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Multidimensional Roles of Microorganisms

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Tel: 011-2696771
Fax: 011-2694754
E-mail: vidurava@nsf.gov.lk

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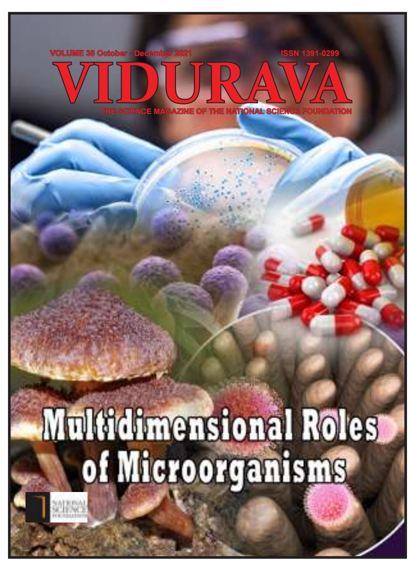

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Editorial

The Invisible Invincibles

The range of acts performed by a group of actors called microorganisms or microbes, is the main theme of this number of *Vidurava*. The themes of the feature articles include, 1) Application of Microbes for the Food Industry, 2) Living Microbes: An Underutilized National Wealth, 3) Microbes and Medicine, 4) Microorganisms in Extreme Environments, 5) Living Microbes: Ciliated Protoists that Flourish in Rice Field Habitats Serving as Natural Parasites of *Culex tritaeniorhynchus* Mosquito Larvae, and 6) Worlds Within Worlds: Hidden Role of Microbes in our World.

It has been claimed that microorganisms are the largest group of living organisms in the world, that have co-evolved with plants and animals. Although microbes are known to play a vital role in ecology, medicine, engineering, and agriculture, in our living memory, we are aware of how the world in recent times was stricken by a deadly microbial virus which traversed trans-continently causing immense damage to the global population. Microorganisms are said to have dominated the biosphere for 3.5 billion years, inhabiting virtually every niche available on the planet. Nevertheless, it has been claimed that 99 % of microbes cannot be readily cultivated under laboratory conditions.

However, what strikes us most painfully is the devastating role played by a virus in recent times under the name of COVID 19, that demonstrated to us in no uncertain terms that microbes can be more powerful, and more vicious than any class of human beings.

M. Asoka T. De Silva

Living Microbes: An Underutilized National Wealth

Dr Upeka Rajawardana, Prof. Ilmi G.N. Hewajulige and Prof. Chandrika Nanayakkara

Microorganisms are the largest group of living organisms on earth while the total number of prokaryotic cells on earth has been estimated to be 4–6 × 10^{30} comprising around 10^6 to 10^8 separate species. These include bacteria, fungi, algae, some parasites like protists and archaea, and viruses which vary in shape, size, and surface morphologies (Fig.1). These organisms are highly diverse in their physiology, biochemistry and also in their nutritional needs. They often appear in nature as complex ecological interactive networks within the ecosystem rather than existing as single planktonic cells. These interactions among microorganisms can be between same species, with different species, or even among completely different genera and families. The interactive patterns within these webs are either positive (win), negative (loss), or neutral, where there is no effect at all on the interacting species. The different win, loss, and neutral associations occurring between interacting partners provide a foundation for diverse forms of interactive patterns. Most microorganisms reproduce rapidly, and the plasticity of their genome helps them to adapt to changing environmental conditions easily. The above mentioned traits allow them to perform a number of essential ecosystem functions, on which several agriculture, food, pharmaceutical and chemical productions depend.

These microbial communities are very important to life on earth as they play an enormous practical role in ecology, medicine, engineering and agriculture.
The microbial diversity on earth also presents a massive, largely unexploited genetic and biological resource that could be exploited

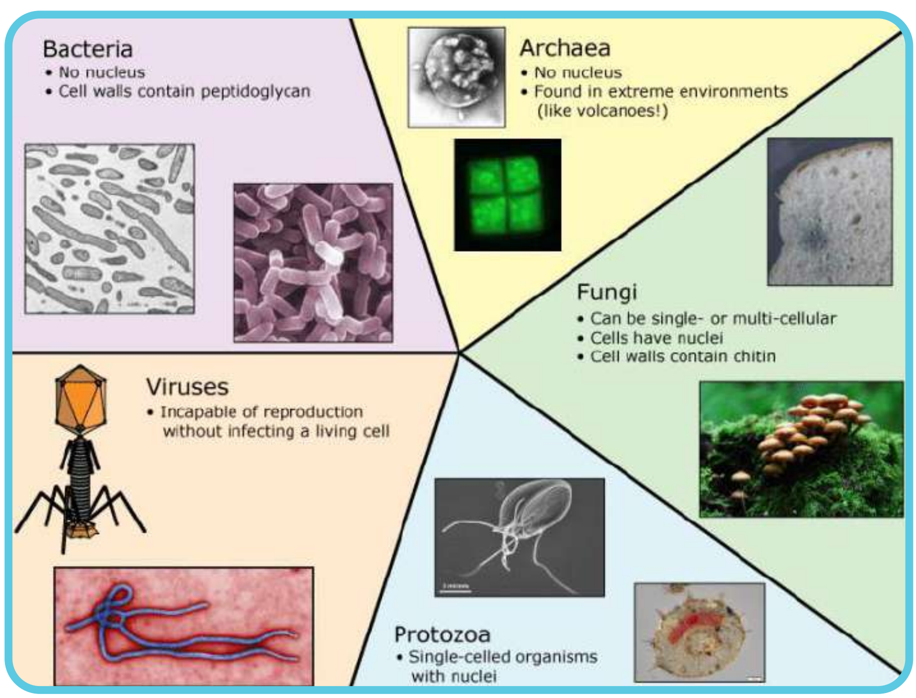


Figure 1: Different types of microbes have distinct characteristics that distinguish them from one another. These differences in the microbes lie in the presence of the central storage space for their DNA (nucleus), if and what type of wall surrounds their cell (cell wall), and whether they are a living thing and can reproduce themselves

for the recovery of new genes, metabolic pathways and valuable products. But unfortunately, more than 99% of the microbes in the environment cannot be readily cultivated under laboratory conditions. Hence, most microbial species have not yet been described for their role or accessed for their industrial potential.

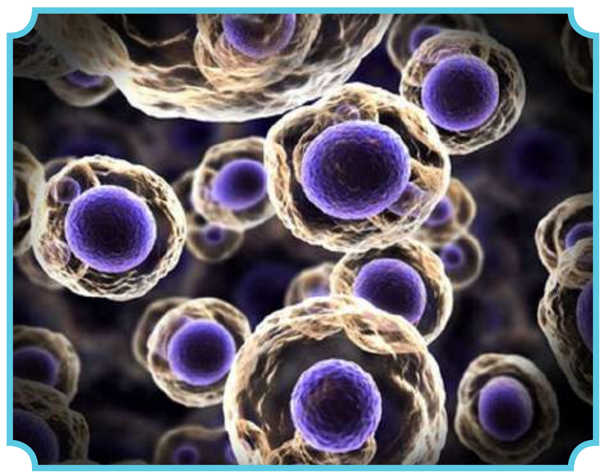
As a result, microorganisms were being used in industrial processes even before their existence was known, as for example in bread making, production of fermented foods and beverages like wine, vinegar, cheese and curd. Today, microorganisms are widely used in various industrial applications including food, pharmaceuticals, biofertilizer, pest control, bioremediation, biodegradation, biofuel processes, plant symbiosis and growth stimulation and in many others. In the lengthy list of possible applications, medicinal, food industrial, agricultural and chemical applications appear at the top, and hence deserve further discussion.

Medical applications of microorganisms

By the early 1900's, approximately 80% of all medicines were synthesized from plant sources. In 1928, Alexander Fleming discovered penicillin from *Penicillium notatum* causing a shift of emphasis of natural products as a source from plants to microorganisms. At the beginning of antibiotic research, streptomycin from *Streptomyces griseus*, chloramphenicol from *Streptomyces venezuelae*, chlortetracycline from *Streptomyces aureofaciens*, and cephalosporin C from *Cephalosporium acremonium* were discovered. Large pharmaceutical companies continue to invest in this traditional realm, and to-date approximately 60% of approved small molecule medicines and 69% of all antibacterial agents originate from natural products.

Food industry applications of microorganisms

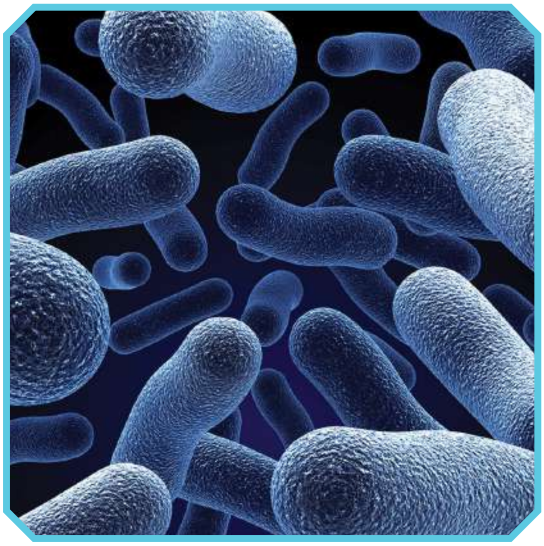
Through the whole agro-food chain, application of microorganisms is used for



upgrading food safety and security. This includes the improvement of food quality by microbe-based feed modifications and reduction of foodborne hazards by pre-harvest interventions, preventing of food spoilage as well as extending shelf-life, probiotics in feed and food development and autochthonous and commercial starter cultures etc. According to FAO (2009), the main functional groups for food processing are beneficial microorganisms (starter and probiotic bacteria, fungi and yeasts). By the use of microorganisms bulky, perishable and frequently inedible raw materials could be converted into safe, shelf-stable and palatable foods or beverages. These microorganisms determine the quality and characteristics of fermented food (acidity, flavour, texture, aroma, nutrients and also health benefits). The recent chemical engineering achievements in fermentation technology facilitate food manufacturers to produce hundreds of types of dairy products such as cheese and fermented milks, vegetable products such as pickles and olives, fish and meat products such as fermented sauce and sausages, bakery products, alcoholic beverages like beer, wine and cider, vinegar, food acids and oils involving microbes.

Agricultural applications of microorganisms

Some of the most commonly used beneficial microorganisms in agriculture worldwide, include *Rhizobia, Mycorrhizae, Azospirillum, Bacillus, Pseudomonas, Trichoderma, Streptomyces* species. These beneficial microbes confer multifunctional plant growth promoting attributes such as nitrogen fixation, up taking major nutrients, solubilization of micronutrients (phosphorus, potassium and zinc), and production of siderophores, promoting root and shoot growth, disease control or suppression by producing antagonistic substances such as antibiotics, auxin, and gibberellins and improving soil structure etc. These microbes could be applied as biofertilizers for native as well as crops growing at extreme habitats. Agriculturally, important microbes with Fe- and Zn-solubilizing attributes can be used for biofortification of micronutrients in different cereal crops.



Chemical applications of microorganisms

In fermentation processes, microorganisms are used to prepare single cell protein (SCP), silages and microbial pesticides. Microbial cultures are also used to produce several food additives like stabilizers, emulsifiers, enzymes, flavors and fragrances etc. Microbial rennets which are cheaper than animal rennet (chymosin), are being produced commercially since the 1970s and have proved satisfactory for the production of different kinds of cheese. Lactases obtained from *Aspergillus niger, Aspergillus oryzae, and Kluyveromyces lactis*, are considered safe and present a wide range of applications. Microbial lactase is used in ice cream, yoghurt and frozen desserts to improve scoop and creaminess, sweetness and digestibility, and to reduce sandiness due to crystallization of lactose. Microbial lipases with region and fatty acid specificity are of immense importance and used for retailoring of vegetable oils, upgrading cheap oils to synthesize nutritionally important structured triacyl-glycerols, like cocoa butter substitutes, low calories triacylglycerols and oleic acid enriched oils. Microbial alkaline proteases are used in the preparation of protein hydrolysates of high nutritional value. Microbial Glucoamylase and β-amylase are used commercially in the production of low-calorie beer etc.

However, very limited work has been done on the isolation and identification of the indigenous microbial strains with industrial prospects in Sri Lanka. As a result, native microbial strains are not available for commercial or basic research applications. The commercially accessible imported starter cultures are expensive which cannot be propagated and utilized for multiple applications, as they not fitting for household and small scale food processing applications etc. Further, a very limited range of fermented foods are available for the Sri Lankan consumers, while and the functional propects of these products are also not scientifically proven. Hence, numerous opportunities exist for market oriented new product developments, in order to enhance the colonic and general health status of consumers and thereby satisfy the market demand through the introduction of locally developed indigenous functional starter cultures. Hence, fundamental and applied research on new microbial strains, and their application to obtain quantitative data, is of a great importance to develop indigenous functional starter cultures, and their technological application process design. This may result in better process control, enhanced food safety and quality, and reduction of economic losses.

Dr Upeka Rajawardana
Senior Research Scientist
Food Technology Section
Modern Research and Development Complex
Industrial Technology Institute

Prof. (Ms.) Ilmi G. N. Hewajulige
Research Professor
Additional Director General - R&D
Industrial Technology Institute

Prof. (Ms.) Chandrika Nanayakkara
Department of Plant Sciences
Faculty of Science
University of Colombo

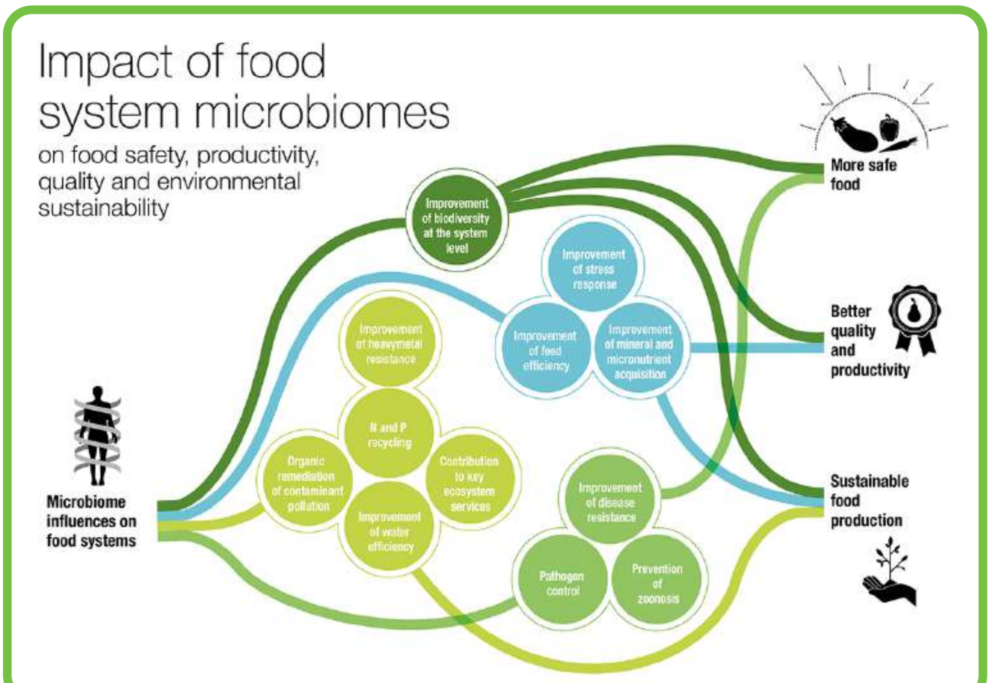
Worlds Within Worlds: Hidden Role of Microbiomes in Our World

Dr Erandi Pathirana

We are in an era, where we no longer can talk of human beings having sole control of everything in the blue planet we live in. The current pandemic of the Corona Viral Disease (COVID-19) taught us, showed us, and reminded us the good lesson that power and wealth are dominated by health factors. It also proved that microbes are more powerful than human beings, having taken control of everything that we do in this world. Thus it is clear that it is not the human-beings that control the earth but the microbes that inhabit diverse environments of this world. Microbes are found almost everywhere in the world, starting with air, in every inch of land, in water bodies, (not excluding the ocean), in plants and in every animal not leaving even human beings. In other words, all animals and plants act as hosts for microbial communities. The human body is inhabited by a huge array of micro-organisms, from both inside and out-side. While the human body is made up of about 10 trillion cells, it is said to host 100 trillion more microbes. We call this our **"microbiome"**.

What is a Microbiome?

Microbiome is the collection of all microbes living in our body, or in/on another organism or even in an outside environment such as a pond, lake, lagoon or even the ocean. This is true for all the other environments in our world. While bacteria play a key role in almost all microbiomes that we know, there are also viruses, fungi, and even archaea, a newly classified group of single-celled microbes.

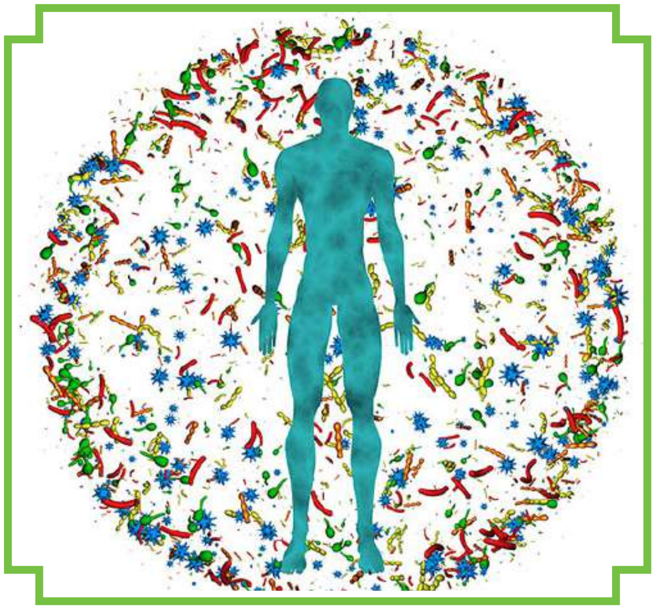


History of Microbiomes

Plants and animals have lived on this earth for about 1.2 billion years. The history of interactions between microbes and higher organisms extend far beyond this period. Since then, knowingly, or unknowingly, we have been living with microbes, or with microbiomes. These relationships are thought to have shaped the evolution of higher organisms in many different ways. Moreover, the microbes that were associated with an animal or plant are thought to have co-evolved with the particular animal or plant. The stomach-associated bacterium *Helicobacter pylori* is a classic example of co-evolution between microorganisms and human beings. Imitating from this point, scientists have been successful in tracing the migration pattern of *Homo sapiens* from Africa and throughout the globe by studying the strain diversity of *Helicobacter pylori*.

Why are Microbiomes Important to Us?

While the microbes living in our gut facilitate digestion and prevent colonisation of our body by harmful bacteria and viruses, the ocean microbiome is responsible for 50% of the primary food production occurring on our earth. The soil microbiome plays a key role in the proper functioning of almost all terrestrial ecosystems. Feeding the ever-growing global population has always been a challenge, and continues to do so with the ever-decreasing availability of land, climate change, and disease pandemics that affect the human resource. Use of beneficial plant microbiomes to enhance plant growth, to increase the efficiency of nutrient use, and to develop resistance against diseases in plant crops, has been overlooked and needs much attention. Given the impact that microbes create on human lives in the present-day world, it is time for us to explore and tap the enormous potential of microbiomes that exist in this world.



Focusing on us, the microbiome of a person plays a key role in that person's health. In other words, the microbiome of an individual determines the overall health of that individual. The microbiome of a person, animal or a plant is colonized by microorganisms coming from the outside environment. The microbes living in our environment largely determine the microbes that live within us. It is not only our diet, but also the persons with whom we closely associate with, that influence our microbiome, or our gut microbiome in particular. Our emotions also affect the composition of our gut microbiome. Our gut microbiome gets disturbed when we are stressed, resulting in ill health.

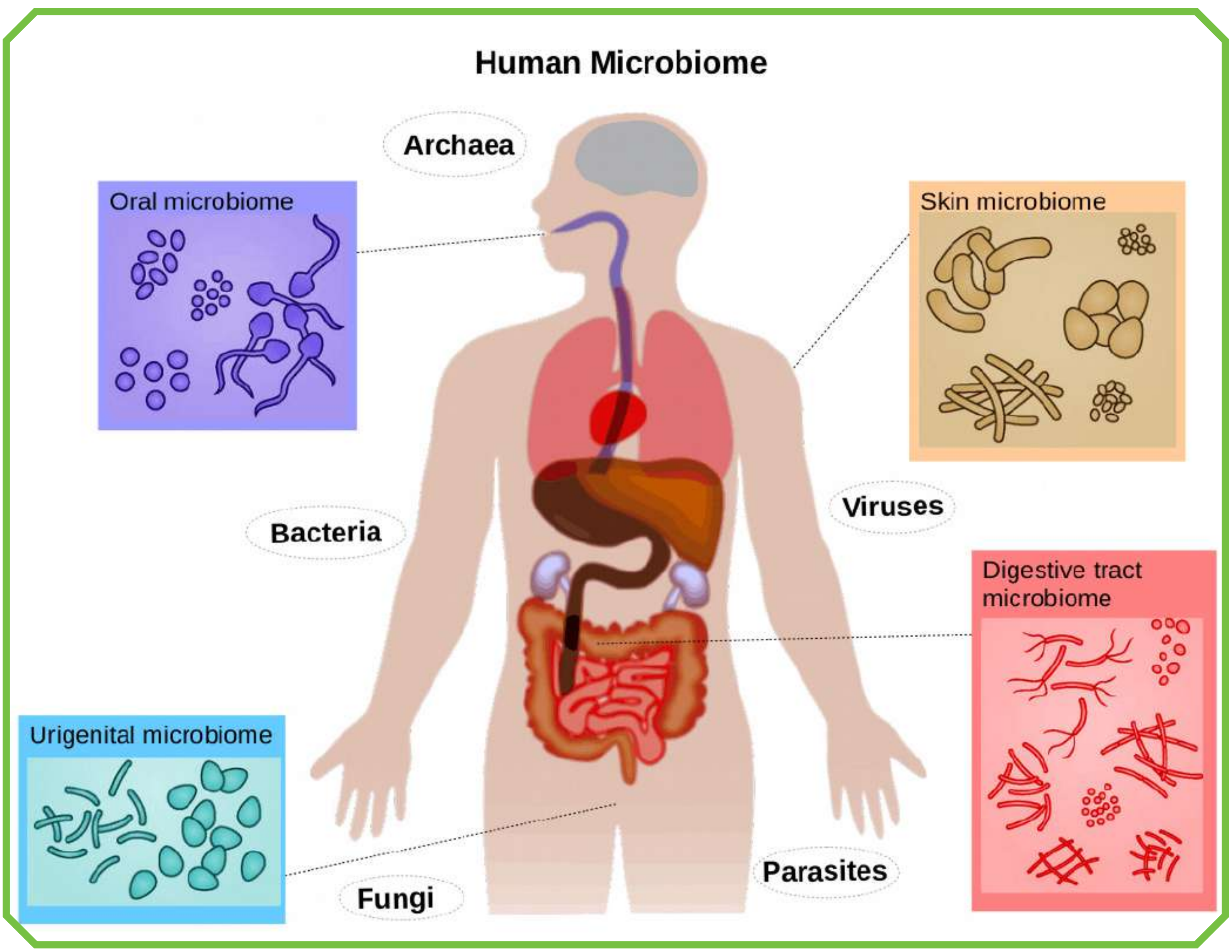
In addition to the role of microbes in human and animal health, the role they play in the availability of food cannot be overlooked. Feeding a world where the population increases within a limited area of cultivable land, has left us with a challenge of finding adequate quantities of food for every one of us, including the animals that share this planet with us. In this background, plant-based foods make up the bulk of food needed by large herbivorous animals such as cattle. Gut microbes become indispensable in this regard, facilitating the digestion of roughages/the cellulose in roughages by producing the enzyme cellulase which is not produced by their herbivorous hosts.

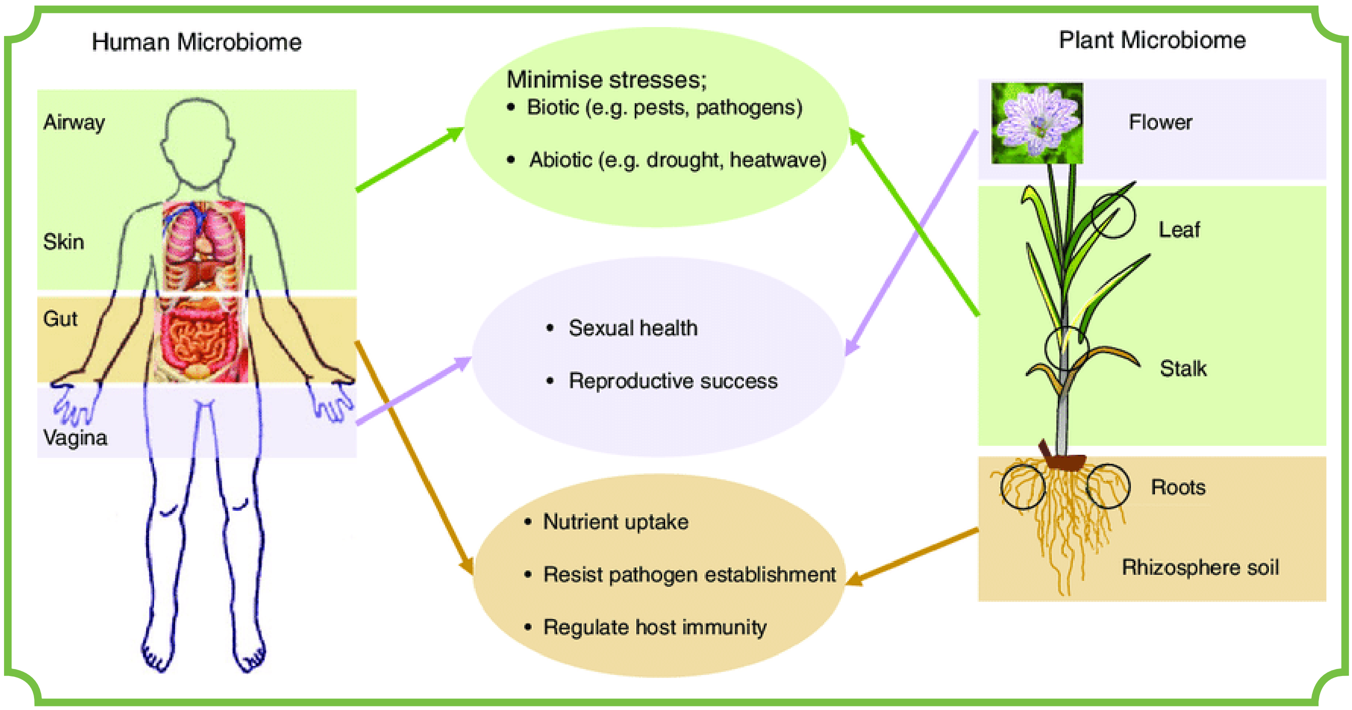
Factors that Determine a Microbiome

Research on microbiomes have shed light on some of the factors that determine the composition of the microbiome. It is a well-known fact, that the microbes in a microbiome originate from the environment in which the host lives. However, not all microbes in the environment become eligible to be a part of the host microbiome. Some microbes have special features that make them special members in a special microbiome. For example, the ability of *Helicobacter* to tolerate the acidic pH inside our stomach has made it an important member in our stomach.

While some interactions between hosts and microbes are competitive, parasitic or predatory, others are mutualistic in creating benefits to both the host and the microbe. In a world stricken by a deadly pandemic caused by a tiny virus, we are well aware about the damaging interactions between a host and its microbes. On the other hand, we are all familiar with the "good" bacteria such as *Lactobacillus* and *Lactococcus* that greatly help in the dairy industry. Then there are the friendly bacteria such as *Rhizobium* and *Mesorhizobium* that lives in the root-nodules of legume plants, fertili of the soil by making nitrogen available to plants.

Interestingly, there are also microbe-microbe interactions within a microbiome, which again shape the composition of the microbiome. Sometimes the commensal bacteria living in/on our body will help reduce the pathogenicity of a bacterial pathogen, by making the environment hostile to the latter. The microbial community associated with one person do not live and act in isolation. Most of the time these microbial communities interconnected with the microbial communities of other hosts that closely interact with the first host. A classic example is where members of one family share more microbes with each other than with non-related individuals. This type of sharing of microbes, crosses species' boundaries, making one to share certain microbes in your microbiome with your pet dog at home. There are always good and





bad things associated with these interactions.

Moving to the microbiome in our bodies, we rely on microbes for digestion and nutrition, to resist pathogens invading our body and to awaken our immune system during such an invasion. With the developments in medical research, and the vast amount of knowledge available on human microbiome, we are well aware that the composition of our microbiome changes with disease. On the other hand, factors such as stress that influence the composition of our microbiome will predispose us to disease.

Social behaviour of animals is thought to influence their microbiome composition. The maternal behaviours in animals, particularly in mammals have a great influence on the microbiome composition of the newborn. Research has shown that babies that are born naturally acquire microbes from the mother's vagina making them ready to face the microbial hazards and threats of the outside environment. This has made medical surgeons and doctors to apply maternal vaginal swabs on babies born by caesarean sections. Similarly, members of the cat family introduce a protective layer of salivary microbes on their kittens and cubs through grooming and licking.

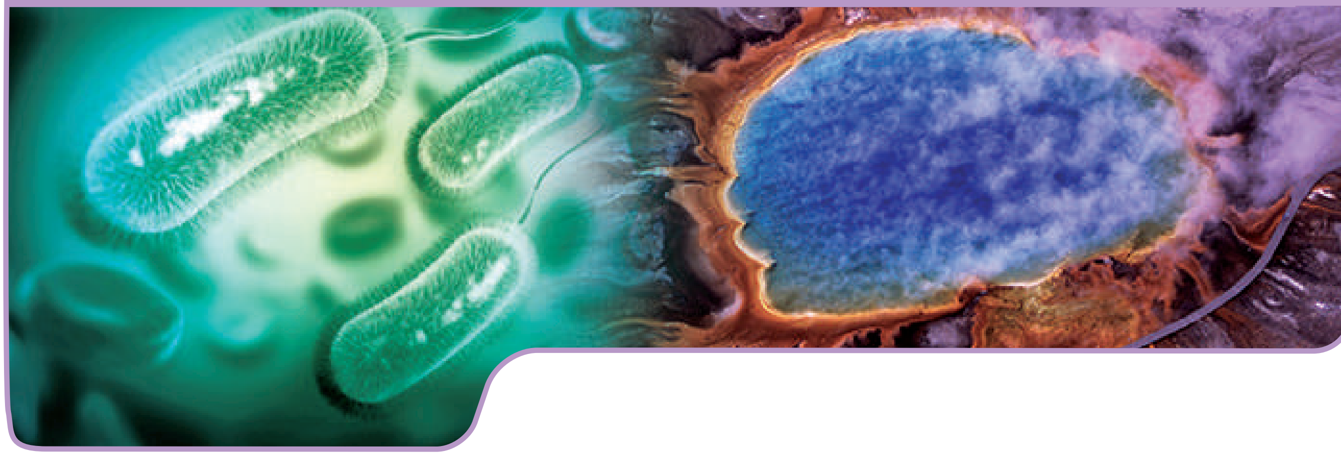
Preserving the Diversity of Microbiomes

In a world where biodiversity is rapidly declining, mostly due to human interventions, microbial diversity is no exception. It is scary to think that the microbial diversity in certain biospheres would be lost even before we study them. Research studies have shown that microbial communities are sensitive to global climate changes. Our own research have shown changes in the microbiome composition of marine organisms in the face of increased rise of seawater temperature. Scientists have warned about potential extinction of microbes that are in symbiosis with critically endangered hosts. Suggestions have even been made to preserve microbial DNA from environments and animals that are at risk.

Dr Erandi Pathirana
Senior Lecturer,
Department of Aquatic Bioresources,
Faculty of Urban and Aquatic Bioresources,
The University of Sri Jayewardenepura,
Gangodawila, Nugegoda
Email: erandi@sjp.ac.lk

Microorganisms in Extreme Environments

Prof. S. Chandrani Wijeyaratne



Introduction

Microorganisms have dominated the biosphere for 3.5 billion years, inhabiting virtually every niche available on this planet, but our knowledge about all of them is limited, as only less than 1% of them can be cultured and identified in the laboratory. Therefore, less information is available on these unculturable microorganisms. Majority of identified microorganisms have been recorded from moderate environmental conditions by culturing them in the laboratory. e.g., temperatures of 37°C, salinity of 3%, normal atmospheric pressure, presence of oxygen, etc. However, development of new tools such as metagenomics have allowed us to analyze DNA or RNA of microorganisms directly from environments that had not been explored earlier without culturing them by conventional methods. Most of these unexplored environments have extreme or harsh conditions, for example, volcanic hydrothermal vents, hyper saline environments such as large salt lakes or Dead Sea, glacial polar environments and ocean floors, which are exposed to very low temperatures and high-pressure conditions, surfaces in biofilms and anaerobic environments.

Microbes present in extreme environments are collectively referred to as extremophiles (love extreme conditions). They not only tolerate harsh environmental conditions, but even thrive in them. Extremophiles are classified according to the conditions in which they grow: thermophiles and hyperthermophiles (high or very high temperature loving, respectively), psychrophiles (organisms that prefer low temperatures), acidophiles and alkaliphiles (optimally adapted to acidic or basic pH values, respectively), barophiles or piezophiles (grow best at high pressure environments), and halophiles (require high NaCl concentrations for growth). Microorganisms, which can thrive under multiple extreme conditions are called ployextremophiles. The Table 1. summarizes the different extremophile groups, the conditions prevailing in those environments and their locality.

Figure 1 : Boiling Hydrothermal System

Diversity of extremophiles

Extremophiles include members of all three domains of life, *i.e.*, bacteria, archaea, and eukaryote. Most extremophiles are microorganisms and most of them belong to the group Archaea, whose molecular characteristic are clearly different from the other two groups of life forms. Extremophiles also includes algae, fungi, protozoa and even few multicellular organisms.

Different types of extremophiles

Thermophiles

Thermophiles thrive in high temperature environments

Table 1: Different types of extremophiles and their environmental conditions

Type	Environmental condition	Locality
Thermophiles	80°C – 113 °C	Deep sea hydrothermal vents, Hot springs.
Psychrophiles	< 0 °C	Antarctic and glacial ice
Halophiles	30 % NaCl	Salterns , salted foods Salt lakes
Acidophiles	pH 0.5	Hydrothermal pools and acid mine drainage
Alkaliphiles	pH 12.5	Soda lakes
Radiophiles	High radiations	Nuclear reactors
Barophiles	High pressure	Mariana Trench

such as hydrothermal and volcanic vents and terrestrial hot springs (Fig. 1). Their optimum growth temperatures fall between 55-65 °C , while minimum temperature is around 20 °C. *Thermus aquticus, Sulfolobus solfataricus, Sulfolobus acidocaldarius* are few examples of thermophiles recorded from different high temperature environments (Fig. 2). Hyperthermophiles grow above 80 °C, where several maxima have been recorded for different microorganisms e.g. *Methanopyrus kandleri* strain 116 grows at 122 °C (252 °F) and *Pyrolobus fumarii*, holds the upper temperature limit for life at 113 °C (235 °F). *Pyrococcus abyssii* and *Pyrodictus occultum* are marine hyperthermophiles. Many hyperthermophiles are members of archaea that can utilize H_2 as the energy source. Thermoacidophiles are found in the areas with very high temperatures and extremely acidic conditions.

Cyanobacteria, the pioneer inhabitants of earth, have been reported in abundance from high temperature environments such as hot springs, e. g. Yellowstone Park (Fig. 3). They perform biological processes under extreme temperatures, pH, high salinity, desiccation, and other hostile environments. Cyanobacteria often form microbial mats with other bacteria, developing highly organized communities in hot springs, stratified lakes in summer and in tropical oceans. *Calothrix, Oscillatoria, Phormidium, Synechococcus* and *Chloroflexus* are examples of thermophilic cyanobacteria.

Figure 2 : Thermophilic bacteria
a) *Thermus aquaticus* b) *Thiobacillus ferrooxidans*

Among eukaryotes, fungi (alone or in symbiosis) are the most adaptable and ecologically successful group of organisms on earth. The majority are cellulolytic, saprobic, often common in soils of temperate and tropical regions. *Thermoascus auranticus, Rhizomucor miehi, Humicola insolens, Chaetomium thermophile* are few examples of fungi found in decomposing organic matter, where the temperatures rise to 60-70 °C. Apart from hyperthermophily, they adapt well to other extreme environments such as acidic and metal-enriched waters from mining regions, alkaline conditions, hot and cold deserts, the deep ocean, and to hypersaline regions like the Dead Sea.

Lichens survive in most of the inhospitable environments on earth, as they possess stress tolerant features such as, slow growth rates, low demand for nutrients and morphological and physiological adaptations. Reindeer moss, *Cladonia rangiferina* dominates the Arctic tundra, which is one of the harshest biomes in the world. Likewise, lichens flourish mountains that have extreme temperature fluctuations, high UV levels, high wind speeds and in

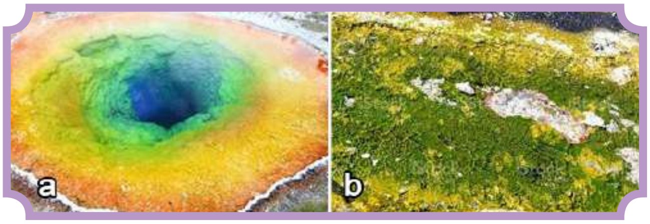


Figure 3 : a) Cyanobacteria and b) bacterial mat in hot springs in Yellowstone National Park

variable snow cover, due to their adaptability to extreme conditions. *Xanthoria elegans* and *Lecanora polytropa* have been recorded at extreme elevations in Himalayas (Fig. 4). Lichens can survive even in arid desert regions as they can absorb water from damp air e. g. *Ramalina maciformis.*

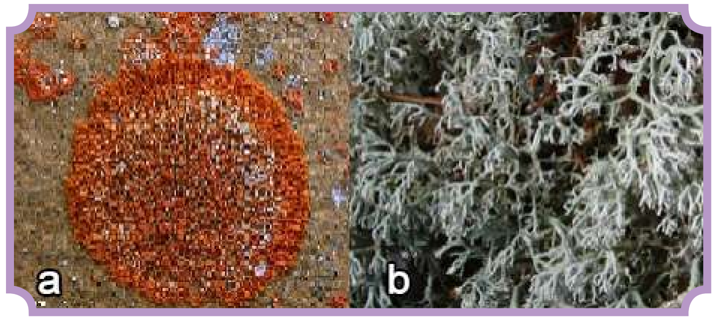


Figure 4 : Lichens in extreme environments a) *Xanthoria elegans* b) *Cladonia rangiferina*

The most heat tolerant animal known to science is the Pompeii worm living close to the hydrothermal vents in the ocean floor. It can survive temperatures up to 113 °C . Tardigrades or water bears can survive several types of extreme conditions. They live in hot springs and Antarctic ice. *Artemia salina* is a primitive arthropod living in salt lakes tolerate high salt concentrations (Fig. 5).
In contrary to thermophiles, psychrophile or cryophiles can survive and proliferate in conditions of extremely low temperatures of around -20 °C. Their maximal temperature for growth is at about 20°C and minimal temperature for growth is at 0°C or lower. *Arthrobacter sp. Psychrobacter sp., Hyphomonas, Sphingomonas* are few examples for psychrophiles. *Methanogenium frigidum*, a psychrophilic, slightly halophilic, H_2-using methanogen, found in the perennially cold lake in Antarctica.

Prolific growth of cyanobacterium, *Calothrix parietina* has been discovered in snow covered areas in Greenland. These bacteria can also develop in hypersaline and alkaline lakes, support high metal concentrations, and tolerate xerophilic conditions (Low availability of water) also.

Acidophiles

Naturally, acidic environments are created when inorganic sulphur rises to the surface and gets oxidized to sulphuric acid (Fig. 6). Acidophiles are organism, which grow with optimal growth at pH 3 or below. Their optimum pH varies between pH 0- pH 5.5. The genus *Picrophilus* (e.g., *Picrophilus torridus*) includes the most acidophilic organisms currently known, with the ability to grow at a pH of 0.06. Among archaea, many sulphur oxidizers, e. g. *Ferroplasma acidiphilum* and *sulphur reducers* e. g. *Pyrodictium abyssii* live in extreme acidic environments. These organisms are isolated from geothermal springs and deep -sea hydrothermal vents. Among the eubacteria, *Thiobacillus ferrooxidans* can metabolize iron or sulphur, producing sulphuric acid. *Helicobacter pylori* is a Gram-negative bacterium that lives in the extreme acidic environment of the stomach and causes ulcers in humans (Fig. 7). Several lichens such as *Pseudevernia furfuracea* and *Bryoria spp.* can also tolerate acid conditions that originate due to acid rains.

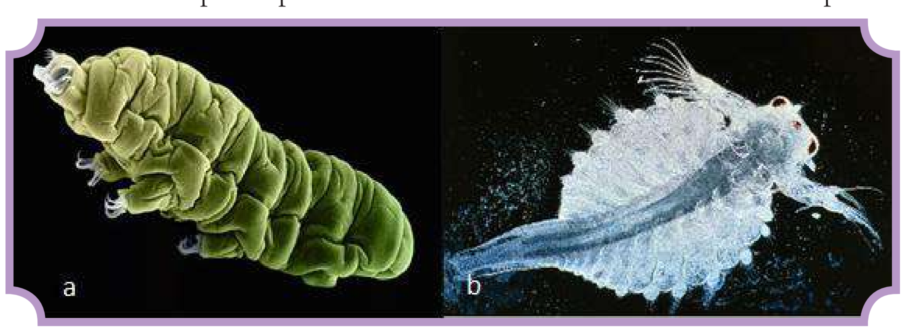


Figure 5 : Extremophile animals: a) Tardigrades or water bears , b) *Artemia salina* (Sea monkey)

Alkaliphlies

Alkaliphlies are organisms which can thrive under alkaline environments. Aerobic alkalipohiles including *Bacillus, Micrococcus, Pseudomonas* and eukaryotes such as yeast and fungi have been isolated from different alkaline habitats e.g. Soda lake in Africa (Fig. 8). Alkaliphiles has optimum growth pH between pH 8- pH 11.5. Extreme alkaliphile e. g. *Alkalihalobacillus alcolophilus* (formally *Bacillus alcolophilus*) isolated from alkaline wastewater grows at pH 9- 10.5.

Halophiles

Halophiles are extremophiles that require high salt concentrations for their survival and growth. There are two types of halophiles; obligate halophiles that require NaCl concentration of 3% or more and halotolerant that survive at both average salt concentrations and higher. *Halococcus salifodinae, Halobacterium salinarum* (Fig 9), *Limimonas halophilia, Lentibacillus kimchii, Sporohalobacter salinus* are few examples of halophilic bacteria recorded from various saline environments. *Artemia salina* (sea monkey) can live in conditions with extremely high salt concentrations. These extremophiles inhabit salt lakes, salt swamps, seas, and rocky coasts (Fig . 10).

Figure 6 : Acid mine drainage

Barophiles/ Piezophiles

Barophiles or piezophiles can survive high pressure environment such as ocean floor. Sea beneath 1000 m is characterize by a high hydrostatic pressure mainly having coldness, darkness, and shortage of organic matter, which is not conducive for the growth of most of other microbes. All barophiles cultured from the cold deep sea are psychrophilic as well. *Photobacterium profundum and Schewenella benthica* are examples of barophilic bacteria. *Shewanella benthica* is an obligate piezophilic bacterium isolated from the sediment of Mariana Trench at depth of 10,898 m (Fig. 11).

Figure 7 : Acidophilic bacterium: *Helicobacter pyroli*

Radiophile:

These organisms thrive in conditions with high levels of radiation, including ultraviolet and nuclear radiation. Some common examples of radiophiles are *Deinococcus radiodurans, Rhodococcus, Halomonas, Herbaspirillum, Rhodobacter*

Anaerobic environments

Anaerobic environments are also considered as extreme environments as oxygen is required for the survival of majority of living beings on the Earth. As the primordial earth had an anaerobic environment, the primitive life forms that existed were methanogenic bacteria. They are strictly anaerobic and harvest energy by converting H_2 and CO_2 into methane gas. Some of these organisms use a fluorescent pigment for energy harvest to drive this reaction. They are found in marshes and also in some animals such as cattle, who then blech large quantities methane into the atmosphere e.g., *Methanococcus* and *Methanobacterium* (Fig. 12).

Ammonia-oxidizing archaea (e.g. *Nitrososphaera, & Nitrosopumilus*) in aquatic and terrestrial environments e.g. *Nitrosoarchaeum*, are identified as being involved in nitrification. They are capable of oxidizing ammonia at much lower concentrations than other ammonia oxidizing bacteria, and present in sediments and hot springs.

Figure 8 : Soda Lake

Figure 09 : *Halobacter salinarum*

Figure 10 : Halophilic bacteria in saltpans

Adaptation to extreme environmental conditions

How do extremophiles survive these unique environments? Although strategies employed for survival are not fully understood yet, it is known that there are three ways in which an organism can adapt to an extreme environment. They are, by developing a mechanism for excluding the factor from its structure, by having a mechanism for detoxifying the factor or by learning to live with the factor.Often these organisms have specialized biochemical pathways and adapted biomolecules to overcome the stress conditions they encounter in extreme environments.

The greatest success of thermophiles is due to the presence of heat stable enzymes and proteins, which remain catalytically active under extremes of temperature, salinity, pH, and solvent conditions. Some of these enzymes display polyextremophilicity that make their wide use in industrial biotechnology possible. Some hyperthermophiles have an enzyme called DNA gyrase that changes the topology of the DNA and enhances its stability.

Heat stable proteins have more hydrogen and other non-covalent bonds to stabilize their structure. In addition, proteins are stabilized and aided in folding by special group of proteins called chaperones.

Figure 11 : Mariana trench

Nucleotide associated proteins appear to stabilize the DNA of thermophilic bacteria. Further, the membrane lipids of thermophiles are more saturated, more branched with higher molecular weight, which increase the melting point of membrane lipids. Archaea have membrane lipids with ether linkages and such lipids are resistant to hydrolysis at high temperature. Thermophiles utilize elemental sulfur as an electron acceptor in their electron transport chain in respiration, when compared to mesophilic organisms, that have oxygen driven respiration. This is the reasons for thermophiles to thrive in hot, sulfur-rich locations such as volcanoes, deep sea vents, hot springs, etc.

At extreme low temperatures cytoplasm and DNA of thermophiles are protected from freezing by producing antifreeze proteins that maintain the fluidity of the plasma membrane. This characteristic has recognized psychrophiles as a topic of interest from an astrobiological point of view.

Organisms living in acidic environments, where the hydrogen ion concentration is high, pump out poisonous hydrogen ions fast enough so that damage to DNA is prevented. On the other hand, alkaliphilic microorganisms maintain internal pH close to neutral by exchanging internal Na^+ ions for external protons.

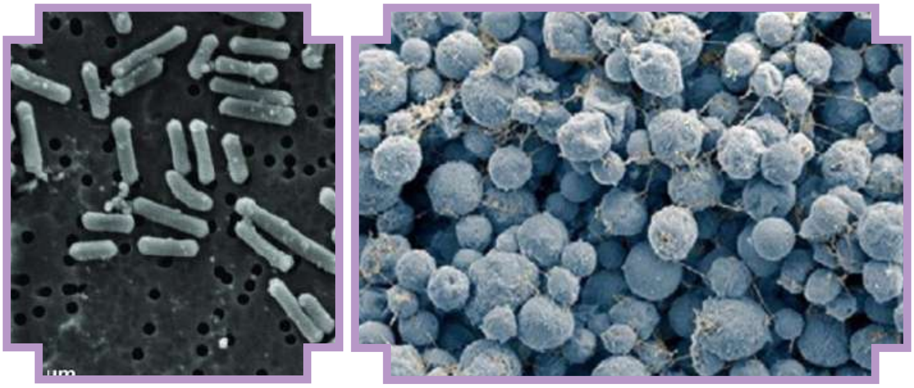


Figure 12 : ***Methanococcus sp.***

Halophiles use two distinct strategies to increase the osmotic activity of their cytoplasm with the external environment. They either produce compatible organic solutes, or accumulate large salt concentrations in their cytoplasm to reach an equilibrium state, so that the overall salt concentration within cells correlate with that of the environment.

In the case of barophiles, the fluidity of the cell membrane is reduced by producing increased levels of unsaturated fatty acids in the lipids for the normal functioning of the cell at high pressure, low temperature, or both.

Applications of extremophiles

At present studies are done extensively to detect, and to make use of microorganisms in extreme environments, to satisfy the future requirements of the human population. In this regard, many novel enzymes that have biotechnological interests due to their thermostability, halotolerance, cold-adaptability and other unique abilities, have been identified from different extremophiles. Several lipolytic and cellulolytic enzymes, have been identified from environments with extreme temperatures and pH values.

Alkaline active proteases, amylases, cellulases and lipases are used in the formulation of heavy-duty laundry and dishwashing detergents, as they are efficient in removing stains effectively at low temperatures. Taq polymerase enzyme from *Thermus aquaticus* bacterium is an ideal enzyme for use in the polymerase chain reaction due to its ability to withstand high temperatures.

In addition to novel enzymes, some extremophiles may comprise of large reservoirs of therapeutic agents such as antibiotics and other pharmacologically important biochemicals.

Bioleaching is another biotechnological application of extremophiles, where metal ions are removed from their respective insoluble sulphide ores biochemically. Microorganisms can extract various metals e.g. copper, tin, nickel, zinc, etc. from sulphur-based ores, by catalyzing biochemical oxidation-reduction processes. Acidophilic microorganisms (e.g., *Thiobacillus* group) are capable of metabolizing metal sulphides. Mesophilic but highly acidophilic (pH 1.5-2.0); *Thiobacillus ferrooxidans, Thiobacillus thiooxidans*, and *Leptosprillum ferroxidans*, can reduce sulphur compounds, and the latter use only ferrous ions. Together they can rapidly degrade pyrites (FeS_2). However, still little is known about the roles of these extremophiles in the ecosystem. Recent studies have shown that they contribute to the production of greenhouse gases, to the major carbon, nitrogen and nitrate cycles. Further, their genomes contain more than 90% of genes encoding proteins of unknown functions. Possibly these macromolecules may be playing an important role in the adaptation of these extremophiles to the physico-chemical conditions, nutritional and energy resources specific to these environments.

Thus, extremophiles, which can perform vital biological functions under one or more extreme stress conditions, are important for the scientific world for their potential application in various industrial ventures.

Prof. S. Chandrani Wijeyaratne
Emeritus Professor
Department of Botany
University of Sri Jayewardenepura
Nugegoda

Application of Microbes for the Food Industry

Prof. Upali Samarajeewa

All living beings including micro-organisms metabolize complex organic molecules to generate simple molecules and obtain energy for their activities. Micro-organisms are unique in fermenting various food materials, to generate new products of industrial value, or to impart preferred sensory characteristics to foods. The term fermentation was used originally to describe processes that exhibit effervescence, releasing carbon dioxide. It was also described as agitation or effervescence due to bubbling of gases, and causing disturbances in a liquid. In modern science, fermentation is described as a process where complex molecules are broken down into simple molecules, that end up as inorganic molecules at times. This term has a wider meaning today which is beyond visible fermentation of sugars to ethanol and evolution of carbon dioxide. The ability of the micro-organisms to bring about fermentations is used industrially for the benefit of the mankind, in the food industry.

The reactions generated by the microbial enzymes are utilized in the bakery, brewing, dairy, vegetable, fish and meat industries to produce new sensory properties in the foods including texture, colours and flavours. Some fermentations bring in the nutritional benefits to foods such as easy digestibility and added vitamins. These metabolic changes are brought about by yeasts, bacteria and molds (fungi). Some fungi produce fruiting bodies in edible forms, which are commercially cultivated. This article describes the different microbiological processes used in the food industry and their benefits to mankind.

Fermentation of sugars

Fermentation of sugars to produce beer and wine has a history that gose back to 7000 years. Louis Pasteur, the French chemist and microbiologist studied the fermentation of sugars in 1850, and discovered that the yeast *Saccharomyces cerevisiae* was responsible for the fermentations. The conversion of glucose to ethanol and carbon dioxide by yeasts is shown in the following chemical equation.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + CO_2$$

When this balanced equation is examined on a molecular weight basis, it indicates that 180 g of glucose produces 92 g of ethanol, and 44 g of carbon dioxide. Forty-four grams of carbon dioxide is substantial, which is used in the bakery industry to produce ready-to-eat foods preferred by consumers.

Bakery industry

In the bakery industry, cane sugar (sucrose) is fermented to get the benefit of the fast evolving carbon dioxide to puff up bread and other baked products. The spaces created by the pressure of carbon dioxide (CO_2) trapped within carbohydrate structures cause puffing, providing the soft texture. This reaction is a little more complex than the simple fermentation of glucose. Initially the sucrose is broken down into glucose and fructose by the yeast enzymes. Both sugars are next converted to ethanol and carbon dioxide by the yeast enzymes. The ethanol being volatile, get released from bread during baking, while carbon dioxide applies the pressure in the dough prior to baking and during baking to generate the new soft texture. The residual sugars left unfermented and provide a rich body in the taste, and an increased sweetness than that of sucrose by itself to the bakery products.

Fermentation in carbohydrate matrices to develop textural characteristics has been a practice in South Asian countries since ancient times. The *hoppers, idli* and *thosai* mixtures are fermented for 8-12 hours to allow carbon dioxide to get accumulated in the dough leading to soft textures and new flavours. The new flavours are due to hundreds of secondary metabolites produced by the yeasts during fermentation. The use of a few milliliters of fermenting toddy as the yeast culture in preparing the dough for hoppers is well known. Scientific investigations have shown that these natural cultures carry lactobacillus species generating characteristic flavours.

Brewing industry

The same chemical reaction of converting the sugars (sucrose, glucose or maltose) to ethanol and carbon dioxide is used in the brewing industry with the same yeast *Saccharomyces cerevisiae*. Although it is the same yeast, the cultures selected for the two processes differ. The baking yeasts are more active in producing carbon dioxide, whereas the brewing yeasts are geared to produce high concentrations of ethanol. Even among the *Saccharomyces cerevisiae* subspecies used in the brewing industry, the amount of ethanol produced could vary.

The sugar used as the raw material for fermentation may differ. The sugar in barley used in the beer industry is maltose. Wine industry uses glucose from the grapes. The coconut, kitul and palmyrah industries ferment sucrose. Sugar cane molasses contain a mixture of sucrose, and its breakdown products are glucose and fructose. The final ethanol contents produced can vary resulting in 4-6% in beers, 12-14 % in grape wines, 6-9 % in palm toddies and 2-3% in apple ciders. The concentration of ethanol achieved industrially, depends on the initial amount of fermentable sugar and the capacity of the selected *Saccharomyces* strain to ferment.

The industrial alcohol fermentation system has its own problems in getting the best performance of the selected *Saccharomyces* subspecies. There are other micro-organisms that contaminate the fermenting sugars by competing with *Saccharomyces* and producing acids, higher alcohols and esters, there by altering the flavour of the fermented products. Such organisms are collectively described as 'wild yeasts". The entry and activity of such "wild yeasts" is controlled in the industry by various means.

In the beer industry, a mixture of barley and hops are fermented. Hops provide the bitterness as well as a check on "wild yeasts" to a certain degree. The *Saccharomyces* strains used in each brewery differ from each other, providing specific flavour characteristic associated with its brand. The top breweries in the world keep their *Saccharomyces* strain as a trade secret, and claim that the same strain had been used unchanged for hundreds of years. A sub species of *Saccharomyces calsbergensis*, is one such yeast. The initial breakdown of carbohydrates in barley to the sugar maltose is carried out by initiating the grains to germinate. This process is called malting. Peptidases and proteases in the grain generate peptides and amino acids, break down the walls of endosperm by beta-glucanases. Amylases convert starch to sugars. The grains are then kilned to arrest further enzymatic reactions and then powdered. Kilning also provide the environment for the liquid called "wort" produced by using heat on water to prevent proliferation of "wild yeasts". Saccharomyces species providing the desirable characteristics are then introduced for efficient fermentation. Fermentation is a biotechnological process, where adjustments of temperatures and pH are done to achieve the final sensory characteristics unique for each commercial brewery or in home brewing. A science discussing the aroma and flavour of alcoholic beverages has become an art in comparing the satisfactions associated with different brewing practices.

The check on spoilage by "wild yeasts" in the wine industry is done by applying predetermined concentrations of sodium metabisulphite. Sodium metabisulphites inactivate the "wild yeasts", providing a competitive advantage for the *S. cerevisiae* strains identified for the purpose. The final ethanol content depends on the ability of the *S. cerevisiae* strain to withstand the ethanol and carbon dioxide concentrations produced by itself, and the availability of sugar for continued fermentation.

In the palm toddy industries of Sri Lanka (Coconut, kittul and palmyrah), and in the African palm wines (Sendi, tari, nareli etc.), the sap from the inflorescences, or the trunk of the palm tree is allowed to ferment by air-borne natural microflora deposited by visiting bees. The resulting microflora contain mainly lactic acid bacteria and yeasts. The sugar available for fermentation is sucrose. Initially the micro-organisms convert the sugars in to lactic acid by bacteria, there by reducing the pH of the sap from 7 to 4. The reduced pH provides a more conducive environment for ethanol producing enzymes of the yeasts. The ethanol producing yeast identified from fermenting toddy in Sri Lanka is different from *S. cerevisiae* used in other fermentation industries. The microorganism is *Saccharomyces chevaleri*, different from *S. cerevisiae* in its inability to ferment maltose. The presence of *S. chevalieri* indicates an adoption by the yeast to ferment sucrose more efficiently with the loss of the ability to ferment maltose in the original *S. cerevisiae*.

Fermentation of alcohols using natural microflora results in decreased ethanol yields. Coconut sap contains sufficient sucrose to produce about 10% ethanol on fermentation. However, the yields in the Sri Lankan toddy industry are about 6%. Scientific investigations have shown the possibility of introducing calculated quantities of sodium metabisulphite to check the "wild yeasts", and the consequently possibility of reaching more than 9% yield with the yeast strain *Saccharomyces chevalieri* Y18. This industry needs to commit itself for better profits than by being satisfied with traditional practices.

Industrially fermented ethanol is a good raw material for a second step of microbial fermentation, either as it is, or for other industrial uses after concentration by distillation.

Vinegar fermentation industry

Ethanol produced by fermentation by yeasts, continue to become acidic due to the production of acetic acid by *Acetobacter acetii* and *Gluconobacter* species under aerobic conditions. They produce 4-6% of acetic acid popularly known as vinegar. This fermentation produces riboflavins, Vitamin B1, minerals and salts of potassium and sodium imparting special flavours. Vinegar is an important condiment used in food preparations providing a preservative effect and flavour. In Sri Lanka coconut vinegar is produced by circulating toddy containing ethanol in large barrels packed with maize cobs after removal of the seeds. The cobs hold the bacteria on its surface allowing interactions between oxygen from the atmosphere, and fermenting toddy with bacteria for efficient industrial production. There are large wooden barrels with the capacity of several hundred liters, packed with cobs or wood chips for producing coconut vinegar. This oxidation reaction is used to prepare vinegar from apple cider, sugar cane juice, rice, grape wines, dates and other fruits in other countries.

Fermented ethanol concentrated by distillation is a major industrial raw material. Up to 20% of ethanol can be mixed with vehicle fuels as an economic measure. Ethanol diluted with water at different concentrations, are used as beverages, perfumes, aftershaves and colognes, medicinal liquids, mouthwashes, liniments, and some rubbing alcohols. Ethanol at 70% became an important antiseptic in hand cleaning to protect humans from COVID-19 virus.

Dairy industry

Milk is a rich source of nutrients contributing lactose, proteins and fats to human diet. Fresh milk is fermented using micro-organisms to a variety of foods with new sensory characteristics, nutritional value and to be preserved. Some population groups in South Asia and Africa lack the enzyme lactase among the adults, resulting in indigestion problems with fresh milk. Microbiological fermentation of milk has been practiced since ancient times to overcome this problem. The *Lactobacillus* species ferment the milk converting lactose to the simple sugars, glucose and galactose. The *lactobacilli* also produce a variety of other compounds including acetic acid, diacetyl and acetaldehyde. They contribute to new flavours in fermented milk products. The flavour profiles of the products depend on the species ands strains of microorganisms used in each dairy processing.

Milk from buffaloes and cows are fermented to produce curd. It is a traditional home-industry in Sri Lanka and many other countries. The starter cultures for fermenting obtained from the previous day is introduced to boiled and cooled milk which is free of pathogenic micro-organisms to make curd. The curd inoculum

is a mixture of micro-organisms dominated by *Lactobacilli* species. The inoculum from the previous fermentation is active and rich with proteins, and vitamin B12 to initiate fermentation of fresh milk. The lactobacilli create the acidity necessary in curdling of milk and solidifying milk proteins, mainly casein. The mixed cultures used in curd may contain *Lactococcus lactis, Streptococcus diacetylactis, Streptococcus cremoris, Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* as single cultures or mixed cultures.

Fermentation of yoghurt is similar to preparing curd, but the inoculum is a well-established mixture of two micro-organisms in the ratio of 1:1. The *Lactobacillus bulgaricus* in the mixture produces lactic acid. The *Streptococcus thermophiles* produces the flavours specific to yoghurt. The rates of growth of the two micro-organisms in milk differ. It is therefore necessary to transfer the yoghurt after initial fermentation at 45 ^{o}C to refrigerated temperatures to prevent over activity of the microorganisms leading to increased acidity spoiling its flavour. It is not possible to reuse the yoghurt inoculum compared to this situation in curd, as the original population balance changes during fermentation. Yoghurt may be flavoured with mango, strawberry or other flavouring agents. Yoghurt is also marketed in other forms such as stirred yoghurt, drinking yoghurt and frozen yoghurt. Microbial fermentation of yoghurt is well established in large commercial scale operations as well as in small industries.
In other countries milk from goats, mare, etc. are fermented and these are refereed to by names such as Ymir, kefir, cultured buttermilk, filmjolk (Scandinavian sour milk), cultured cream and koumiss (a product based on mares' milk). Kefir is fermented by a mixture of lactic acid bacteria and yeasts. Butter milk forms the basis for production of butter by churning and other processes.

Cheese produced by initial fermentation of milk to a curd. Once the curd is produced, it is separated from the liquid fraction. The separated solid is salted and further processed using a variety of micro-organisms to produce cheeses of different aroma and appearance. The micro-organisms used for specific cheese brands include,

1.*Penicillium camemberti* for Camembert cheese
2.*Penicillium roqueforti* for Roquefort cheese with blue veins
3.*Penicillium glaucum* to produce greenish blue veins in cheese
4.*Brevibacterium linens* to produce red colour in Limburger cheese
5.*Propionibacterium freundenreichii* creating holes inside Swiss cheese due to carbon dioxide

The cheese industry uses the above micro-organisms to produce specific appealing characters, flavours and textures to satisfy varying customer preferences.

Fish fermentation

Fish are fermented by keeping them packed anaerobically by submerging in salt water. Other additives such as goraka (*Garcinia cambogia*) and potassium nitrate are also used. These additives and salt check the growth of spoilage micro-organisms, providing the opportunity for lactic acid bacteria to ferment. These more common micro-organisms in fermenting fish include *Lactococcus* spp., *Lactobacillus brevis*, and *Pediococcus* spp. The micro-organisms provide specific flavours due to breakdown of lipids. Natural enzymes in the fish muscles bring about proteolysis, and also play a fermentative role in changing the texture of the muscles. *Jadi* is one such product available in Sri Lanka. Fermented fish are quite popular in countries like Philippines and Indonesia. Fish fermentation is done mostly home-based small industries targeting specific consumer markets. Similar microbiological applications are used in preparation of fish sauces, especially by keeping fish buried in underground pots with salt for long durations till the whole mass breakdown to a liquid. Fermentation of fish sauces occurs due to *Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas* species.

Fermentation of meat products

Meat is fermented to produce acids aiming for preservation. The common fermented meat products include sausages, hams, salami etc. In fermentation of meat, naturally occurring microflora were used traditionally. In this industry, starter cultures consisting of a single or multiple species of lactic acid bacteria, *Staphylococci*, and *Micrococcus* are employed. These micro-organisms produce acids, peptides, amino acids, amines, organic acids and aldehydes due to proteolysis of meat. These compounds impart specific flavour characteristics. In fermenting meats, nitrites and nitrates are added to arrest the growth of meat poisoning microorganism, *Clostridium botulinum*. The nitrates and nitrites impart a pink colour, and new flavours, thus improving sensory characteristics of the fermented meats. Among lactobacilli, *Lactobacillus plantarum, Lactobacillus curvatus*, and *Lactobacillus sakei* have been identified as the predominant bacteria in traditional sausage ripening. In commercial fermenting, the meat is separated from bones, minced and mixtures of pepper, salt and nitrites are added. The microbial cultures identified for fermenting consisting of *Lactobacillus, Micrococcus* and *Staphylococcus* are then introduced. The product inserted into casings, are then allowed to ripen through fermentation at low temperatures under controlled relative humidity. In some fermentations, yeasts and fungi (mostly *Penicillium* species) are allowed to grow on the surface of fermenting meats, imparting flavours due to lipolysis and proteolysis. The specific micro-organisms used in meat fermentations are given in table 1. In actual practice, more variations within similar groups of micro-organisms, but specific to the processing situation could be expected.

Table 1: Microorganisms predominantly used in fermenting meat products.

Lactobacillus	Pediococcus	Micrococcaeae	Yeasts	Molds
L. plantarum	*P. acidilactici*	*M. varians*	*Debaromyces hansenii*	*Penicillium nangiovense*
L. sake	*P. pentosaceus*	*Staphylococcus xylosus*		
L. curvatus				
L. pentosus		*Staphylococcus carnosus*		

Fermentation of vegetable products

Fermented vegetables are used widely in Asia. The vegetables are heat treated to blanch and then packed in salt solutions to discourage activities of spoilage bacteria. Lactobacillus cultures are used during fermentation to create an acidic pH. Of the fermented vegetables, sauerkraut produced from cabbage is the most popular in the European countries, while kimchi is more popular in Korea and countries in the East Asian region. In fermenting Kimchi, a mixture of garlic, ginger and red chilli powder are used in addition to the main vegetable, cabbage or cucumber (gherkins). The microflora in fermenting vegetables is dominated by *Enterobacteriaceae, Lactic acid bacteria and yeasts. Leuconostoc mesenteroides, Pediococcus pentosaceus, Pediococcus acidilactici, Lactobacillus brevi, Lactobacillus plantarum and Lactobacillus pentosus* have been reported more commonly in fermenting vegetables. These micro-organisms survive and actively ferment under the low salt concentrations and low acidity.

Fermented cucumber is a popular food in many countries. In cucumber fermentations the bacteria of the species *Torulopsis, Brettanomyces, Zygosaccharomyces, Hansenula, Torulospora and Kloeckera* along with oxidative yeasts of the genera Debaryomyces, Pichia, and Mycoderma have also been observed. Some of these organisms produce films on the surface of cucumbers. This lengthy list of micro-organisms represents those present naturally in vegetables, and continue to be active in the presence of salt and low pH. The dominance of micro-organisms during fermentation, changes with their ability to survive under changing salt and carbohydrate concentrations and acidities. There are many health claims linked with fermented vegetables, especially with kimchi as benefiting digestion.

Fermentation of Soya beans

Fermented soya products, especially miso, suffu and nato are popular in many countries. Non-fermented soya milk and tofu have gained popularity market as milk and meat substitutes for the vegetarians. Soy milk is fermented to produce yoghurts, butter and cheese of soya origin by methods similar to fermentation of dairy products discussed earlier. These microbial fermentations remove two antinutrients, lectins (a protein) and phytic acids present naturally in soya beans. The phytic acids bind minerals and make them unavailable for the body. Some of the common fermented soya products are described below.
Miso is a thick paste made by fermenting soya beans with a ‘koji’ microbial culture of mixed organisms. In miso production *Aspergillus oryzae* initially breakdown the soya bean constituents to form what is described as koji. Koji continue to undergo fermentation in a second stage by a mixed flora of bacteria and yeasts in an environment containing salt. The term koji is at times used to describe the culture used for fermentation. The production takes about 2 years. Miso is used as a constituent in soups or to pickle vegetables and meats as a flavouring agent. It is high in protein, vitamins, and minerals.
Tempeh is used widely in Indonesia. The microbiology of tempeh fermentation was studied in detail by an American scientist, Professor K. H. Steinkraus who subsequently and converted the practices to generate modern foods, meeting international regulatory and food safety standards.
Tempeh is fermented in solid phase from whole soya beans using the mold *Aspergillus oryzae*, and pressing it to a cake. It is a meat substitute as the fermentation gives it meat-like nutritional and textural properties. It is often sliced and fried, battered and fried, roasted, grilled, or crumbled to make edible forms similar to meat.

Soy Sauce has a history that goes back to 2500 years linked to the vegetarian concepts of Buddhist culture. Soy sauce is often used as a condiment to season rice dishes in Asia, especially in China where it originated. Soy sauce is widely used everywhere in the world. It is fermented in two steps. In the first step a microbial culture called koji, which contains predominantly *Aspergillus oryzae* with few other molds at times is used. The molds break down the complex molecules in macerated soya beans to simpler molecules. The molds release proteases and amylases to ferment the soya beans. In the second step fermentation is done in brine solutions predominantly by the bacteria *Tetragenococcus halophilus* and the yeast *Zygosaccharomyces rouxii*. They produce ethanol and lactic acid and various compounds contributing to a characteristic flavour. The second microbial culture is called moromi. Soya sauce is rich in monosodium glutamate arising from the breakdown of proteins. Monosodium glutamate is a flavour enhancer contributing to the rich flavour of soy sauce.

Table 2 – Colours of microbial origin that are currently used or having a potential to be used as natural food colours.

Name	Colour and (approved use)	Microorganism
Astaxanthin	Red-orange (Fish and animal foods)	*Basidiomycetous yeasts*
Beta-carotene	Red-orange (In a variety of foods)	*Dunaliella salina, Blakeslea trispora*
Canthaxanthin	Orange-deep pink (Salmon and poultry feed)	*Bradyrhizobium sp.*
Lycopene	Brilliant red (Meat)	*Fusarium, Sporotrichioides, and Blakeslea trispora*
Melanin	Black (Many foods)	Several micro-organisms
Phycocyanin	Blue pigment from Chlorophyll A (Sweets and ice cream)	*Aphanizomenon flos-aquae and Spirulina*
Prodigiosin	Red pigment (Carbonated drinks, milk and yoghurt)	*Serratia marcescens*
Riboflavin (Vitamin B2)	Yellow (Dairy items, breakfast cereals, baby foods, sauces, fruit drinks, and energy drinks)	Produced by several micro-organisms
Violacein	Purple (Has potential as a food colour)	*Chromobacterium violaceum*

Production of soy sauce is a major condiment industry supporting the global food industry.

Natto is a traditional staple food based on soya bean in the Japanese diet. It is fermented with a mixture of micro-organisms among which *Bacillus subtilis*. Natto is often consumed at breakfast. Natto is rich in proteins, vitamins, and minerals, especially vitamin K. Natto has a strong flavor and odor and a bit of a stringy texture. It is popular in Japan as a highly nutritious food.

Fermentation of mono-sodium glutamate

Monosodium glutamate is a flavour enhancer originally isolated from seaweeds in 1908. It is now produced industrially by fermenting hydrolyzed manioc starch, beet sugar, sugar cane or molasses with the addition of ammonium hydroxide. Fermentation is brought about by *Micrococcus glutanicum* or *Corynebacterium glutanicum*. It is currently a USD 7.2 billion industry. The basic reaction for producing monosodium glutamate is described by the equation below.

$$C_{12}H_{22}O_{11} + 3O_2 + 2NH_3 \rightarrow 2C_5H_9O_4N + 2CO_2 + 5H_2O$$

Monosodium glutamate is considered a natural product as many food items contain it in tracer amounts. The purified product has a white crystalline appearance. It is the most widely used food additive in the World.

Food colours of microbial origin

There is wide interest among consumers to move from synthetic food colours to natural food colours. Micro-organisms provide an opportunity that could be explored for food colours in the same way as flavour enhancers. Micro-organisms already produce industrially useful natural colorants such as carotenoids and anthocyanins. Micro-organisms also produce a variety of pigments that can be used as food colors including carotenoids, flavins, melanins, quinines, monascins, violacein, amongst others. The microbial colours may also serve as antioxidants, color intensifiers, and functional ingredients. Food colours already in use and those with a future potential to be used from micro-organisms are given below.

New industries may develop in time for production of food colours using microorganisms in the same way as monosodium glutamate production. However, the challenges for these industries are high, as use of the pigments

need regulatory clearance, and already established synthetic colours are produced at a much lower cost. Natural colours also tend to change with time in their intensities and shades in different foods unlike synthetic colours. However, modern science has developed technologies such as microencapsulation, nano emulsions and nano-formulations that could protect the microbial colours against effects from food matrices.

Mushroom and food industry

Edible mushrooms are fruiting bodies of molds consumed by human beings and cultivated at commercial level in Sri Lanka and other countries. They are low in calories, carbohydrates and fats, and are cholesterol-free. Mushrooms provide nutrients, selenium, potassium, riboflavin, niacin, vitamin D and proteins. The most frequently cultivated mushrooms commercially for edible processes include *Agaricus bisporus, Lentinus edodes, Pleurotus spp., and Flammulina velutipes*. Mushrooms could become a source of new antimicrobial compounds, produced as secondary metabolites, such as terpenes, steroids, anthraquinones, benzoic acid derivatives, and quinolones. They also could serve as sources for oxalic acid, peptides, and proteins. Mushrooms are an important source of the essential fatty acid linolenic acid. There is much interest in the production of powder formulations of mushrooms as important food ingredients. The Shiitake mushroom, *L. edodes* is used to isolate functional compounds as pharmaceuticals. With minimum space required and high protein content, mushrooms form another food of microbial origin that could be produced commercially. It is already happening in Sri Lanka.

Food fermentation and biotechnology

In biotechnology, natural sciences are integrated with engineering sciences to harness the potentials of micro-organisms, cells, parts thereof, and molecular analogues to generate new products and services. There is much that can happen with the application of biotechnology to harness and improve the potential of micro-organisms to produce food colours and other ingredients for foods. The potential to begin with micro-organisms, and then to move into large scale industries to produce foods is high, but require time and heavy scientific inputs.

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Prof. Upali Samarajeewa
Emeritus Professor in Food Science and Technology
University of Peradeniya

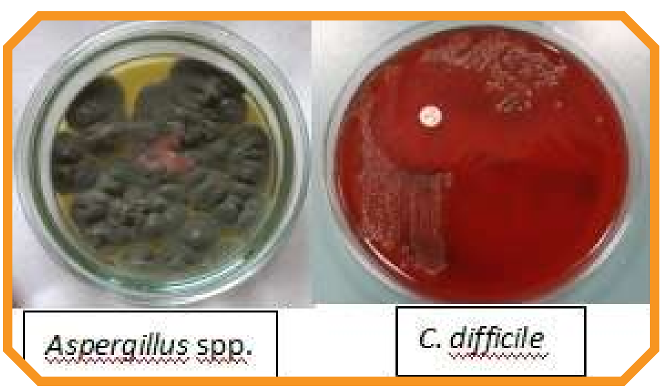
Microbes and Medicine

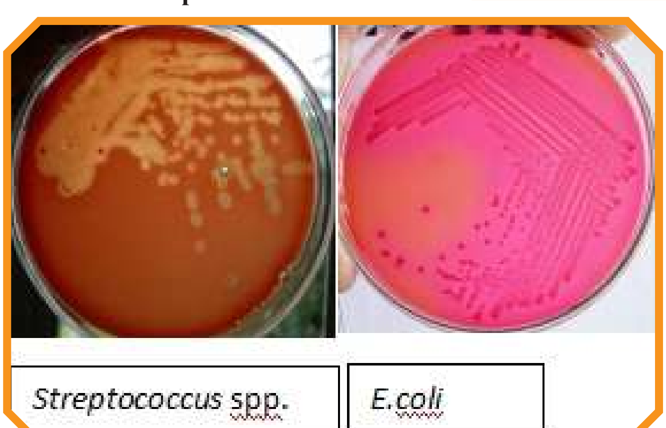
Dr S. S. Wickramasinghe

1. Introduction

Microorganisms which are ubiquitous, are essential to all other life forms. They are either prokaryotes or eukaryotes and exist as a single cell or as clusters. Prokaryotes consist of bacteria and archaebacteria. Eukaryotes which have a true nucleusarealgae, are protozoa and fungi.

Bacteria are simple unicellular organisms with no nuclear membrane, mitochondria, golgi bodies, or endoplasmic reticulum, and they can be seen with a light microscope (Figure 1). The bacterial cell wall is complex. Some bacteria lack this cell wall structure and compensate by surviving only inside host cells. The size (1 to 20 µm or larger), shape (spheres, rods, spirals), and arrangement (single cells, chains, clusters) of the cells are used for the preliminary classification of bacteria, and the phenotypic and genotypic properties of the bacteria form the basis for the definitive classification.



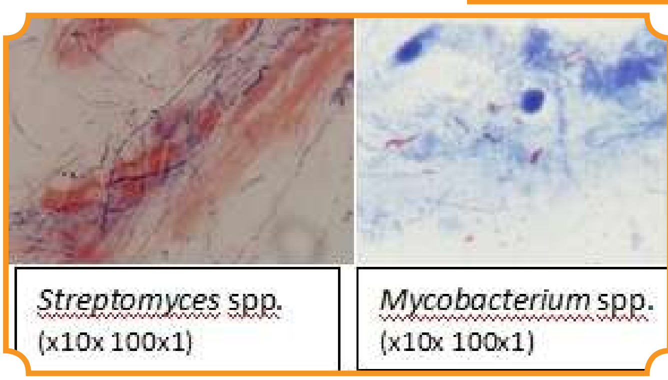


Microorganisms grown in culture plates

Viruses are the smallest infectious particles, ranging in diameter from 18 to 600 nanometers (most viruses are < 200 nm and cannot be seen with a light microscope). They typically contain either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) but not both (Figure 2). The viral nucleic acids required for replication are enclosed in a protein shell with or without a lipid membrane coat. They are essentially intracellular organisms, requiring host cells for replication. More than 2000 species of viruses have been described, with approximately 650 infecting humans and animals.

Fungi in contrast to bacteria are eukaryotic organisms that contain complex cellular structures, a well-defined nucleus, mitochondria, golgi bodies, and endoplasmic reticulum. They can exist either in a unicellular form (yeast) or in a filamentous form (mold). However, some fungi can assume either morphology at two different temperatures. These are known as dimorphic fungi and include organisms as *Histoplasma*, *Blastomyces,* and *Coccidioides*[1].

Although all parasites are classified as eukaryotic, some are unicellular and others are multicellular. They range in size from tiny protozoa as small as 4 to 5 μm in diameter to tapeworms that can measure up to 10 meters in length. Their life cycles are equally complex, with some parasites establishing a permanent relationship with humans and others going through a series of developmental stages in a progression of animal hosts[1].

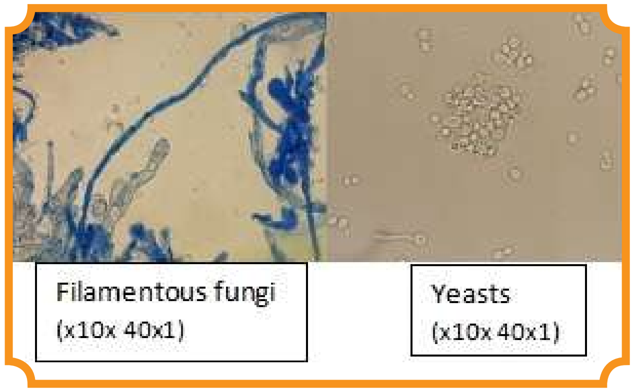


The surfaces of the skin, nose, mouth, gut, and genitourinary tract are covered with bacteria, as well as some fungi and parasites. Some are living transiently, others in a permanent parasitic relationship. These organisms are critical for the maturation of our immune system, important metabolic functions such as the digestion of food products, and protection from infection by unwanted pathogens. These organisms are referred to as our **normal flora** or **microbiome**.

1.1 Benefits of Microbes

Among the millions of microbes on the planet, disease-causing microbes or pathogens make up only a very tiny fraction whereas the majority of microbes, are no threat to human life as well as they are beneficial for us in many ways.

Microorganisms visualized under the microscope



1.2 Production of antimicrobials

Antimicrobials are chemicals that kill or inhibit the growth of microorganisms and are used to treat infections caused by them. They consist of antibiotics (which act against bacteria), antivirals (which act against viruses) and antifungals (which act against fungi).

Antibiotics are produced in nature by soil bacteria and fungi. They act on different structural components of a bacterium inhibiting formation of cell wall and membrane, or synthesis of bacterial protein and nucleic acid.

The microbial drug era began with the discovery of penicillin by Alexander Fleming in 1929. Penicillin was the first antibiotic discovered which was isolated from the mold *Penicillium notatum.* Within a few years of its discovery, penicillin was being used to treat common bacterial infections. Soon, other naturally occurring antibiotics such as streptomycin and chloramphenicol were isolated and are still being used to treat bacterial infections.

Those that are currently of the greatest use have been derived from a relatively small group of microorganisms belonging to the genera *Penicillium*, *Streptomyces*, *Cephalosporium*, *Micomonospora* and *Bacillus* spp. Compounds such as polymyxin, colistin, and circulin exhibit antibacterial activity whereas bacillomycin, mycobacillin, and fungistatin are effective agents against molds and yeasts.

More recently, microorganisms living in the sea and ocean have shown promising effects for being sources for antibiotics as well as anticancer agents.

1.3 Production of vaccines:

Vaccination or immunization is the deliberate induction of protective immunity to a pathogen thereby preventing the infection in the immunized person. These vaccines contain either killed, weakened (live-attenuated), or a part of a pathogen (Table 1). Immunization has eradicated dreadful diseases from the world (eg: smallpox) or eliminated from certain areas (eg: polio).

Not only prevention of infections, certain vaccines can be used for other purposes.
Eg: BCG (*Bacillus Calmette–Guérin*): This vaccine was designed to protect against tuberculosis,

Table 1. Some Vaccines Used in Sri Lanka

Vaccine	Active component in the vaccine	Disease
BCG	Live attenuated (weakened) bacilli of *Mycobacterium bovis*	Tuberculosis
DT	Neutralized toxins (toxoid) of diphtheria and tetanus	Diphtheria , tetanus
Hepatitis B (Recombinant vaccine)	Surface antigen of Hepatitis B virus	Hepatitis B
Inactivated polio (IPV) (Salk vaccine)	Killed polio virus	Polio
Oral polio (OPV)(Sabin vaccine)	Live attenuatedpolio virus	Polio
Hib (*Haemophilus influenzae* type b)	Capsular polysaccharide of *Haemophilus influenzae* type b	Infections due to H. *influenzae* type b
MMR	Live attenuated (weakened) viruses of mumps, measles and rubella	Mumps, measles and rubella

but is also effective in treating bladder cancer. It consists of a live-attenuated strain of *Mycobacterium* that stimulates antitumor immune responses leading to tumor regression and prevention of a relapse.

1.4 Production of hormones, vaccines and vitamins by genetic engineering

Genetic engineering is the manipulation of genes. It is also called as Recombinant DNA Technology. In genetic engineering, pieces of DNA (genes) are introduced into a host by a variety of techniques. The foreign DNA becomes a permanent feature of the host, and is replicated and passed on to daughter cells along with the rest of its DNA.This technique can be used to transfer of DNA into different species, synthesis of novel hormones and foreign proteins (recombinant proteins).

Escherichia coli which can be found in the gut, are used for commercial preparation of riboflavin and vitamin K. These are involved in nutritional supplementation used to treat deficiencies of different diseases. The human papillomavirus (HPV) vaccine is a structural mimic of the HPV capsid manufactured in genetically engineered Saccharomyces. This vaccine protects against several types of cancer caused by high-risk strains of HPV.

2.0 Exploring new possibilities on utilization of microbes

Even though finding of antimicrobials was a major revolution in the treatment of infectious diseases, a very few antibiotics have been introduced lately, and microbes have rapidly shown resistance to current antimicrobials. This has led to a growing interest in alternative treatments, including the potential use of bacteriophages in phage therapy, and prevention strategies, such as biocontrol of insect vectors to limit the spread of diseases such as dengue.

2.1 Use of bacteriophages to treat infections caused by drug resistant bacterial pathogens

Bacteriophages are viruses that only infect and kill certain bacteria (Figure 3). With the emergence of multi-drug resistant microorganisms, phages are being studied as an alternative means for treating infections caused by these bacterial pathogens. Phage therapy offers many advantages over antibiotics for treating bacterial infections. One is that these lytic phages usually kill the targeted bacteria whereas some antibiotics merely stop bacteria

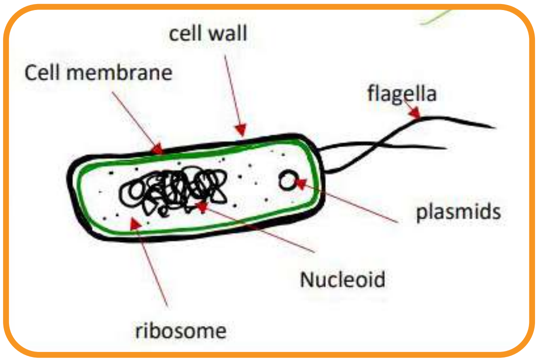


Figure 01: Structure of a bacterium

from replicating. Also, when the bactericidal antibiotics may take hours or days to kill the pathogen, lytic phages typically eliminate them within several minutes, bringing quick relief to patients. The disadvantage is that these

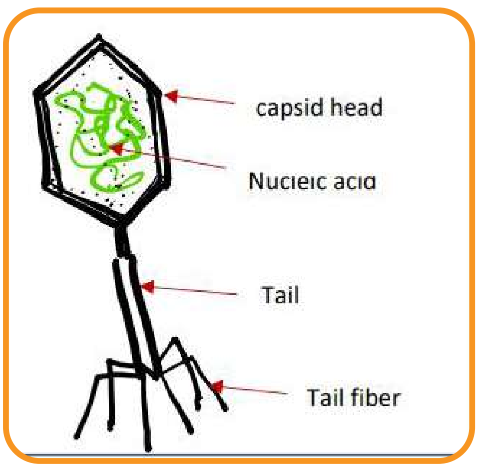


Figure 03: Structure of a bacteriophage

fast-acting phages can release toxin from bacteria leading to development of shock response in the patients.

2.2 Biocontrol of insect vectors in the prevention of disease transmission

Biocontrol of insect vectors is used to limit the spread of diseases. Dengue is a mosquito borne disease transmitted by *Aedesaegypti* mosquitoes.When these mosquitoes get infected with bacteria, Wolbachia, they are less susceptible to get infected with Dengue virus. This results in prevention of transmission of dengue among humans. Sri Lanka being an endemic country, the risk of Dengue exists throughout the island, and transmission occurs year-round. Recently, as a pilot project, in some parts of country Wolbachia injected *Aedes* mosquitoes were

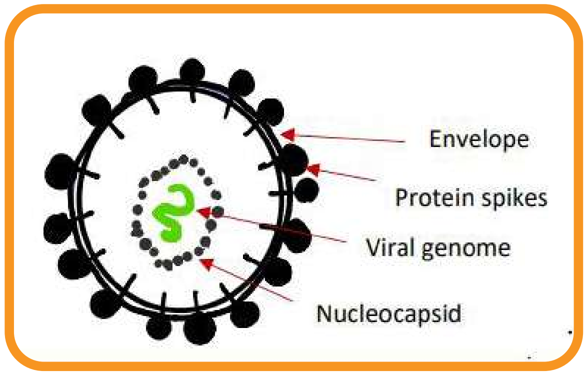


Figure 02: Structure of a virus

released into the environment to combat the spread of dengue.

3.0 Faecal transplantation in the management of resistant gut infections

Modified bacteria and carefully formulated microbial communities could form the basis of new living treatments, eg, faecal microbiota transplantation (FMT). Here, feaces or stools from healthy donors are used, and given as a treatment for people with a devastating, recurrent gut infection caused by the bacterium *Clostridium difficile*.It has been found that the gut infection results from a disturbance of the normal bacterial flora (microbiome) of the colon, which is followed by the colonization with C. *difficile* leading to an infection.

3.1 Gene therapy

A new experimental approach called **gene therapy** is currently under investigation in research laboratories and in clinical trials. The technique involves the delivery of nucleic acid (DNA or RNA) into a patient's cells to treat a disease or genetic disorder. Several viruses (eg adenoviruses, retroviruses), have been genetically engineered to act as the delivery vehicle, or vector, for the therapeutic nucleic acids. Diseases that could be treated in this way include certain types of cancer, viral infections (e.g. HIV/ AIDS) and inherited genetic disorders.

Dr S. S. Wickramasinghe
Senior Lecturer/ Consultant Microbiologist
Department of Microbiology
Faculty of Medicine
University of Ruhuna

Living Microbes: Ciliated Protists that Flourish in Rice Field Habitats Serving as Natural Parasites of *Culex tritaeniorhynchus* Mosquito Larvae

Prof. L. D. Amarasinghe and Dr H. A. K. Ranasinghe

When we consider the life cycle of a mosquito, there are four different stages; egg, larvae, pupae and adult. Immature stages of mosquitoes are aquatic and majority of their life stages are spent in water habitats. Therefore, the selection of a suitable oviposition site is critical for survivals of both immature stages and population dynamics of mosquitoes. Mosquito habitat ecology plays an important role, determining the larval densities, proliferation, species assemblage, and species succession in there. The quality of the larval habitat is an important determinant of mosquito abundance and temporal and spatial distribution. Therefore, studying the ecological and environmental factors associated with the mosquito life cycle is a vital necessity, since mosquito-borne diseases are one of significant public health concerns in Sri Lanka. Many mosquito species tend to select both natural and artificial containers as their breeding places.

Rice field habitats in Sri Lanka are one such natural mosquito breeding habitat type which have significantly influenced the distribution of mosquito populations, including vector mosquitoes, thereby facilitating disease transmission. The larvae of *Culex* and *Anopheles* species are mainly found in rice fields, nursery paddy beds, and stagnant water collected in places in rice fields in Sri Lanka.

Distribution, abundance, and individual fitness of mosquitoes in any particular breeding habitat are known to be dependent on biotic factors associated with those mosquito breeding habitats. Such biota include several species of macrobiota and microbiota including bacteria, fungi, and protists that can be categorized into autotrophs, heterotrophs, and detritivores. There are some microbiota species associated with mosquito breeding habitats serving as parasites, pathogens, predators, competitors, non-competitors, and food items for developing larvae in them. Therefore, there are naturally occurring microbiota species that serve as potential controlling agents against mosquito larvae, causing lethal effects on them. Therefore microbiota composition in a mosquito breeding habitat and their interactions with mosquito larvae influence immature survivorship. Moreover they influence the larval developmental rate which can ultimately alter the adult traits and population dynamics, and thereby, more importantly, the vector competence and disease transmission ability. Identification of such naturally occurring microbiota and their interactions on mosquito larvae, in terms of parasitic, pathogenic, competitive or predatory organisms against mosquito larvae as controlling agents would be beneficial for potential larval controlling approaches in an environmental friendly manner. On the other hand, this sort of approaches would provide beneficial solutions to the limitedly trained staff, as well as funding, through the rational use of finances and human resources in order to achieve better productivity and fruitfulness of mosquito controlling programs. Hence, this warrants vector control entities with environmentally-friendly country wide control approaches for medically important disease vectors.

When considering paddy field habitats, several species/taxa of unicellular organisms such as ciliate protists, bacteria, fungi, algae are known to cause negative or lethal effects on developing larvae. Meanwhile, comprehensive studies or checklists on associated microbiota, specifically targeting a variety of mosquito breeding habitats has been developed in Sri Lanka recently. Among those recorded ciliated protists species, three species; *Vorticella microstoma*, *Zoothamnium* sp, and *Chilodinella* sp. were identified as possible mosquito larval parasitic, pathogenic or epibiont species that inhabit rice field habitats(Figure 1). The important point is that all three species were found to be effective on *Culex* genera mosquitoes which were found most prominently and abundantly in paddy fields. Natural infection of immature stages of mosquitoes by epibiont parasitic ciliate, *V. microstoma* indicate that host species, and the degree of mortalities are different. Mosquito species that were found to foster the *V. microstoma* infection (Figure 2), had variations in the degree of parasitism. The mortality rate in *Cx. tritaeniorhynchus* was found to be higher in the Gampaha and Kurunegala Districts of Sri Lanka. *Culex quinquefasciatus* larvae were found to be associated with abandoned paddy fields, where 26.78% were found to be dead from the infected larvae with *V. microstoma*, indicating relatively low susceptibility of larvae to ciliate infection compared to other species like *Cx. tritaeniorhynchus*. The mosquito larvae infested with *V. microstoma* were observed under a microscope (40× magnification) and identified as the live sessile stalked trophont stage. Higher densities of this organism were attached to the head,

Figure 1 : Ciliated protists species associated with rice field habitats; a-*V. microstoma*, b- *Zoothamnium* sp., c- *Chilodinella* sp.

Figure 2 : Infection of the parasite (*V. microstoma*) to 3rd larval instars of *Cx. tritaeniorhynchus* anal papillae region (X 40 magnification), and attached trophonts of *V. microstoma* (X 100 magnification).

saddle, and abdominal regions of the body of dead mosquito larvae. *V. microstoma* usually did not get attach to the siphon region of live mosquito larvae; instead, they got attached to other regions of the body. However, *V. microstoma* were found to be attached to the siphon and head regions when the larvae died. *Culex tritaeniorhynchus* was the most preferred host for the trophont stage of *V. microstoma*, causing 100 % death of the mosquito larvae. However, the precise reason for this situation is still unknown. However, the biopolymer glue used for the surface attachment may damage the sensory systems, as well as pore formation of infected larvae, thereby interfering respiration.

V. microstoma was the majorly encountered from paddy fields. After the paddy is harvested, vector breeding habitats became limited with the restricted distribution of parasitic or pathogenic ciliates in host mosquito larvae. Thus the survival of the parasitic agent should face dry conditions until the next paddy transplantation season begins, coupled with monsoon rains, and await the return of the high vector density situation. Thus the encystation of these ciliates seems to be a possible way for the time-lap. After excystation, trophonts (free-swimming stage) of these ciliates could be increased easily when the optimum environmental conditions re-occurred. Desiccation resistance of *Chilodinella uncinata* have also been recorded.

Figure 3 : Infection of parasite (*Zoothamnium* sp.) to 3rd larval instars of *Cx. tritaeniorhynchus* abdominal region (X 100 magnification) and attached colonies (X 400)

Zoothamnium sp. has been also recorded as an epibiont in *Cx. tritaeniorhynchus* mosquito larvae (Figure 3) in the Gampaha, Kegalle, Kandy, and Kurunegala Districts of Sri Lanka. *Zoothamnium* sp. has one main stalk with many branches ending in zooids, which is a major difference from *Vorticella*, which has only a single stalk and zooid. Upon stimulation, *Zoothamnium* contracts the entire colony into one large globule and then folds the main stalk. Further, **Zoothamnium** spp has been reported as a ciliated protist associated with rice fields in Sri Lanka, which while acting as a parasitic species on *Cx. tritaeniorhynchus* mosquito larvae causes lethal effects. *Zoothamnium* sp. also serves as external parasites for many freshwater crustacean shrimps and some fish species. Thus the possibility of field application and risk assessment in the usage of these agents at higher densities should be further evaluated.

Chilodinella sp. was identified as another ciliate causing pathogenic effects (Figure 4) under natural environmental conditions on the *Cx. tritaeniorhynchus* mosquito larvae collected from a paddy field in the Gampaha District. Due to the infection of pathogenic ciliate, 4.58% mortality of mosquito larvae was observed. Due to infection, the body cavities of dead and transparent larvae were found packed with thousands of the motile endoparasitic stage of *Chilodinella* sp. They attack the mosquito larvae and invade the host hemocoel by dissolving the host cuticle, while forming cuticular cysts. After the death of the host larvae, the ciliate continues to reproduce for some more time, and fill up almost the entire body cavity of the susceptible host. At this stage, the infected larvae turn transparent, with thousands of motile microscopic endoparasitic stages become visible inside the host body cavity. Moreover, this ciliate was found to be highly virulent, desiccation resistant, and had a high reproductive potential with the capability to dispersing in the environment by transovarial

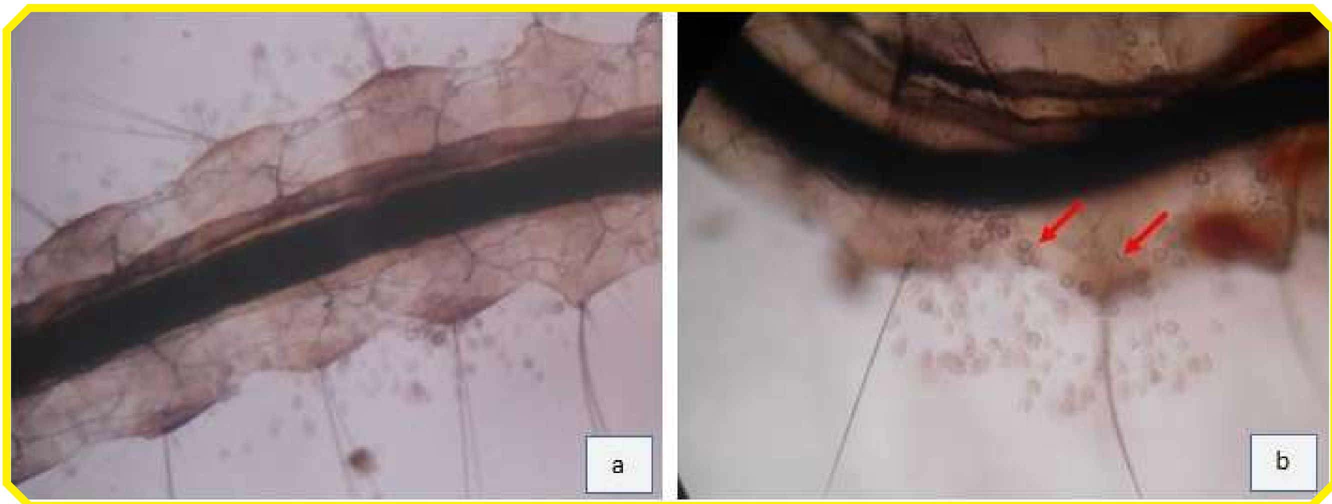


Figure 4 : Transparent *Cx. tritaeniorhynchus* larvae (magnification x40) showing endoparasitic ciliates (*Chilodina*) in the host body; showing cuticular invasive cysts of the pathogen (arrows, *Chilodinella*) on the cuticle of host body (magnification x100).

transmission through the mosquito host.

Therefore, it is evident that there is a potential of these naturally occurring microbiota for vector control interventions in an environmentally friendly manner, after proper evaluation of the scenarios that occur to non-target populations, through the application of these potential species. Nevertheless, a higher potential of such naturally occurring ciliated protists are there, specifically targeting container-type of mosquito breeding habitats.

Identification of naturally occurring ciliated protists in mosquito breeding habitats and their interactions on mosquito larvae, in terms of parasitic, pathogenic, competitive or predatory organisms against mosquito larvae as controlling agents would be therefore beneficial for potential larval controlling approaches in an environmental- friendly manner, while in the meantime, stepping towards a bio-economy. On the other hand, this sort of an approach would be a beneficial solution to limited trained staff, and funding through rational use of finances and human resources in order to achieve better productivity and fruitfulness of mosquito controlling programs in Sri Lanka.

Insecticide-based interventions are the most common methods of controlling mosquito populations in Sri Lanka from early times. As a result of the reliance on a few active ingredients (pesticides) registered and used in public health, resistance has now evolved in most of the regions to all four classes of insecticides. Apart from the higher costs involved, training of health staff for insecticide application, cost for protection equipments used during insecticide applications, impacts on other non-target organisms, bioaccumulation of chemicals along food chains in non-environmentally friendly situations, have been challenges in the use of insecticides for controlling mosquito populations. Consequently, nowadays scientists are targeting the use of other alterative measures, particularly usage of biological components for vector control interventions.

Prof. DeepikaAmarasinghe
Professor of Zoology
Department of Zoology and
Environmental Management
University of Kelaniya

Dr H.A. Koshila Ranasinghe
Lecturer
Department of Biomedical Sciences
Faculty of Health Science
CINEC Campus
Malabe

What have you learnt from the Vidurava 2021 October - December Q_4 Issue? Scan your own memory!

1] Living Microbes: An Underutilized National Wealth

True or False?

1.The different win, loss and neutral associations occurring between interacting partners provide a foundation for diverse forms of ineractive patterns.

2.These microbial communities are very important to life on earth as they play an enormous practical role in ecology, medicine, engineering and agriculture.

3.Through the whole agro-chain, application of microorganisms is used for downgrading food safety and security.

4.In fermentation processes, microorganisms are used to prepare single cell protein, silages and microbial pesticides.

5.Lactases obtained from *Aspergillus niger*, *Aspergillus oryzae*, and *Kluyveromyces lactis*, are considered unsafe for a wide range of applictions.

2] Worlds Within World : Hidden Role of Microbiomes in Our World

True or False?

1.Microbes are found almost everywhere in the world, starting with air, and every inch of land.

2.Soil microbes play a key role in the dis-functioning of almost all terestial ecosystem.

3.The microbes of a person, animal or plant is coloniged by microoganisms coming from the outside environment.

4.Some members of microbes have no special features that make them special member in a special microbiome.

5.Interestingly, there are also microbe - microbe interactions within a microbiome, which shape the composition of the microbiome.

3] Microoganisms in Extreme Environments

True or False?

1.Microbes present in extreme environments are collectively referred to as extremophiles.

2.Thermophiles thrive in low temperature environments such as hydrothermal and volcanic vents and terrestrial hot springs.

3.Lichens survive in most of the hospitable environments on earth, as they possess stress tolerant features such as high growth rates, low demand for nutrients, and morphological and physiological adaptations.

4.The most heat tolerant animal known to science is the Pompeii worm living close to the hydrothermal vents in the ocean floor.

5.The greatest success of thermophiles is due to the presence of heat stable enzymes and proteins which remain catalytically active under extremes of temperature, salinity, pH, and solvent conditions..

4] Applications of Microbes for the Food Industry

True or False?

1.The reactions generated by the microbial enzymes are utilized in the bakery, brewing industries, vegetable, fish and meat industries to produce new sensory properties.

2.The ethanol being involatile, fails to get released from bread during baking.

3.The use of a few milliliters of fermenting toddy as a yeast culture in preparing the dough for hoppers is well known.

4.Coconut sap contains sufficient sucrose to produce about 10 percent of ethanol on fermentation.

5.Sodium metabisulphite activates the wild yeasts, providing a competitive advantage for the *S. cerevisiae* strains.

5] Microbes and Medicine

True or False?

1.Viruses are the smallest infectious particles, ranging in diameter from 18 to 600 nanometers.

2.Fungi in contrast to bacteria are eukaryotic organisms that contain simple cellular structures, a ill-defined nucleus, mitochondria, Golgi bodies, and endoplasmic reticulum.

3.Among the millions of microbes on the planet, disease-causing microbes or pathogens make up a major fraction whereas the majority of microbes, are a minor threat to human life.

4.The microbial drug era began with the discovery of penicillin by Alexander Fleming in 1929.

5.Vaccination or immunization is the deliberate induction of protective immunity to a pathogen thereby preventing the infection in the immunized person.

6] Living Microbes: Ciliated Protists that Flourish in Rice Field Habitats Serving as Natural Parasites of *Culex tritaeniorhynchus* Mosquito Larvae

True or False?

1. The quality of the larval habitat is an important determinant of mosquito abundance and temporal and spatial distribution.

2. When considering paddy field habitats, several species/taxa of unicellular organisms such as ciliate protists, bacteria, fungi, algae are not known to cause negative or lethal effects on developing larvae.

3. Natural infection of immature stages of mosquitoes by epibiont parasitic ciliate, *V. microstoma* indicate that host species, and the degree of mortalities are different.

4. After the death of the host larvae, the ciliate continues to reproduce for some more time, and fill up almost the entire body cavity of the susceptible host.

5. Insecticide-based interventions are the most uncommon methods of controlling mosquito populations in Sri Lanka from early times.

Answers

01)1. True,	2. True,	3. False,	4. True,	5. False
02)1. True,	2. False,	3. False,	4. True,	5. True
03)1. True,	2. False,	3. False,	4. True,	5. True
04)1. True,	2. False,	3. True,	4. True,	5. False
05)1. True,	2. False,	3. False,	4. True,	5. True
06)1. True,	2. False,	3. True,	4. True,	5. False

National Science Foundation
47/5, Maitland Place
Colombo 07