

# JOURNAL OF HYGIENE SCIENCES

Committed to the advancement of Clinical & Industrial Disinfection & Microbiology

VOLUME - III

ISSUE - I

JAN-FEB 2010

## Contents

■ Editorial	1
■ Mini review	2
■ Encyclopedia	6
■ Current Trends	7
■ In Profile	9
■ Relax Mood	10
■ Bug of the Month	11
■ Did you Know	13
■ Best Practices	14
■ In Focus	16

## Editorial

Well to Jump start with this issue we have the section on 'Mini Review' giving us a description of 'Soil Microbiology' and its various aspects that make soil an important entity consisting of diverse features; a dynamic, constantly changing phenomenon. Microbes residing in the soil greatly influence the cycling of nutrients in the environment. Biodegradation being an important essential of the topic is mentioned about in the Encyclopedia.

Biovalidation of commonly used lab instruments such as the Autoclave and Oven is a very important practice since these instruments when used in the pharmaceutical sector play a key role in the process, which is a major necessity in product quality 'Current Trends' takes a look at this important approach.

Ferdinand Julius Cohn is 'In Profile' for his important contributions to the field of Microbiology. Though a naturalist and a botanist, he is considered as one of the founders of bacteriology. His contributions include systemic classification of bacteria, the discovery of bacterial spore amongst others, which crowned Cohn a founder member of 'Modern Microbiology'.

'Bug of the Month' have the *Lactobacillus* species which comprises of Gram positive anaerobic or microaerophilic bacteria. They are common, usually benign, are present in the vagina and the gastrointestinal tract, where they are symbiotic. *Lactobacillus* species are also prominent plant decomposers.

'Did You Know' mentions Endoclens which is a specially designed system which is used to provide rapid, automated, point – of – use chemical sterilization of flexible endoscope reprocessing machine and a new proprietary liquid sterilant that uses performic acid.

'Relax Your Mood' as usual helps you to unwind, laugh and think about what could fill the void, in track your brains.

Best Practices sheds light on the importance of a proper diet and healthy food intakes. To maintain a good health without those rigorous diet regimes is not a task we need to worry about, we just ought to have the correct foods that are enriching and energizing, which simultaneously give us the vigor and vitality for our daily activities and of course for the year ahead!

As we bid adieu to the year that was, we thank our esteemed customers, well wishers and readers for their continual support and look forward to your cooperation in the year ahead. Marcus Aurelius was once quoted as saying, 'Do every act of your life as if it were your last' well then if we go by this quote; we ought to give all our tasks our very best shot and Give *Lives* the special touch that makes every moment worth living and a fulfilling experience that we can fondly cherish! Wish you all a Happy New Year 2010...

# Soil Microbiology: Important Aspects

Soil Microbiology greatly influences the cycling of nutrients in the environment and this in turn affects the outcome of nature and the atmosphere. Therefore the lithosphere, hydrosphere and the atmosphere are integrally related and dependent on the functions of each other.

## **The study of biota that inhabit the soil and the processes that they mediate**

The soil is a complex environment colonized by an immense diversity of microorganisms. Soil microbiology focuses on the soil viruses, bacteria, actinomycetes, fungi, and protozoa, but it has traditionally also included investigations of the soil animals such as the nematodes, mites, and other microarthropods. These organisms, collectively referred to as the soil biota, function in a below ground ecosystem based on plant roots and litter as food sources. Modern soil microbiology represents an integration of microbiology with the concepts of soil science, chemistry, and ecology to understand the functions of microorganisms in the soil environment.

The surface layers of soil contain the highest numbers and variety of microorganisms, because these layers receive the largest amounts of potential food sources from plants and animals. The soil biota form a below ground system based on the energy and nutrients that they receive from the decomposition of plant and animal tissues. The primary decomposers are the bacteria and fungi.

## **Soil Microbiology Basis**

Soil factors such as organic matter content, soil structure, nutrient content, nutrient cycling, nutrient availability, and water holding capacity are all influenced by, or dependent upon, soil microorganisms. These microbes can be classified into major groups such as microarthropods, nematodes, protozoa, fungi, algae and bacteria, and all of these groups can be further subdivided based on genetic relationships, specific functions or habitats in which they survive. Important soil microbial functions such as organic matter breakdown and nitrogen fixation are fairly well understood, but this is only a small fraction of microbial capabilities.

Soil testing using genetic analysis methods has estimated that a single teaspoon of soil may contain as many as 10,000 distinct species of bacteria. Of these only about 1% or less can be cultured, and even fewer of these are known to have specific soil functions.

A fascinating and relatively new area of soil microbiology is the study of how these microscopic life forms communicate and interact. Imagine any major city in the world being reduced to something that would rest in the middle of a teaspoon. All the different activities of the people in that city would be analogous to what the community of bacteria and other microorganisms perform in a teaspoon of soil. Thus interactions of

microorganisms in the soil is equal to, if not more complex, than the population of a large city.

On a microscopic scale, the highways and roads in the soil are thin films of water on and between soil particles. These water films not only enable the microbes to get to their food source, but they can also carry food to the organisms. The nutrient sources for soil organisms are as broad and diverse as the organism community itself. Soil microorganisms proliferate when their "food of choice" is available and conditions are just right. They must also develop survival mechanisms (or perish) when food is scarce. Many soil microbes are a food for other organisms and their populations are kept in check by these predators.

Soil microbes are able to survive by communication with their environment on a bio-chemical basis. For bacteria, nutrients are absorbed through their cell walls. Some of these nutrients are readily available in water films; other nutrients must be obtained by the excretion of enzymes or other compounds in order to release the needed nutrients from organic or mineral particles. Nutrient availability signals the bacteria to multiply; but they are also receptive to biochemical signals indicating that the food reserves are limited or that conditions are developing which indicate the need for protective survival strategies. Through such mechanisms of communication and adaptation, the community or organisms work effectively and collectively to cycle nutrients within the soil.

## **Early colonizers and nutrient cycling**

Microorganisms, especially algae and lichen, are pioneering colonizers of barren rock surfaces. Colonization by these organisms begins the process of soil formation necessary for the growth of higher plants. After plants have been established, decomposition by microorganisms recycles the energy, carbon, and nutrients in dead plant and animal tissues into forms usable by plants. Therefore, microorganisms have a key role in the processing of materials that maintain life on the Earth. The transformations of elements between forms are described conceptually as the elemental cycles.

In the carbon cycle, microorganisms transform plant and animal residues into carbon dioxide and to the soil organic matter known as humus. Humus improves the water-holding capacity of soil, supplies plant nutrients, and contributes to soil aggregation. Microorganisms may also directly affect soil aggregation. The extent of soil aggregation determines the workability or tilth of the soil. A soil with good tilth is suitable for plant growth because it is permeable to water, air, and roots.

Soil microorganisms play key roles in the nitrogen cycle. The atmosphere is approximately 80% nitrogen gas (N<sub>2</sub>), a form of nitrogen that is available to plants only when it is transformed to ammonia (NH<sub>3</sub>) by either soil bacteria (N<sub>2</sub> fixation) or by humans (manufacture of fertilizers). Soil bacteria also mediate

denitrification, which returns nitrogen to the atmosphere by transforming  $\text{NO}^{-3}$  to  $\text{N}_2$  or nitrous oxide ( $\text{N}_2\text{O}$ ) gas. Microorganisms are crucial to the cycling of sulfur, phosphorus, iron, and many micronutrient trace elements.

In addition to the elemental cycles, there are several interactions between plants and microbes which are detrimental or beneficial to plant growth. Some soil microorganisms are pathogenic to plants and cause plant diseases such as root rots and wilts. Many plants form symbiotic relationships with fungi called mycorrhizae (literally fungus-root). Mycorrhizae increase the ability of plants to take up nutrients and water. The region of soil surrounding plant roots, the rhizosphere, may contain beneficial microorganisms which protect the plant root from pathogens or supply stimulating growth factors. The interactions between plant roots and soil microorganisms is an area of active research in soil microbiology.

The incredible diversity of soil microorganisms is a vast reserve of potentially useful organisms. Many of the medically important antibiotics are produced by filamentous bacteria known as actinomycetes. The soil is the largest reservoir of these medically important microorganisms.

The numerous natural substances that are used by microorganisms indicate that soil microorganisms have diverse mechanisms for degrading a variety of compounds. Human activity has polluted the environment with a wide variety of synthetic or processed compounds. Many of these hazardous or toxic substances can be degraded by soil microorganisms. This is the basis for the treatment of contaminated soils by bioremediation, the use of microorganisms or microbial processes to detoxify and degrade environmental contaminants. Soil microbiologists study the microorganisms, the metabolic pathways, and the controlling environmental conditions that can be used to eliminate pollutants from the soil environment.

Microbiologists traditionally isolate pure strains of microorganisms by using culture methods. Methods that do not rely on culturing microorganisms include microscopic observation and biochemical or genetic analysis of specific cell constituents. The rates or controlling factors for microbial processes are studied by using methods from chemistry, biology, and ecology. Typically, these studies involve measuring the rate of production and consumption of a compound of interest. The results of these studies are commonly analyzed by using mathematical models. Models allow the information from one system to be generalized for different environmental conditions.

### **The microbiology of soil and of nutrient cycling**

Soil is a dynamic habitat for an enormous variety of life-forms. It gives a mechanical support to plants from which they extract nutrients. It shelters many animal types, from invertebrates such as worms and insects up to mammals like rabbits, moles, foxes and badgers. It also provides habitats colonized by a staggering variety of microorganisms. All these forms of life interact with one another and with the soil to create continually changing conditions. This allows an on-going evolution of soil habitats. The activity of living organisms in soil helps to control its quality,

depth, structure and properties. The climate, slope, locale and bedrock also contribute to the nature of soil in different locations. The interactions between these multiple factors are responsible for the variation of soil types. Consequently, the same fundamental soil structure in different locations may be found to support very different biological communities. These complex communities contribute significantly to the continuous cycling of nutrients across the globe.

The digging or burrowing activities of animals contribute to the mechanical breakdown of soil. Microbial activity by thermoacidophilic bacteria, such as those found in coal slag heaps, results in an extremely acid environment. Leaching of acid from slag heaps may cause chemical changes in bedrock.

Plants are the major producers of organic matter to be found in soil, and this plant matter accumulates as litter. Animal feces and decomposing bodies of dead animals complement this organic supply. Artificially added fertilizers, herbicides and pesticides all affect the biological component and hence the organic content of soils. Horse dung and chicken manure which are very commonly used by gardeners aid in the recycling of such materials which is carried out by microbes, soil microbes are also responsible for the chemical degradation of pesticides. However there are some chemicals that cannot be decomposed by microbes, as a result of which these accumulate in the environment, such compounds are referred to as 'recalcitrant pesticides'.

During the evolution of a soil habitat, its organic content may eventually become predominant. The ultimate organic soil is found in a bog. Bogs are waterlogged and consequently form an anaerobic environment. Any dissolved oxygen is quickly used up by facultative organisms. This provides a very inhospitable environment for fungi and aerobic bacteria. Since these organisms tend to be responsible for the decomposition of organic structures, bogs provide excellent sites for the preservation of organic matter.

Both bacteria and fungi provide an abundant source of food for soil protozoa. The most commonly encountered soil protozoa include flagellates and amoebas. The abundance of such creatures depends upon the quantity and type of organic matter present in the soil sample. Protozoa play a key role in the regulation and maintenance of the equilibrium of soil microbes. Whereas many microbes obtain their nutrients from solution, protozoa are frequently found to be of a scavenging nature, obtaining their nutrients by devouring other microbes.

The distribution of microbes throughout the soil is not even. Microorganisms tend to cluster around the roots of higher plants. This phenomenon is referred to as the rhizosphere effect (rhiza: Greek for root; hence the rhizosphere is the region surrounding the roots of a plant). The majority of microorganisms found in the rhizosphere are bacteria, but fungi and protozoa also congregate in this region. Microorganisms are thought to gain nutrients from plants, and auxotrophic mutants requiring various amino acids have been isolated from the rhizosphere. Plants may also derive benefit from this arrangement. Bacteria may fix nitrogen in a form that can be taken up and used by plants. In certain circumstances, the association between microorganisms and

higher plants can become very intimate. Mycorrhizas are formed when roots become intimately associated with fungi. Root nodules provide another important example of the close association between leguminous plants and nitrogen-fixing bacteria. In this instance bacteria rather than fungi are involved in the association with plants.

### Types of soil microbes

Soil micro-organisms come in many shapes, but are by definition small in size — microscopic, that is. There are bacteria, algae, viruses, actinomycetes, and protozoa. Fungi are generally multicelled organisms; many are microscopic but they may have structures visible to the eye (e.g. mushrooms). Other very small organisms such as nematodes and microarthropods inhabit the soil but are usually not considered “microbes.”

### Classification of soil microbes

#### 1. Mode of nutrition

Microbes can be classified in various ways. Those that do not need to “eat” like we do are called autotrophs. They can derive their energy from the sun (through photosynthesis) or from chemical reactions (as with the nitrifying bacteria). All others are called heterotrophs and must get their energy from consuming the fixed carbon in living or dead organisms.

#### 2. Oxygen requirement

Another classification has to do with the oxygen requirement. Aerobes require oxygen for their metabolic processes, whereas anaerobes do not. Some anaerobes are obligate in that they cannot survive with oxygen. Others are facultative, and can function in both high and low oxygen situations.

This illustrates the fact that there are exceptions to most rules and to classifications of microbes. Soil microbial communities generally consist of all these groups. For example, most agricultural soils contain both aerobic and anaerobic locations within the pore structure of the soil particles; thus, other than with a rice paddy, it can be misleading to call a soil aerobic or anaerobic.

### Factors affecting soil microbes

All the factors that affect soil formation also affect the microbes. These are climate, parent material and texture (especially clay), vegetation, topography, and time. Both long-term and short term factors have shaped the soils differently, particularly the influence of moisture on vegetation growth and the resulting soil organic matter levels. Other factors include the chemical status of the soil (pH, salt levels, redox potential) and physical conditions (water potential, temperature, soil structure). Soil organisms, especially microbes, influence the same factors that influence them. Management actions dramatically affect soil microbes. These include the choice of crop rotation, and tillage, and the use of irrigation, fertilization, or fumigation. Research is increasingly pointing to certain practices that almost always enhance the microbial status, such as the use of composts and cover crops. Of

all factors, soil organic matter can be considered the primary determinant of microbial status. It is the “fuel” for the soil microbes. The organic matter is often described as consisting of three components, each differing in composition and function. The Biologically Active fraction is the smallest and turns over every 1-5 years. It is the most important for microbes, nutrient cycling, and disease suppression. The Protected fraction is intermediate in size, turns over every 5-30 years, and plays a key role in soil structure and water retention. The Stable fraction is the largest, turning over every 50-10,000 years, and contributes to the cation exchange capacity, other chemical properties, color, and microaggregation. A number of researchers are looking for analytical tests beyond simple soil organic matter to better understand the more active fraction and the role it plays in support of soil biology. Particulate organic matter (POM) is one of these. Microbial biomass carbon generally makes up 1-5% of the soil carbon. For instance consider that soil has 3.5% organic matter as reported from a lab test. Because soil organic matter contains carbon, nitrogen, oxygen, phosphorus, etc., we need to divide the organic matter by 1.75 to determine the % soil organic carbon (organic matter is about 57% C). So this soil has 2% soil carbon. Then multiply that by the approximate weight of the top 6-7 inches of soil, called the 'acre-furrow slice'. This is 2 million lb. So 2 % soil carbon x 2 million lb = 40,000 lb of organic carbon per acre. It is referred to as organic, since some soils may contain significant amounts of inorganic carbon in carbonate compounds that are not part of the soil organic matter equation. Then, if 3% of the organic carbon is in the microbes, that equals about 1,200 lb of microbial carbon per acre, similar to the weight of a cow gazing in a one-acre field.

### Measuring soil microbes

There are large numbers of microbes in a small amount of soil. Bacteria and fungi dominate both in terms of number and biomass (total weight of organisms) in a cubic inch of soil. Microbial numbers can be extremely variable, making measurements difficult. Most researchers feel that there needs to be at least a 10-fold difference in microbial number to be meaningful because of variability in measuring organisms. Sometimes we see “desirable” levels of microbes proposed. For example, a productive agricultural soil might be expected to contain 10100 million bacteria per gram of soil. So if you compared two soils, one with 15 million bacteria and the other with 90 million, they have a similar number of bacteria and would perform the same if all else was equal.

There are several common techniques for measuring the number or biomass of soil microbes. The most basic is the direct count, where microbes are extracted from soil in a liquid, sometimes dyed to illustrate particular groups, and then counted on a slide under the microscope. This method tends to overestimate since dead organisms cannot always be distinguished from live ones. Also, sampling error can be magnified by the small sample size and the potential for uneven microbial distribution in the extract. Another method for determining microbial numbers is the plate count. Again, an extract is made from soil and a small amount is placed in a Petri dish and incubated. Various growth media can be used in the dish to select for or against certain organisms. The

growth of the microbes into visual colonies is then measured. Plate counts tend to underestimate microbes because only 1-10% of soil organism types can grow on artificial medium, and thus many go undetected.

Microbial biomass methods were developed to try to address the problems with these first two methods. The Fumigation Method (which has been adapted for use with a microwave) kills about 99% of the microbes. But there are always the few that remain and then proliferate by feasting on the dead bodies of their former neighbors. The carbon dioxide that is released by this feeding frenzy correlates well with the original size of the microbial biomass, particularly the bacteria.

Another method, the Respiratory Response Method, relies on the fact that soil microbes are generally starved for carbon-based energy. So they are fed a big shot of glucose, which leads to a huge increase in respiration in a short period of time, which can be correlated to the original biomass size.

Soil respiration and enzyme tests are common, but probably yield less useful information than other methods as they can experience large fluctuations in short time periods and can be influenced by many transient management effects. Newer tests have focused on the microbial community, both its structure and function. These include fatty acid analysis, substrate utilization, and DNA analysis. Often it is useful to know what important functions these microbes are performing in the soil, so tests such as nitrogen mineralization may be used to look at specific processes of importance to agriculture.

Microbial measurements can vary tremendously with space, time, weather, and management activities.

### Biological Soil Crusts

Biological soil crusts are the community of organisms living at the surface of desert soils. Major components of which are cyanobacteria, green algae, microfungi, mosses, liverworts and lichens.

### Agricultural Practices

Cultivation can reduce soil organic matter and organic nitrogen concentrations 25% to 60%, with corresponding decreases in nitrification rates and quantity of organic N nitrified. The type of cropping system also influences the rate of organic carbon and nitrogen loss.

Cultivation also changes the soil environment affecting the number and kinds of soil organisms. Clearing forested or grassland areas for cultivation drastically alters the soil environment. First, the quantity and quality of plant residues (food for soil organisms) is significantly reduced.

Second, the number of species of higher plants is reduced. Monoculture or even common crop rotations provide a much narrower range of plant materials than forests and grasslands.

Generally, agricultural practices such as monoculture and pesticide application also contribute to a general reduction of

species diversity and total organism population. However, some agricultural practices positively affect soil microbiological activity. For example, the application of organic fertilizers such as manure or compost increases microbe activity.

### Soil Aeration

Soil aeration is an important factor in the degree of soil fertility. It is the trapped air that is present in the soil that brings about decomposition of material in the subsurface regions of soil. Though aeration may be sometimes taken for granted it is the most vital factor in the type of microbial growth that is obtained. The lack of air in the subsurface regions of the soil will result in anaerobic conditions which in turn deplete the microaerophilic bacterial population which also comprise of quick decomposers.

### Soil Porosity

Soil Porosity depends from one soil type to another, and differs also from region to region since there are different factors that affect soil porosity like tillage, moisture, bacterial activity, texture etc. Soil porosity in turn affects also the fertility, microbial population and other aspects of the crops and soil in general.

A common practice among farmers is that of ploughing which is practically to increase the porosity of soil and also the aeration.

In cases where the pore size of the soil particles is too large the soil is unable to hold the water and allows the water to drain out thereby leaving the soil apparently dry for instance, sand, which is better for xerophytic plants. On the contrary clayey soils retain a lot of water, are rich in microbial population and black soils are ideal for cotton plantations.

### Size of soil particles

Particle size is directly proportional to the aeration and inversely proportional to the moisture retained. Therefore the bigger the particle size the more the air penetration and the bigger the particle size the lesser is the moisture retained by the soil. For instance the particle size of sand is larger as compared to the particle size of clay and thus water retained by clay is much more than water retained by sand.

### Soil Productivity

The productivity and stability of soil as a medium for plant growth depends greatly on the balance between living and nonliving components. Energy from the sun and nutrients essential for growth are stored in crop plants and recycled through decomposition by micro- and macroorganisms in soil. The soil organic matter formed during this process serves as a continuous nutrient supply and a factor stabilizing the soil's physical environment. To maintain productivity, soluble nutrients removed from soil by plant growth must be replaced. In natural systems, the action of soil microbes and fauna are major determinants of efficient nutrient cycling and plant growth. Therefore, biological decomposition of plant residue is the largest source of nutrients.

Microbial biogeochemical cycling activities and interactions

between microbial populations have a direct and major influence on agriculture. A controlled balance of microbial activities is important for success in agriculture. Such activities in soil become very important. Soil is the natural habitat for all organisms and this is a center of important ecological processes. Addition of fertilizers and pesticides to soil makes the study of its microbiology even more sophisticated.

Soil has to be preserved and maintained such that the fertility of soil does not drop below the required threshold, and soil is not polluted with biohazardous chemicals which will accumulate and

concentrate in soil and alter the flora and fauna, thus should not affect the overall fertility of the soil.

### To Conclude

It is best that we use manure, fertilizers and pesticides that are more eco-friendly. We should choose methods that are not harmful to the soil microbes and ensure that we don't just rip the soil of its nutrients but at the same time replenish the soil such that we can maintain it for the future.

## Encyclopedia

**Biodegradation** is nature's way of recycling wastes, or breaking down organic matter into nutrients that can be used by other organisms. "Degradation" means decay, and the "bio-" prefix means that the decay is carried out by a huge assortment of bacteria, fungi, insects, worms, and other organisms that eat dead material and recycle it into new forms.

Biodegradation is the chemical breakdown of materials by a physiological environment. The term is often used in relation to ecology, waste management and environmental remediation (bioremediation). Organic material can be degraded aerobically with oxygen or anaerobically, without oxygen. A term related to biodegradation is biomineralization, in which organic matter is converted into minerals. Biosurfactant, an extracellular surfactant secreted by microorganisms, enhances the biodegradation process.

Biodegradable matter is generally organic material such as plant and animal matter and other substances originating from living organisms, or artificial materials that are similar enough to plant and animal matter to be put to use by microorganisms. Some microorganisms have the astonishing, naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclides and metals. Major methodological breakthroughs in microbial degradation have enabled detailed genomic, metagenomic, proteomic, bioinformatic and other high-throughput analyses of environmentally relevant microorganisms providing unprecedented insights into key biodegradative pathways and the ability of microorganisms to adapt to changing environmental conditions.

### Methods of Measuring Biodegradation

Biodegradation can be measured in a number of ways. The activity of aerobic microbes can be measured by the amount of oxygen they consume or the amount of carbon dioxide they produce. Biodegradation can be measured by anaerobic microbes and the amount of methane or alloy that they may be able to produce.

### Microbial Degradation

Interest in the microbial biodegradation of pollutants has intensified in recent years as humanity strives to find sustainable ways to clean up contaminated environments.

The elimination of a wide range of pollutants and wastes from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. Biological processes play a major role in the removal of

contaminants and they take advantage of the astonishing catabolic versatility of microorganisms to degrade/convert such compounds. In the field of Environmental Microbiology, genome-based global studies open a new era, providing unprecedented silico views of metabolic and regulatory networks, as well as clues to the evolution of degradation pathways and to the molecular adaptation strategies to changing environmental conditions. Functional genomic and metagenomic approaches are increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds and they will certainly accelerate the development of bioremediation technologies and biotransformation processes.

### Oil Biodegradation

Petroleum oil contains aromatic compounds that are toxic for most life forms. Episodic and chronic pollution of the environment by oil causes major ecological perturbations. Marine environments are especially vulnerable since oil spills of coastal regions and the open sea are poorly containable and mitigation is difficult. In addition to pollution through human activities, millions of tons of petroleum enter the marine environment every year from natural seepages. Despite its toxicity, a considerable fraction of petroleum oil entering marine systems is eliminated by the hydrocarbon-degrading activities of microbial communities, in particular by a remarkable recently discovered group of specialists, the so-called hydrocarbonoclastic bacteria (HCB). *Alcanivorax borkumensis* was the first HCB to have its genome sequenced.

In nature, there is no waste because everything gets recycled. The waste products from one organism become the food for others, providing nutrients and energy while breaking down the waste organic matter. Some organic materials will break down much faster than others, but all will eventually decay.

By harnessing these natural forces of biodegradation, people can reduce wastes and clean up some types of environmental contaminants. Through composting, we accelerate natural biodegradation and convert organic wastes to a valuable resource. Wastewater treatment also accelerates natural forces of biodegradation. In this case the purpose is to break down organic matter so that it will not cause pollution problems when the water is released into the environment. Through bioremediation, microorganisms are used to clean up oil spills and other types of organic pollution. Composting and bioremediation provide many possibilities for research studies. Biodegradation is important to maintain soil fertility and hence the lithosphere.

# Biovalidation of commonly used lab instruments

Biovalidation of commonly used lab instruments is very important, since it is this validation that can finally result in the sterilization process quality and hence the quality of the material that is sterilized.

## Biological Indicators

Biological indicators are characterized and standardized preparations of specific micro-organisms having known stable high resistance to one or more sterilization procedures. A biological indicator is used to (a) assist in the qualification of the physical operation of sterilizer, (b) develop and establish a validated sterilization process for a particular article and for the sterilization of equipment, materials and packaging components for aseptic processing, (c) monitor an established sterilization cycle and (d) revalidate established and documented sterilization cycles.

A biological indicator is in one of two main forms, each of which incorporates a viable culture of a known species of micro-organisms. In one, the organisms (spores) are added to a carrier (disc or strip of filter paper, glass or plastic) and packed so as to maintain the integrity of the inoculated carrier but when used appropriately in the individual immediate package allows the sterilizing agent to exert its effect. In the other, the spores are added to representative units of the lot to be sterilized (inoculate product) or to similar units (inoculated similar product). An inoculated product should not adversely affect the performance characteristics of the viable spores. If the material to be sterilized is a liquid and if it is not practicable to add a biological indicator to selected units of the lot, viable spores may be added to a simulated product but in such a way that the resistance of the simulated product to the sterilization process does not differ from the resistance to sterilization of the product to be sterilized.

The following factors govern the choice of indicator organisms:

- The test strain should be stable and non-pathogenic.
- The resistance of the test strain to the particular sterilization process should be greater as compared with the resistance of all species of micro-organisms likely to contaminate the product including, where possible, the ambient flora in the production environment.
- The recovery of the test strain should be reproducible when cultivated under carefully standardized conditions.

A biological indicator used for monitoring of a sterilization process may not be suitable, and may even be satisfactory, for validation of sterilization cycles, which may differ in their needs for particular applications. The proportion of test organisms surviving the sterilization process should be quantified and related to the expected lethality of the process. The effective use of an indicator for the monitoring of a sterilization process requires knowledge of the product being sterilized and its component parts and a general idea of the probable types and numbers of micro-organism constituting the microbial burden in the product immediately prior to sterilization. A biological indicator is characterized by the strain of test organisms constituting the microbial burden in the product immediately

prior to sterilization.

A biological indicator is characterized also by the strain of test organism, the total viable spore count per carrier (test piece of the indicator), the D-value (Decimal Reduction Value), the Z-value and the expiry date. Information on the recovery medium and the conditions of incubation should also be known. The D-value is a measure of the resistance of a micro-organism to a particular type of sterilization process. It is the value of the appropriate parameter of the process (duration or absorbed dose) required to reduce the number of viable micro-organisms to 10% of the original number. In the case of steam sterilization, the D-value is expressed by the time in minutes at a defined temperature, e.g. D<sub>121</sub>, indicates the temperature of 121-170°C sterilization. In the case of radiation sterilization, the D-value is expressed by the absorbed dose and subscripts are often used to show the log system used, e.g. D<sub>10</sub>. In the case of ethylene oxide sterilization, the D-value is expressed by the time in minutes and is only of significance under precisely defined sterilization conditions. In case of steam and dry heat sterilizations, the Z-value relates the heat resistance of a micro-organism to changes in temperature. The Z-value is the change in temperature required to alter the D-value by a factor of 10. Biological indicators with indeterminate labeled spore counts or without such labeled information at all, or with a vague description of the sterilization method for which the indicator is to be used, are unsatisfactory unless the user determines the required resistance characteristics and the total spore count per carrier with the necessary precision under the user's sterilization conditions.

The selection of a biological indicator is critical and requires that due weight be given to a knowledge of the resistance of the indicator to the specific sterilization process so that when it is used within its performance characteristics it provides a challenge to the sterilization process that exceeds the challenge of the natural microbial burden in or on the product. The indicator should be placed at the locations presumed or, wherever possible, found by previous physical measurements to be least accessible to the sterilizing agent. Even in placing the indicator in any selected location, attention should be paid to its positioning, e.g. vertical, sideways, to assure maximum penetration of the sterilizing substrate. The performance of a biological indicator is a function of both its initial viable spore count and the resistance of the viable spores to the sterilization process. It is therefore important that the indicator maintains its numbers of viable spores and resistance characteristics throughout its shelf-life.

## Validation of Autoclaves

### Material

Soyabean Casein Digest Medium (SCDM), Spore strip (*Geobacillus sterothermophilus*), Glass vials.

### Equipments

Laminar Air Flow, Incubator (60°C + 2°C), Autoclave.

### Procedure

(1) Depending upon the loading capacity of the autoclave, the position of exposure of the bacteriological spore strip are decided i.e. for small Autoclave— two sites, for large autoclave – six sites.

(2) Place the bacteriological spore strips in a clean sterile test tube/vial as its two sites i.e. bottom and top of the fully loaded small autoclave and its six sites for larger autoclave. (3) Operate the respective Autoclaves as per the operating procedure (121 /15 psi/151 min). (4) After autoclaving remove and transfer the strips aseptically in sterile 50 ml SCDM. Carry out the operation inside Laminar Air Flow. (5) Label them accordingly. (6) Incubate at  $60^{\circ}\text{C} + 2^{\circ}\text{C}$  for 7 days with a positive and negative control. (7) Note: (1) Positive control = Sterile 50 ml SCDM incubated with unexposed bacteriological spore strip to steam sterilization. (8) Note: (2) Negative control = Sterile 50 ml SCDM incubated without bacteriological spore strip. (9) Observe for growth everyday till 7 days. (10) Validation using bacteriological spore strip is carried out once in 6 months. (11) Sterilize all cultures before discarding.

#### Interpretation

(1) Growth within 3 days in positive control vials, no growth in negative control vials and test vials till 7 day indicates proper steam sterilization. (2) Growth within 3 days in positive control vials, no growth in negative control vials and growth in test vials in 7 days indicated failure in steam sterilization, hence faults have to be rectified. (3) Autoclave should be validated again after correction.

#### Validation of Ovens

##### Material

Soyabean Casein Digest Medium, Spore strip (*Bacillus atrophoeus*), Glass vials/ petridishes.

##### Equipments

Laminar Air Flow, Incubator ( $35^{\circ}\text{C}+2^{\circ}\text{C}$ ), Oven.

##### Procedure

(1) Place the *Bacillus atrophoeus* spore strips in petri dishes/ glass vials and cover with aluminum foil. (2) Arrange petri dishes/ glass vials in the fully loaded oven (for Big oven use 10 strips, for medium size oven use 6 strips and for small oven use 2 strips each). (3) Carry out standard cycle of sterilization ( $160^{\circ}\text{C}$  for 2 hours /  $180^{\circ}\text{C}$  for 1 hours). (4) Remove petri dishes containing spore strips from oven when temperature falls below  $60^{\circ}\text{C}$  and transfer it to Laminar airflow unit. (5) Let them cool down to room temperature. Conduct further experiment under laminar air flow. (6) Aseptically transfer each strip into separate tube containing 50 ml of sterile soyabean casein digest medium. Incubate the tubes at  $35^{\circ}\text{C} + 2^{\circ}\text{C}$  for 7 days. Check the tubes for turbidity / pellicle formation every day till end of 7 days. (7) Inoculate the unheated strip in another sterile Soyabean Casein Digest Medium tube under aseptic conditions that acts as positive control. (8) Keep one tube containing sterile SCDM as negative control without adding spore strip. (9) Incubate the test tubes along with positive control and negative control at ( $35^{\circ}\text{C} + 2^{\circ}\text{C}$ ) for 7 days. (10) Sterilize all cultures before discarding.

#### Interpretation:

(1) Positive control - Within 18-24 hours growth should be observed. (2) Negative control - No growth should be present till the end of 7 days. (3) Test media tubes containing spore strips exposed to sterilization cycle should not show any growth till the end of 7 days.

#### It is important to know:

##### Loading and processing instruments in sterilizers (autoclave and oven)

The sterilizer should be loaded so that steam or hot air can

circulate and reach all packages. Autoclaves or ovens should not be overloaded by jamming instruments or packages into the sterilizing chamber as this will cause failure of the sterilizing process. Once the sterilizing cycle has commenced, a note should be recorded of the time taken to reach an effective sterilizing temperature (this is the holding temperature and the load should be held at this temperature for as long as required to achieve sterilization. In addition to the minimum holding time the sterilizing cycle will need additional time for heat penetration of the load being sterilized, and additional time to provide a safety margin. For example, using an autoclave with steam pressure at a pressure of 206 kilo pascals (30 pounds per square inch) at  $134^{\circ}\text{C}$ , the minimum holding time is 3 minutes. To this minimum time of three minutes (two minutes to allow for heat penetration of the load plus one minute safety margin) will need to be added to the autoclaving cycle. In this example the total time for the autoclaving cycle is six minutes. The effectiveness of any sterilizing cycle may vary with the sterilizer (autoclave or oven) used and other conditions. Effectiveness of a sterilizing cycle should be established using biological resting, the tests should be repeated regularly. The autoclaving cycle (time, pressure, and temperature) has to be validated by testing with biological monitors to establish its effectiveness and each cycle must be monitored to ensure that it conforms to the validated cycle. Biological validation should be repeated regularly, usually weekly, to ensure the sterilizing cycle is effective. In order to monitor the sterilizing process, some autoclaves are equipped with a printer to provide a printed record of the cycle. If there is no printer available details of the autoclaving cycle should be obtained and recorded manually. When using an autoclave a drying cycle is the preferred method for drying instruments and packages. This process immediately follows the sterilizing cycle, taking place before the autoclave door is opened after sterilization. Alternatively, after pressure reduction in the autoclave chamber, the door of the autoclave may be left slightly open for several minutes after evacuation of steam at the end of the cycle. To minimize possible thermal damage to sensitive instruments (for instance hand pieces) when the sterilizing time (holding plus penetration and safety times) has elapsed the pressure should be evacuated and the instruments removed as soon as is practicable. This also minimizes the length of time instruments are exposed to high temperatures. Evacuation of steam pressure may be part of an automatic autoclave cycle. When liquids, for instance saline, or bacteriological media, are sterilized in an autoclave allow the pressure to drop slowly to avoid the liquid boiling over into the chamber. (Some cycles, when adjusted to sterilize liquids incorporate this procedure automatically).

#### Dry heat sterilization (sterilizing ovens)

Dry heat is used to sterilize anhydrous items and items sealed in non-permeable containers which cannot be sterilized by steam under pressure. Metal instruments not susceptible to heat damage during dry heat sterilizing can be sterilized by this process. The sterilizing cycle will need validation and also monitoring to ensure the cycle conforms to the validated cycle. Dry heat sterilizers require convection and circulation of heated air, and instruments should be packed within the sterilizing chamber to permit easy air circulation inside the oven. It is important not to over pack or jam instruments into the sterilizing chamber. Once the oven has been closed it should not be opened until completion of the sterilizing cycle. The sterilizing cycle must not be interrupted.



### Ferdinand Julius Cohn

Birth: January 24, 1828

Death: June 25, 1898

Nationality: German

Known for: Foundation of Modern Microbiology

German naturalist and botanist known for his studies on algae, bacteria and fungi. He is considered as one of the founders of bacteriology. His contributions include systemic classification of bacteria, discovery of bacterial spore, help in disproving the fallacy of spontaneous generation, and establishing a journal "Beitrage zur Biologie der Pflanzen" which served as an important vehicle for the publications of many bacteriological papers. Cohn's work also helped establish the recognition of bacteria as a separate group of living organisms different from plants and animals.

Ferdinand was born on January 24, 1828 in Breslau (now Wroclaw), Lowre Silesia, now in Poland. His father Isaac Cohn, was poor and lived in Jewish ghetto when Ferdinand was born. But Isaac became a successful merchant and he cared very much about the education of his children. To his great joy, Ferdinand was a genius, he could read at the age of two and was interested in natural history at a very young age. He first attended school at the age of four. In 1835, he entered the Breslau Gymnasium (equivalent to high school) and did very well in all courses. Unfortunately at the age of ten or eleven, he developed for an unknown reason a hearing defect, which slowed his incredible pace of learning. It was probably due to this defect that he became shy, studious and sensitive who also suffered an acute physical and emotional retardation, which he did not begin to overcome since his last year at high school. In 1842, he entered the University of Breslau. During this period, he developed an interest in botany. Young Cohn finished all the requirement for graduation at the University of Breslau but, because he was a Jew, he was barred from taking the final examination, therefore Cohn went to the more liberal University of Berlin in October of 1846, and received his doctorate degree in botany on November 13, 1847 when he was only 19 years old. In Berlin he was very much inspired by the teaching of Eilhard Mitscherlich, Karl Kunth, Johannes Muller and Christian Ehrenberg, who introduced him the study of microscopic organisms. But because he was sympathetic to the revolutionaries of 1848, his academic career in Berlin was not prosperous.

In 1849, he returned to Breslau and stayed there for the rest of his life. In 1850, he became a Privatdozent at the University of Breslau. In 1859, he was appointed an extraordinary professor and in 1871, an ordinary professor. Cohn was a great inspiring teacher. His academic career and research findings were all accomplished at this University.

Because he was influenced by Matthias Schleiden's cell theory and Hugo von Mohl's description of protoplasm in plant cells, he began to focus on lower plants---microscopic organisms. His tedious observations on the unicellular algae *Protococcus pluviialis* led to his early fame. He found that the protoplasm in plant cells and "sarcode" in animal cells were very similar. He suggested that the distinction between animals and plants should not merely be based on the fact that animals possessed differentiated organ systems or a contractile substance peculiar to themselves. He drew an explicit attention to the identity between the contractile contents of plant and animal cell. Cohn's work on *P. pluviialis* confirmed and expanded the suggestions of Karl Wilhelm von Nageli, Hugo von Mohl, Alexander Braun, Max

Schultze, and others, that the essential constituents of the cell was its protoplasmic contents. All these findings eventually led to the "protoplasm theory of life", which was first published by Max Schultze in 1861. The protoplasm theory of life was a big advance step to the understanding of life.

In 1848, Cohn's former teacher Goeppert asked him to devote himself to algae and hoped that he would contribute to the flora of the cryptogamous plants of Silesia. Cohn diligently accepted this assignment and published the first two volumes of the cryptogamous plants in 1876. This work alone is a significant contribution to the plant science in general.

Cohn made detailed study of microscopic algae, fungi and bacteria. Of particular interest was the way he treated bacteria, generally called Vibrionia. At that time, bacteria were considered animals primarily because of their active, apparently voluntary movement. Cohen pointed out that the ciliated swarm cells of algae and fungi performed similar movement. He suggested that bacteria followed the same developmental course as algae, and that some large bacteria belonged to the plant kingdom and displayed an especially close relationship to the Oscillaria. But he studied mainly *Bacterium Termo* Dujardin.

Between 1856 and 1866, Cohn did some work on the contractile tissues of plants, and also pioneered the phototrophic studies of microscopic organisms. At his urging an Institute of Plant Physiology of the University of Breslau was ultimately created in 1866. That was the first Institute of Plant Physiology in the world. In 1872, he became the director of the that institute. In this institute, he installed a marine aquarium that yielded materials for much of his later work.

He cultured marine plants, and studied the classification of lower plants. In 1870, he founded a journal entitled *Beitrage zur Biologie der Pflanzen*, designed primarily to publish the work that came out of his institute. This journal became well known because many pioneer papers of modern bacteriology were published in this journal.

After 1870, Cohn turned his attention primarily to bacteria. He defined bacteria as "chlorophyll-free cells of spherical, oblong, or cylindrical form, sometimes twisted or bent, which multiply exclusively by transverse division and occur either isolated or in cell families"

He divided bacteria into four groups based on their morphology: i. Sphaerobacteria (spherical), ii. Microbacteria (short rods or cylinders), iii. Desmobacteria (longer rods or threads) and iv. Spirobacteria (screws or spirals). He insisted putting bacteria into the plant kingdom because of their similarity with well-known algae.

He explained that the reason why in boiled infusions or hay and cheese there could be resumed microbial growth was because they contained heat resistant spores.

Cohn was a well known botanist, but his work was closely related to microbiology. He published nearly 200 papers and books. Cohn received many honors in his life. He held an honorary doctorate from the faculty of medicine at the University of Tubingen, and was named a corresponding member of the Academia dei Lincei in Rome, the Institut de France in Paris, and the Royal Society of London. In 1885, he was awarded the Leeuwenhoek Gold Medal, and in 1895 the Gold Medal of the Linnean Society.

He married a former student Pauline Reichenback in 1867, apparently he had a wonderful marriage and she wrote his biography in 1901 (Ferdinand Cohn: Blatter der Erinnerung). Ferdinand Cohen passed away on June 25, 1898 in Breslau.

#### Reference:

[www.pnf.org/compendium/Ferdinand\\_Julius\\_Cohn](http://www.pnf.org/compendium/Ferdinand_Julius_Cohn)

# Enjoy the humour

QUESTION: If a devil catches your wife, What will you do?

ANS: U can do nothing.....

If the devil has committed a mistake let him face the consequences.

The teacher was more than disappointed, she was annoyed. Not a boy in the class could name the two birds she had drawn on the blackboard. "Come now", she pleaded, in a final effort, "one of them is a robin and the other is a thrush. Can any boy tell me which is the robin?"

A voice droned up from the last boy in the back row: "the one beside the thrush, miss!"

Birdy birdy in the sky dropped a poopy in my eye, I don't worry I don't cry, I'm just happy that cows can't fly.



## Thoughts to live by

- Nowhere can a man find a quieter or more untroubled retreat than in his own soul. (Marcus Aurelius).
- Any existence deprived of freedom is a kind of death. (Michael Aoun).
- Being deeply loved by someone gives you strength, while loving someone deeply gives you courage. (Lao Tzu).
- Before God we are all equally wise - and equally foolish. (Albert Einstein).
- What does it profit a man if he shall gain the whole world but loses his own soul. (St. Ignatius of Loyola).



### Track your brain

1. The area immediately around the roots of the plant is referred to as the \_\_\_\_\_.
2. \_\_\_\_\_ are the community of organisms living at the surface of desert soils.
3. Microorganisms that prepare their own food are referred to as \_\_\_\_\_.
4. \_\_\_\_\_ of Autoclaves and Ovens is a very important pharmaceutical practice.
5. Ferdinand Cohn described \_\_\_\_\_ as "chlorophyll – free cells of various shapes".
6. \_\_\_\_\_ spp. are used as probiotics and biotherapeutics
7. Vaginal ecosystem is referred to as "\_\_\_\_\_".
8. \_\_\_\_\_ acid is a mixture of hydrogen peroxide and formic acid.
9. Formic acid is also referred to as \_\_\_\_\_ acid.
10. \_\_\_\_\_, \_\_\_\_\_ and \_\_\_\_\_ are best consumed on a hungry stomach.

Check your Answers on Page 16



## *Lactobacillus* species

*Lactobacillus* is a genus of Gram positive facultative anaerobic or microaerophilic bacteria. They are a major part of the lactic acid bacterial group, named as such because most of its members convert lactose and other sugars to lactic acid. They are common and usually benign. In humans they are present in the vagina and the gastrointestinal tract, where they are symbiotic and make up a small portion of the gut flora. Many species are prominent in decaying plant material. The production of lactic acid makes its environment acidic, which inhibits the growth of some harmful bacteria.

### Cell Structure and Metabolism

Lactobacilli are rod-shaped, fermentative, organotrophs. They are usually straight, although they can form spiral or coccobacillary forms under certain conditions. They are often found in pairs or chains of varying length. Lactobacilli are classified as lactic acid bacteria, and derive almost all of their energy from the conversion of glucose to lactate during homolactic fermentation. In this process 85-90% of the sugar utilized is converted to lactic acid. They generate ATP by non oxidative substrate-level phosphorylation (SLP).

### Ecology

Lactobacilli are commonly associated with plant herbage. They have a generation time ranging from 25 minutes to several hundred minutes, and grow optimally between the temperatures of 30 and 40 degrees Celsius, although thermophilic strains can be comfortable at temperatures as high as 60 degrees Celsius. They are also commonly associated with the gastrointestinal tract of animals and humans. As natural gastrointestinal microflora they are believed to perform several beneficial roles including immunomodulation, interference with enteric pathogens, and maintenance of healthy intestinal microflora. *Lactobacillus gasseri* appears to be the main species of lactobacilli that inhabits the human gastrointestinal tract.

### Lactobacilli are used for some of the following purposes:

#### 1. Food Production

Some *Lactobacillus* species are used industrially for the production of yogurt, cheese, sauerkraut, pickles, beer, wine, cider, kimchi, chocolate, and other fermented foods, as well as animal feeds, such as silage. Sourdough bread is made using a "starter culture," which is a symbiotic culture of yeast and lactic acid bacteria growing in a water and flour medium. Lactobacilli, especially *L. casei* and *L. brevis*, are some of the most common beer spoilage organisms. The species operate by lowering the pH of the fermenting substance by creating the lactic acid, neutralizing it to the desired extent.

#### 2. Probiotics and Biotherapeutics

Some *Lactobacillus* spp. and other lactic acid bacteria may possess potential therapeutic properties including anti-

inflammatory and anti - cancer activities, as well as other features of interest. Research studies have demonstrated the protective effects of some strains of these bacteria for anti – tumor and anti-cancer effects. Dietary administration alleviated the risks of certain types of cancers and suppressed colonic tumor incidence, volume and multiplicity induced by various carcinogens. For a few strains oral administration effectively reduced DNA adduct formation, ameliorated DNA damage and prevented putative pre-neoplastic lesions induced by chemical carcinogens in the gastrointestinal tract. Reports also indicated that some cultures administered to animals inhibited liver, colon, bladder, and mammary tumors, highlighting potential systemic effects of probiotics with anti-neoplastic activities.

Lactobacilli are also used to restore particular physiological balance such as in the vaginal eco-system (Gynoflor). Their role is (1) to physically protect the vaginal epithelium by building a thick layer separating the epithelium from pathogens, (2) to physiologically keep the balance of the vaginal ecosystem in maintaining the pH at ~ 4.5 and (3) generating hydrogen peroxide against pathogens.

### Effective Interactions of *Lactobacillus* sp.

#### 1. Surface proteins of *Lactobacillus* involved in Host Interactions

Specific recognition of host components is central in bacterial adhesion and colonization at host surfaces as well as in bacterial interaction with physiological and immunological processes of the host. Isolates of *Lactobacillus* express a variety of adhesive surface proteins, many of which are multifunctional adhesins or also involved in physiological processes in the bacteria. These adhesins can be grouped as S-layer proteins, proteins with the LPXTG surface-anchoring motif, surface-localized housekeeping proteins, as well as transporter proteins. Recognized targets for lactobacillar adhesins include epithelial and phagocytic cells, extracellular matrices, mucins, and circulating components. A more detailed, mechanistic knowledge of lactobacillar adhesion proteins will help to understand their role in colonization and to develop their probiotic use.

#### 2. *Lactobacillus* Stress Responses

Within the lactic acid bacteria group, the genus *Lactobacillus* is widely used for food fermentation and preservation, and as food additives because of its probiotic properties. Lactobacilli are usually exposed to harsh stress conditions such as starter handling and storage (freeze-drying or freezing), during food processing (heat, cold, high concentration of NaCl, and high hydrostatic pressure) and in their passage through the gastrointestinal tract (acidity and bile salts). The optimal performance of these strains depends on the stabilization of their survival potential and their metabolic activity.

### 3. Interactions of *Lactobacillus* with the Immune System

*Lactobacilli* have been used for centuries with the intent to produce a health benefit in the human host. Interactions between the host and *lactobacilli* involve *lactobacilli*-induced alteration of gene expression in gut epithelial and immune cells, with the subsequent modulation of both mucosal and systemic immune function. Effects of *lactobacilli* are concentration and species and strain-dependent. *Lactobacilli* are critical in establishing a state of immunological homeostasis within the host. Beneficial effects exerted by *lactobacilli* bacteria in the treatment of human disease may be broadly classified as those effects which arise due to activity in the large intestine and are related to colonization or inhibition of pathogen growth; and those effects which arise in both the small and large intestine, and are related to enhancement of the host immune response and intestinal barrier function.

### 4. *Lactobacillus* in the Gastrointestinal Tract

Probiotics are living organisms which when consumed have beneficial health benefits outside their inherent nutritional effects. Much has been written in both the medical literature and lay press about their potential benefits, some of it well validated by randomized controlled trials but other claims remain unsubstantiated. There is a growing body of evidence for the role of probiotics in gastrointestinal infections, irritable bowel syndrome and inflammatory bowel disease.

### 5. *Lactobacillus* in the Vagina: Why, How, Which Ones, and What Do They Do?

Of the 50 known species of *Lactobacillus*, at most 20 are able to colonize the intestine, and *L. iners* and *L. crispatus* appear to be the most commonly isolated in women in various countries around the globe. This consistency of isolation is all the more intriguing given the disparity of societal cultures and diet. For the most part, the origin of the *lactobacilli* is the woman's own intestinal microbiota, with passive transfer occurring along the skin from the anus to the perineum, vulva and vagina. The microbiota is disrupted by hormone levels, sexual practices, the types of organisms ascending from the anus, and other factors such as alterations in innate immunity. The exogenous administration of certain strains of *lactobacilli* (probiotics) has been shown to reduce the risk of infection and help eradicate bacterial vaginosis. The mechanisms of action of the most documented probiotic containing *Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14, appear to consist of physico-chemical displacement ability, as well as anti-infective compounds released from the cells, and immune-modulatory factors. Further development of these and other strains will lead to new approaches to help women retain and regain their vaginal health, and reduce their risk of various problematic, and in some cases, life threatening, conditions.

### From Probiotics, Prebiotics and Synbiotics to "Living Drugs"

The human intestine harbors an immense collection of microbes which have co-evolved with us. Recent studies indicate that the gut microbes regulate energy harvest from the diet and participate

in the peripheral body metabolism. Elie Metchnikoff understood that gut microbial dysbiosis severely affects many body functions, including a complex interplay of gut-brain interactions, now under intense study. Most probiotic strains belong to the genus *Lactobacillus*. The promising results of a first generation of probiotic microbes, evaluated in animal models as well as natural infections in animals and humans indicate a promising future for coming generations of probiotics. Antibiotic-associated, travelers' and pediatric diarrhea have been most studied, and more recently, inflammatory bowel disease and irritable bowel syndrome. The probiotic strains should be thoroughly characterized. Probably future probiotics will contain mixes of strains with complementary characteristics, tailor made for different gastrointestinal diseases, vaginosis or as delivery systems for vaccines, immunoglobulins and other protein based therapies.

### To Conclude

*Lactobacillus* sp. encompasses a significant number of different species that are largely diverse. These lactic acid bacteria are used to preserve food and feed. The genus *Lactobacillus* contains several species that make up normal intestinal flora in the human body. They have the ability to derive lactic acid from glucose to create an acid environment that prevents the growth of bacteria that can cause infections.

*Lactobacillus bulgaricus* is one of several bacteria used to produce yogurt. The name *L. bulgaricus* is derived from the country Bulgaria where it was first used to preserve milk. The bacterium feeds on milk to produce lactic acid which is used to preserve milk. *Lactobacillus bulgaricus* naturally exists in the human gastrointestinal tract. The bacteria is helpful to people suffering from lactose intolerance which occurs in individuals who lack the enzyme to break down lactose to simple sugars.

*Lactobacillus plantarum* is a natural inhabitant of the human gastrointestinal tract. The first sample of *L. plantarum* was isolated from human saliva. This strain is one of the largest genome known among lactic acid bacteria. *L. plantarum* significantly reduces intestinal gas and effects last for months after treatment.

*Lactobacillus casei* is found in fermented milk and has beneficial properties for human health. The human digestive tract consists of *L. casei*; a natural flora that prevents an overpopulation of ingested lactic acid bacteria from gaining residence in the gastrointestinal tract. Live *L. casei* reduces diarrhea and helps modify microflora in the body. *Lactobacillus casei* produces DL-lactic acid and amylase that complement the growth of *Lactobacillus acidophilus*.

Deficient growth of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, and *Lactobacillus casei* could be due to the following:

- Antibiotics
- Poor diet
- Chlorinated water
- Fluoride water
- Birth control pills
- Stress.

## Endoclens

In developing countries, like India there is an increasing need to maintain high levels of hygiene since in certain cases factors like illiteracy and poverty play a major role in the spread of disease and hence in causing a large number of deaths. Deaths may occur either due to severity of the disease, malnutrition, improper health care, delay in diagnosis, lack of funds for treatment, or opportunistic infections in cases of immunocompromised individuals.

It is important to mention that medical devices too can act as fomites in the dissemination of diseases, therefore all tools including the endoscope, which is an important tool in diagnosis needs to be maintained such that at the commencement of each diagnostic session the endoscope has to be free from any microbes and after each diagnostic procedure is over; it should be cleansed again so that the accumulation of microbes on the surface of the endoscope is minimum, during storage.

The Endoclens system is designed to provide rapid, automated, point-of-use chemical sterilization of flexible endoscope-reprocessing machine and a new, proprietary liquid sterilant that uses performic acid. The sterilant is produced, as needed by the machine, by automatic mixing of the two component solutions of hydrogen peroxide and formic acid. This sterilant is fast-acting against spore-forming bacteria.

### Significance of Hydrogen Peroxide in the liquid sterilant

The role of Hydrogen peroxide is that of an oxidizer, which appears like water and at times even behaves like water. Formula of hydrogen peroxide is  $H_2O_2$  and it is safe to use it as a disinfectant. In fact Hydrogen Peroxide is considered a better purifier than chlorine compounds and the best part about using Hydrogen Peroxide as a purifier is that it forms Hydroxyl radicals that are quite strong as oxidants in fact even stronger than fluorine. The main advantages of using hydrogen peroxide are innumerable.  $H_2O_2$  is safe to use. It's doses do not produce poisonous gases neither does it leave behind any kind of chemical residues that might be difficult or harmful for earth's atmosphere to decompose. It dissolves with water and does not prove toxic for the aquatic animals as well. There is no problem of osmosis, or rather concentration when Hydrogen peroxide dissolves with water because it completely combines with water. Hydrogen Peroxide is also a versatile chemical, which increases the scope of its usage. Since people have identified the advantages of using hydrogen peroxide, its use has grown manifold and in all kinds of industries ranging from food processing to petrochemicals and detergents. Hydrogen peroxide not only is used by human beings but also by plants which is not only a substitute for water but also acts as an effective insecticide without toxins.

### Role of Formic Acid

The principal use of formic acid (methanoic acid) is as a preservative and antibacterial agent in different sterilizing agents. When sprayed on fresh hay or other silage, it arrests certain decay processes and for instance in the feed to retain its nutritive value longer, and so it is widely used to preserve winter feed for cattle. In the poultry industry, it is sometimes added to feed to kill salmonella bacteria. This chemical is also used as a chemical sterilant in cleansing medical equipment.

### Major features of Endoclens include:

- An automatic cleaning process.
- Capability to process two flexible scopes asynchronously.
- Automated channel blockage and leak detection.
- Filter water rinsing and scope drying after sterilization.
- Hard copy documentation of key process parameters.
- User friendly machine interface, and
- Total cycle time less than 30 minutes.
- The reprocessor can also be disinfected automatically to prevent infection or pseudoinfection.

### Functioning of Endoclens

The reprocessor can independently process two endoscopes at the user's discretion since it has two washing / sterilization bays. The endoscopes are attached to special holders (racks) which slide into the machine bays located in the front of the machine and provide a connection between the reprocessor and the endoscope's inner channels. The endoscope racks are designed to accommodate all types of flexible endoscopes. During washing, enzymatic detergent is automatically dispensed, diluted with warm water ( $45^{\circ}C$ ), and sprayed onto the exterior endoscope surfaces and pumped through the endoscope lumens. The enzymatic detergent is pumped through the lumens with alternating pulses of compressed air to assist in removing any adhering material. Cleaning studies performed by the manufacturer using a synthetic soil show the system can satisfactorily clean and rinse detergents from an endoscope in preparation for point-of-use sterilization.

The concentration and temperature of the mixed chemicals are automatically measured by the machine with refraction and temperature sensors. Once pumped into the washing / sterilization bay, the sterilant is vigorously sprayed over all exterior endoscope surfaces and pumped through all endoscope lumens to sterilize the scope. Simulated-use studies with resistant spores suspended in 5% serum and inoculated on scope surfaces and inside lumens have been used to demonstrate the effectiveness of the sterilant.

All the water used for washing / sterilization and rinsing is filtered through a  $0.2 \mu m$  filter. The scopes are dried when the cycle is completed by using filtered compressed air that is sprayed over the exterior scope surfaces and through the interior lumens through the same connections used for the washing and sterilization steps.

The total cycle time for scope testing, washing, sterilization, and drying is less than 30 minutes. Upon completion of each cycle, the reprocessor prints a hard copy record as well as retaining a record in memory, accessible through its floppy disk drive. Printer parameters are printed at the completion of each cycle and include scope identification, processing date, key cycle parameters, space for insertion of patient name or identification number, procedure type, and date.

### Reference

<http://www.cdc.gov/ncidod/eid/vol17no2/pdfs/rutala.pdf>

# Proper diet - Healthy food intakes

“Health is Wealth” is the common phrase that we have heard people using and have ourselves used so very often that we may never have enough of it.

Well then, the point here would be that we have to act in the direction so as to keep ourselves as healthy as possible. Having meat, fish, vegetables, fruits, pulses, oils is not enough, there is a

need to know the proper time and manner in which we should consume them. Improper consumption of all the healthy stuff would do no good, on the contrary just add bulk to the intake and further strain the digestive system.

In a bid to make our diet more healthier, we neglect the underlying point to ‘think before we act.’

There are plenty of foods that help us have a healthy sustenance; Importance of such foods is mentioned in the table below:

Food	Aids in	Nutrients present
<b>Walnut</b>	Lowering cholesterol, combating cancer, boosting memory, protection against heart diseases	Omega 3 fatty acids, manganese, copper, tryptophan, etc.
<b>Figs</b>	Weight loss, lowering cholesterol, controlling palpitation	Dietary fiber, potassium, manganese, etc.
<b>Fish</b>	Protection against heart disease, boosting memory, fighting obesity, support to the immune system	Tryptophan, vitamins B3, B6, B12, D, omega 3 fatty acids, phosphorus, manganese, protein, etc.
<b>Prawn</b>	Fighting knee and joint pains, improving mood, reducing depression	Tryptophan, omega 3 fatty acids, protein, vitamins B3 and D, minerals, etc.
<b>Olives</b>	Protection of the heart, promotion of weight loss, battling diabetes, fighting body pains	Iron, vitamin E, dietary fiber, copper, etc.
<b>Peanut</b>	Promoting a healthy heart, reducing blood pressure, reducing body weight	Manganese, tryptophan, vitamin B3, folate, copper and protein
<b>Oats</b>	Lowering blood cholesterol, stabilizing blood sugar, prevention of hair fall	Minerals, tryptophan, vitamin B1, dietary fiber, protein, etc.
<b>Apricots</b>	Controlling blood pressure, protecting eyes, slowing aging	
<b>Drumstick</b>	Reducing blood pressure, controlling diabetes, curing asthma and TB	Protein, minerals, vitamins, etc.
<b>Coriander</b>	Improving respiratory health, intestinal health, lowering LDL, increasing HDL, improves digestion	Dietary fiber, minerals, etc.
<b>Cucumber</b>	Improving digestion, facial treatments	Vitamins A, C, minerals, dietary fiber, tryptophan, magnesium, folate, potassium, etc.
<b>Carrot</b>	Lowering cholesterol, fighting obesity, combating diabetes, protecting liver	Minerals, vitamins, dietary fiber, folate, etc.
<b>Soyabean</b>	Staying lean, lowering blood pressure, stabilizing blood sugar, fighting diabetes	Minerals, vitamins, proteins, dietary fiber, omega 3 fatty acids, copper, etc.
<b>Pomegranate</b>	Blood thinning, combating cancer, dental protection, protecting arteries	Minerals, vitamin B complex, vitamin C, etc.
<b>Basil</b>	Protection against chronic disease, enhancing memory, blood purification	Minerals, vitamins, dietary fiber, etc.

The type of lifestyle we have greatly influences the ideal food intake for us, there are some trivial facts about certain foods that we should know:

For instance a certain food contains 50 calories, but the body burns 120 calories to actually digest this food. Examples of such foods include walnuts, figs, soya bean, olives, fish, peanuts, ginger, carrot, cucumber, garlic, papaya, spinach, amla, asparagus, apples, beets, berries, broccoli, cabbage, cauliflower, celery, pineapple, lettuce, onion, lemon, turnip, grapefruit and orange are among the foods that are advisable to folks looking out for a healthy weight loss regime.

However if you want to gain some weight, it is best to eat less of the above and eat other foods that have high calorie levels.

## Vitamins and Minerals and the appropriate intake

Vitamin	Daily Dose	Used for
A (Beta carotene)	10,000 IU	An antioxidant used for skin, eyes, teeth and bones.
B complex		All B vitamins are taken as a group, hence, B complex.
B1 (Thiamine)	50 mg	For nervous system, body growth and body metabolism.
B2 (Riboflavin)	50 mg	Improves in the formation of red blood cells and antibodies and for metabolism.

Vitamin	Daily Dose	Used for
B3 (Niacin)	100 mg	Improves in maintaining good skin and digestive system.
B5 (Pantothenic Acid)	100 mcg	Helps with stress, improves in the release of energy from fats and carbohydrates.
B6 (Pyridoxine)	50 mcg	Helps balance sodium & phosphorus. Improves in formation of antibodies.
B12 (Cyanocobalamin)	200 mcg	Improves in formation of blood cells, helps metabolism and nervous system.
Biotin	200 mcg	Improves in utilization of other vitamins.
Choline	100 mg	Helps in nerve transmission, liver and gall bladder functions.
Dietary fiber	20 – 30 gms	Lowering cholesterol, preventing cancer, constipation, helping weight loss.
Folic Acid	400 mcg	Improves the brain function and for normal cell division.
Inositol	150 mg	Is a necessary component for hair growth.
C (Ascorbic Acid)	2,000 mg	An antioxidant, heals wounds, tissue, bone repair, helps resist infection.
D	400 IU	Required for the body to absorb calcium & phosphorus. Helps nervous system.
E	500 IU	Prevents cancer and cardiovascular disease. An antioxidant helps blood clotting.
K	100 mcg	Necessary for normal blood clotting.
Bioflavonoids	400 mcg	Helps strengthen capillaries and improves in the absorption of vitamin C.
Coenzyme Q10	25 mg	Improves in the effectiveness of the immune system.

Mineral	Daily Dose	Used for
Calcium	1,500 mg	For bones & teeth, nervous system & muscle action.
Chromium	100 mcg	Increases effectiveness of insulin, used in metabolism.
Copper	2 mg	Formation of blood cells, works with vitamin C in healing process.
Iodine	130 mcg	Helps regulate metabolism.
Iron	18 mg	Used in the production of blood, works in the immune system.

Mineral	Daily Dose	Used for
Magnesium	400 mg	Acts as a catalyst in utilization of carbohydrates, fat, protein & other minerals.
Manganese	3 mg	For skeletal development & sex hormone production.
Molybdenum	25 mcg	Helps iron transport from liver, promotes normal cell function.
Potassium	200 mg	Necessary for heart muscle function, kidneys & nervous system.
Selenium	200 mcg	Works with vitamin E to promote antibodies. Keeps tissue and artery elasticity.
Tryptophan	3 grams	An amino acid that brings feelings of calm, relaxation, confident and sleepiness.
Zinc	25 mg	Improves in healing process, used by prostate gland & immune system.

**Tips for Health**

- The best time to eat fruits and vegetables is ideally when we are hungry, since there are plenty of digestive juices that can digest and or segregate the plant fiber adequately, in this manner we ensure that we are reaping all the benefits of eating fiber and consuming all the vitamins and minerals. When we have high fiber foods such as fruits and vegetables on a full stomach, the fiber will just add bulk to the consumed food.
- Water should be had at a time when we are hungry and not immediately before and after meals, since just prior to meals, water dilutes the acid which will increase the time taken by the digestive system to breakdown the food into simpler molecules, and water should not be consumed immediately after meals since food gets mixed with the water and the digestive system gets strained in such a digestion process, therefore it is ideal to drink water at least half an hour before and / or half an hour after meals.
- As for protein consumption, its best to be had at night since tissue repair occurs during the night and carbohydrates are best had in the morning.
- Spice any gravy with less oil but more of ginger, onion, garlic, cinnamon and coriander. This helps since oils are rich in saturated fats, which are responsible for different health problems whereas ingredients like ginger, garlic, onion, cinnamon, coriander help in overcoming heart associated problems, diabetes, menstrual problems, body pains, arthritis, acidity and digestive problems.
- Breathing heavily, especially while climbing helps in removing the residual levels of CO<sub>2</sub> from the lungs and filling the lungs with more oxygen, which helps in certain breathing disorders. Taking a brisk walk will help in clearing the respiratory tract. However, it is not advisable to take a walk immediately after meals.
- It is good to exercise regularly, but even an exercise needs to be done appropriately, preferably on an apparently empty stomach, since exercising immediately after meals puts a strain on the digestive system. Therefore exercising is most beneficial in the morning before breakfast or in the evening, and best to be avoided during conditions like menstruation.

All the food items in the diet are essential and in a proper proportion give us the vigor and vitality to carry on with all our regular chores with good health.

**BioShields offers TRIOSEPT™**

Liquid handrub antiseptic with triple action

**COMPOSITION**

2.5% v/v Chlorhexidine Gluconate Solution IP, 0.5% w/v Triclosan USP, 50% v/v Isopropanyl Alcohol (2-propanol) IP, 25% v/v N-Propanol (1-Propanol) BP, Skin Emollients.

**DESCRIPTION**

TRIOSEPT is a colourless non-perfumed alcohol based hand rub with a powerful triple action; its suitable for surgical & hygienic hand disinfection especially for perfume and colour sensitive areas and work patterns.

**ACTIVITY**

Broad spectrum: Bactericidal, Fungicidal and Virucidal

**CONTACT TIME**

30 Seconds

- BENEFITS** :
- No perfume or colour
  - Potent synergistic formulation
  - Effective against HIV, HBV & Mycobacterium
  - Combined and instant residual action
  - Skin safe
  - Non sticky & Soft feel

**APPLICATION**

**MEDICAL** - In NICU, PICU, OT, OPD, dental setups, Infectious disease departments, In laboratories, Ambulances. **INDUSTRIAL**- In Pharmaceutical industry, In Food processing industry. **GENERAL**- Personal hygiene, Handling and care of patients, old people and infants, Waterless hand disinfection during travel.

**USAGE DIRECTIONS**

**HYGIENIC HAND DISINFECTION:** Rub 3 ml of TRIOSEPT well over clean dry hands and nail groves for at least 30 seconds. **SURGICAL HAND DISINFECTION:** Repeat the above process thrice for 5 minutes.

Track your brain

1. Rhizosphere
2. Biological soil crusts
3. Autotrophs
4. Biovalidation
5. Bacteria
6. *Lactobacillus*
7. Gynoflor
8. Performic
9. Methanoic
10. Water, Fruits Vegetables

**Microxpress's range of media for the cultivation of *Lactobacillus* species and soil microbes**

**Glucose Yeast Extract Agar**

A medium for enumeration of Lactobacilli in pharmaceutical preparations.

**Lactobacillus MRS Agar & Lactobacillus MRS Broth**

A medium for the isolation and cultivation of all *Lactobacillus* species.

**PNY Medium**

A medium for cultivation and isolation of *Lactobacillus* species.

**Rogosa SL Agar & Rogosa SL Broth**

A medium for cultivation of oral, vaginal and faecal Lactobacillus.

**Tomato Juice Agar**

A medium for cultivation and enumeration of *Lactobacillus* species.

**Corn Meal Agar**

A general purpose medium for cultivation of fungi

**Czapek Dox Agar**

A semisynthetic medium for general cultivation of fungi, yeasts and soil bacteria.

**Jensen's Broth & Jensen's Medium**

A medium for detection and cultivation of nitrogen fixing bacteria.

**Pikovskaya's Agar & Pikovskaya's Broth**

A medium for detection of phosphate solubilizing soil microorganisms.

**Sabouraud Dextrose Agar**

A general – purpose medium for the cultivation of yeast, moulds and aciduric bacteria.

**Yeast Mannitol Medium with / without supplements**

A medium for cultivation, isolation and enumeration of soil microorganisms like *Rhizobium* species.

