground mycelial network covers an area of 8.9 km² (2200 acres).

Friendly Fungi: Some fungi are quite useful to us. *Saccharomyces cerevisiae*, baker's yeast, to make bread rise and to brew beer. Fungi break down dead plants and animals and keep the world tidier.

Fungal Enemies: There are some nasty fungi that cause diseases in plants, animals and people. One of the most famous is *Phytophthora infestans*, which caused the Great Potato Famine in Ireland in the mid-1800s that resulted in a million deaths.

Protista: Discover life

Protists were traditionally (for the last 150 years) subdivided into several groups based on similarities to the higher kingdoms: the plant-like algae, the animal-like protozoa, and the fungus-like slime moulds and water moulds.

Protists are eukaryotic creatures, ie; their DNA is enclosed in a nucleus inside the cell. They are not plants, animals or fungi, but they act enough like them that scientists believe protists paved the way for the evolution of early plants, animals, and fungi. Protists fall into four general subgroups:

unicellular algae, protozoa, slime molds, and water molds.

Algae, the Plant-like Protists

Algae gather light energy through photosynthesis. They include many single-celled creatures that are also considered protozoa, such as *Euglena*, which have acquired chloroplasts through secondary endosymbiosis. Others are non-motile, and some (called seaweeds) are truly multicellular, including members of Chlorophytes (green algae, related to higher plants e.g., *Ulva*), Rhodophytes (red algae, e.g., *Porphyra*), and Heterokontophytes (brown algae, diatoms, etc., e.g., *Macrocystis*)

Protozoa, the Animal-like Protists

Protozoa are mostly single-celled, motile protists that feed by phagocytosis, though there are numerous exceptions. They are usually only 0.01-0.5 mm in size, generally too small to be seen without a microscope. Protozoa are ubiquitous throughout aqueous environments and the soil, commonly surviving dry periods as cysts or spores, and include several important parasites. Based on locomotion, protozoa are grouped into Amoeboids (Transient pseudopodia, e.g., *Amoeba*), Ciliates (Multiple, short cilia, e.g., *Paramecium*) and Sporozoa (Non-motile parasites; some can form spores, e.g., *Toxoplasma*)

Fungus-like protists

Various organisms with a protist-level organization were originally treated as fungi, because they produce sporangia. Slime moulds are peculiar protists that normally take the form of amoebae, but under certain conditions develop fruiting bodies that release spores, superficially similar to the sporangia of fungi. Slime moulds generally move only about 1 mm per hour, although some can reach

2 cm per minute. They engulf their food, which can include bacteria, fungi, and decaying organic matter, and can eject inedible material. Water moulds are a group of filamentous, unicellular protists, physically resembling fungi. They are microscopic, absorptive organisms that reproduce both sexually and asexually and are composed of mycelia, or a tube-like vegetative body (thallus). The name "water mold" refers to the fact that they thrive under conditions of high humidity and running surface water.

Viruses: Hijacking the cells

Viruses are non-cellular replicating entities that must invade living cells to carry out their replication. They are obligate intracellular parasites. They are strange things that straddle the fence between living and non-living. On the one hand, if they're floating around in the air or sitting on a doorknob, they're inert. They are about as alive as a rock. But if they come into contact with a suitable plant, animal or bacterial cell, they spring into action. They infect and take over the cell like pirates hijacking a ship.

A virus is basically a tiny bundle of genetic material—either DNA or RNA—carried in a shell called the viral coat, or capsid, which is made up of bits of protein called capsomeres. Some viruses have an additional layer around this coat called an envelope. That's basically all there is to viruses. Viruses can't metabolize nutrients, produce and excrete wastes, move around on their own, or even reproduce unless they are inside another organism's cells. Yet viruses have played key roles in shaping the history of life on our planet by shuffling and redistributing genes in and among organisms and by causing diseases in animals and plants. Viruses have been the culprits in many human diseases, including smallpox,

flu, AIDS, certain types of cancer, and the ever-present common cold.

When viruses come into contact with host cells, they trigger the cells to engulf them, or fuse themselves to the cell membrane so they can release their DNA into the cell. Once inside a host cell, viruses take over its machinery to reproduce. Viruses override the host cell's normal functioning with their own set of instructions that shut down production of host proteins and direct the cell to produce viral proteins to make new virus particles. Viruses may be able to infect and reproduce in more than one kind of animal, but the same virus can cause different reactions in different hosts. For example, flu viruses infect birds, pigs, and humans. While some types of flu viruses don't harm birds, they can overwhelm and kill humans. Plant viruses do not infect animals or vice versa. Viruses that infect bacteria do nothing to animal or plant cells. Hemophilia and muscular dystrophy are two human diseases that researchers now believe resulted from mobile elements that, while skipping about the genome, ungraciously barged right into the middle of key human genes. Viruses exist for one purpose only: to reproduce. To do that, they have to take over the reproductive machinery of suitable host cells.

Other Virus-Like Things

Viroids

Scientists in the 1970s began discovering even simpler and smaller virus-like organisms that can cause disease. Viroids contain only RNA, but lack an envelope and capsid. Agricultural researchers found they caused problems in potatoes, tomatoes, and some fruit trees, and recently a viroid has been linked to hepatitis D.

Prions

Do you recall hearing about Mad Cow Disease? This

is an ailment that affects the animals' brains and is also called bovine spongiform encephalopathy because it makes the brain appear holey, like a sponge. There is a human form of this disease called Creutzfeldt-Jakob disease. Some scientists now believe these brain illnesses are among a few diseases caused by infectious agents called prions. Prions are not even DNA or RNA, but simply proteins. They are thought to be misshapen or abnormal versions of proteins normally found in animals or people. Very little is known about prions. Scientists suggest that they spread when a prion comes into contact with the normal version of the protein and causes the normal protein to change shape and become a prion, too. Unlike other infectious agents such as bacteria, viruses, and viroids, prions do not have the nucleic acids DNA or RNA. Prions are proteins that have the ability to transmit diseases, a finding that defied scientific expectations. There is still much debate about how they work, but scientists think these rogue proteins direct the host to create abnormal proteins that can cause serious neurological disease in animals and humans. Prions are blamed for scrapie in sheep, and bovine spongiform encephalopathy ("Mad Cow Disease") in cattle, and its human variant Creutzfeldt-Jakob disease. Some scientists suspect that prions may be responsible for Alzheimer's disease.

Microbial Mergers: Collaborations on a Minute

Over millions of years of evolution, we humans have worked out a mutually beneficial partnership with the microbes that came to inhabit our guts. In return for their aid in digestion, we give them a stable, protected home and plenty of nutrients via the food we eat. We need them as much as they need us. Microbes

break down food molecules our body's enzymes and acids can't dissolve, helping us squeeze all the nutrients out of our food. Some make valuable vitamins that our body needs.

Beneficial Effects of Micro - organisms

The beneficial effects of microbes derive from their metabolic activities in the environment, their associations with plants and animals, and from their use in food production and biotechnological processes. Bacteria, often in combination with yeasts and molds, are used in the preparation of fermented foods such as cheese, pickles, soy sauce, sauerkraut, vinegar, wine, and yogurt. Using biotechnology techniques, bacteria can be bioengineered for the production of therapeutic drugs, such as insulin, or for the bioremediation of toxic wastes.

Nutrient Cycling and the Cycles of Elements that Make Up Living Systems

The most significant effect of the microorganisms on earth is their ability to recycle the primary elements that make up all living systems, especially carbon, oxygen and nitrogen. Decomposition or biodegradation results in the breakdown of complex organic materials to forms of carbon that can be used by other organisms. There is no naturally occurring organic compound that cannot be degraded by some microbe, although some synthetic compounds such as teflon, styrofoam, plastics, insecticides and pesticides are broken down slowly or not at all. Through the metabolic processes of fermentation and respiration, organic molecules are eventually broken down to CO₂ which is returned to the atmosphere. Nitrogen fixation is a process found only in some bacteria which removes N₂ from the atmosphere and converts it to ammonia (NH₃), for use by plants and animals.

Nitrogen fixation also results in replenishment of soil nitrogen removed by agricultural processes. Some bacteria fix nitrogen in symbiotic associations in plants. Other Nitrogen-fixing bacteria are free-living in soil and aquatic habitats.

Beneficial Associations with Animals and Plants

Microbes invariably enter into beneficial, sometimes essential, associations with all higher forms of organisms, including insects, invertebrates, fish, animals and plants. For example, bacteria and other microbes in the intestines of animals digest nutrients and produce vitamins and growth factors. In the plant world, leguminous plants (peas, beans, clover, alfalfa, etc.) live in intimate associations with bacteria that extract nitrogen from the atmosphere and supply it to the plant for growth.

Production of Foods and Fuels

In the home and in industry, microbes are used in the production of fermented foods. Yeasts are used in the manufacture of beer and wine and for the leavening of breads, while lactic acid bacteria are used to make yogurt, cheese, sour cream, buttermilk and other fermented milk products. Vinegars are an acetic acid fermentation product. Other fermented foods include soy sauce, sauerkraut, dill pickles, olives, salami, cocoa and black teas. Yeast are also involved in fermentations to convert corn and other vegetable carbohydrates into ethanol to make gasohol.

Medical, Pharmaceutical and Biotechnological Applications

In human and veterinary medicine, for the treatment and prevention of infectious diseases, microbes are a source of antibiotics and vaccines. Antibiotics are substances produced by microorganisms

that kill or inhibit other microbes which are used in the treatment of infectious disease. Antibiotics are produced in nature by molds such as *Penicillium* and bacteria such as *Streptomyces* and *Bacillus*.

Vaccines are substances derived from microorganisms used to immunize against disease. The microbes that are the cause of infectious disease are usually the ultimate source of vaccines. Thus, a version of the diphtheria toxin (called toxoid) is used to immunize against diphtheria, and parts of *B. pertussis* cells are used to vaccinate against pertussis (whooping cough). The use of vaccines such as smallpox, polio, diphtheria, tetanus and whooping cough has led to virtual elimination of these diseases in regions of the world where the vaccines have been deployed.

Biotechnology

Microbiology makes an important contribution to biotechnology, an area of science that applies microbial genetics to biological processes for the production of useful substances. Microorganisms play a central role in recombinant DNA technology and genetic engineering. Important tools of biotechnology are microbial cells, microbial genes and microbial enzymes.

Basic research

Microorganisms, in particular the bacterium, *E. coli* and the yeast, *Saccharomyces*, have been used as model organisms for basic research and the study of cellular life. Hundreds of thousands of scientific papers have been published on these two organisms. Because of cell theory and the unity of biological processes in all organisms, this information provides us with insight and understanding of life at all levels, including human. About half the work

force of microbiologists is involved in basic research with microorganisms. These microbiologists are housed in university, government and institutional laboratories, wherein they utilize microorganisms to study and learn about their fundamental biological characteristics that stem from their structure in relationship to function, their physiology or metabolism, and their genetics and genetic systems. Because of the ease of manipulation of microbes in the laboratory, as well as their rapid generation times (as little as 15 minutes, compared to many months or years for animals, for example), many of the principles of molecular and cell biology and genetics are more readily studied in a microbe than in a rat or a human. Basic research is, of course, not without value to society. Many of the discoveries made intentionally or by accidental observation in basic research with microbes, have resulted in huge advances in medicine and agriculture and food science. One part of basic research studies the structure and function of microbial genomes. This field is known as microbial genomics. One way that this information can be applied for the betterment of humanity would be to sequence and identify the genes of microbes which cause illness. It is anticipated that this knowledge will lead to better methods for identification of pathogens, as well as design of therapies against individual pathogenic organisms. This technology is also used to improve understanding of beneficial microbes such as those living in the gastro intestinal tract. Innovative approaches in research, education and training are critical for moving the field of microbiology forward.

Probiotics and its Application in Mariculture

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Introduction

Aquaculture is a multidisciplinary activity, more complex than agriculture due to the multidimensional aquatic medium. The aquaculture boom and increased socioeconomic benefits together with increase in extent and intensity of aquaculture are alleged to have created several problems, particularly those of deteriorating water and soil quality and outbreak of diseases (Kutty, 1999). For example, the global production of farmed shrimp has doubled in the past 15 years. India has also witnessed such spectacular growth in shrimp farming until the mid nineties, after which it became beset with disease and environmental problems. Overstocking, overfeeding and excessive use of antibiotics during farming are some of the reasons attributed to the outbreak of shrimp diseases. But what has been recognised now by scientists and policy makers is that aquaculture must be environmentally friendly so that it can sustain itself without seriously affecting the coastal ecosystem. In other words, the ecological footprint of aquaculture must be sufficiently small so as to ensure sustainability.

The excessive use of antibiotics and the resulting residue in meat products lead to curbing of its usage in animal rearing and production. The farmers then turned to an age-old practice of using beneficial bacteria to quell infectious diseases. Thus, Parker (1974) introduced the modern concept of probiotics more than 25 years ago. Aquatic animals are quite different from land animals for which the probiotic concept was developed, and therefore, the probiotic usage in aquaculture,

especially in shrimp culture has taken a different meaning. The high risk of losing their crop to disease attack has prompted many shrimp farmers all over the world, and especially in India, to use probiotics during their culture operations.

What are probiotics?

The origin of the term probiotic is attributed to Parker (1974) who defined them as organisms and substances, which contribute to intestinal microbial balance. However, the concept of microbial manipulation was first appreciated by Metchnikoff during the early 1900s when he viewed the consumption of yoghurt by Bulgarian peasants as conferring a long span of life. Although evidence for a link between longevity and ingestion of fermented milk products has not been proven yet, some workers have claimed that its therapeutic value is related to viable bacteria, in particular *Lactobacillus sp.* Although a strict definition of probiotics is difficult to come by, Tannock (1997) proposed it as "living microbial cells administered as dietary supplements with the aim of improving health". Gatesoupe (1999) reviewed the state of probiotic usage in aquaculture and stated that the first application of probiotics in aquaculture is relatively recent, but the interest in such environmentally friendly treatments is increasing rapidly.

Types of Aquatic Probiotics

Recognising the conceptual difference of terrestrial and aquatic probiotics, Gatesoupe (1999) suggested a modification in the definition of probiotics as used in aquaculture. He defined probiotics as *microbial cells that are administered in such a way as to enter the gastrointestinal tract and to be kept alive, with the aim of improving health.* He further classified the microbial preparations used in aquaculture into 3 types - biocontrol agents,

probiotics and bioremediation agents. Biocontrol agents are those methods of treatment using the antagonism among microbes to kill or reduce the number of pathogens in the aquaculture environment (Maeda *et al.*, 1997). Those bacterial treatments which improve the water quality and thus indirectly the production were termed as bioremediation agents. The bioremediation agents have also been termed as bioaugmentation agents or water additives (Moriarty, 1998) and probiotics.

It is important to clear the concepts and definitions with regard to the term probiotics. At present the definitions and classifications brought forth by Gatesoupe (1999) serve the purpose and can be applied without confusion in shrimp culture. The commercial availability of probiotics and bioremediation agents in shrimp culture and its widespread usage in India has spawned separate terminologies among shrimp farmers. The strict probiotic agents are known as gut-probiotics and the bioremediation agents are known as water-probiotics.

Biocontrol Agents

Antagonism to pathogens is a characteristic of a good aquatic biocontrol agent. Antagonism may be mediated not only by antibiotics, but also by many other inhibitory substances like organic acids, hydrogen peroxide and siderophores (Gatesoupe, 1999). These compounds produced by the biocontrol agents are highly dependent on experimental conditions that are different *in vitro* and *in vivo* conditions. It was Maeda and Liao (1992) who first isolated a strain "PM-4" (subsequently identified as *Thalassobacter utilis*) from the rearing water of larval *Penaeus monodon* for use as a biocontrol agent.

Bioremediation Agents

The importance of

microbial communities in aquaculture systems and pond productivity cannot be overstressed. Bioremediation agents serve to modify or manipulate the microbial communities in water and sediment such that they reduce or eliminate selected pathogenic microbes and generally improve growth and survival of the targeted species. There are various ways through which bioremediation agents could act in aquaculture systems. These include competitive exclusion of pathogens, enhancing digestion through the supply of essential enzymes, moderating and promoting the direct uptake of dissolved organic materials, active promotion of pathogen inhibiting substances and other possible mechanisms (Jory, 1998).

Probiotics in live feeds - Bio-encapsulation

Marine larval rearing involves feeding the hatched larvae with suitable live feeds (diatoms, rotifers, copepods, nematodes, *Artemia* nauplii and metanauplii, mysids etc). Most often live feeds are the primary source of bacterial contamination in rearing systems. By virtue of their size and feeding habits, most live feeds are size specific filter feeders. Therefore, it is possible to incorporate into the live feed particles (say an antibiotic or therapeutic drug like Romet-30 or a probiotic organism) of the appropriate size. This process called bio-encapsulation is thus an innovative means of delivering drugs and probiotic organisms to the larvae (Mohamed, 2001). Indeed, for fish larvae that are active sight feeders, it is the only effective means of drug delivery and several studies have been made on this aspect.

Microalgal cultures are a virtual storehouse of various microorganisms, and therefore, by feeding marine microalgae to marine larvae, we transfer many potentially pathogenic microorganisms to the culture

medium. The consequences are low survival and poor quality larvae, besides failure of microalgal culture due to overgrowth of microorganisms. Probiotic organisms have been incorporated into microalgal cultures with remarkable benefits. In a recent study it was found that the addition of the probiotic yeast *S. boulardii* as a single addition to *Chaetoceros* culture resulted in significantly ($P < 0.01$) improved (162% increase in maximum algal density) algal growth rates with prolonged stationary period when compared to the control. It also helped in keeping the total aerobic bacterial counts and total *Vibrio* counts on TCBS in the medium to very low levels.

Perspectives and Conclusions

It is well known that microorganisms cannot be avoided in aquaculture operations (Ringo and Birbeck, 1999). The key to successful management of aquaculture operations lies in the manipulation of these microbes through innovative means such as use of probiotics. The state of the art of aquatic probiotics has not reached to the level found in land animals. The application of probiotics for fish and shrimps, either as a biocontrol or as a bioremedial

measure shows promise, but much more research efforts are needed to come to a complete understanding. Gatesoupe (1999) stated that the first question that remains unanswered in most cases is the fate of the probiotic organism in the rearing medium or in the gut. More investigations using molecular and immunological approaches may yield better results.

Even without much research backing, a vast number of commercial probiotic products are being used by shrimp farmers, mostly under pressure from marketing agents and peers. It is essential that proper testing of these products under local environmental conditions be done before they are marketed. Government research laboratories therefore, have to equip themselves for carrying out tests of these products and ascertain the factual in the claims. At the same time, the search for new and better probiotics should continue. Unlike endothermic animals, the ubiquitous environmental microbe *Vibrio* dominates the gut microflora of fishes and shrimps. It is very likely that non-pathogenic *Vibri*os hold the key to isolating and developing a successful probiont for use in aquaculture.

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