



Review Paper

Importance of Exploration of Microbial Biodiversity

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Available online at: www.isca.in

Received 1st June 2012, revised 8th June 2012, accepted 12th June 2012

Abstract

The microbial world is the largest unexplored reservoir of biodiversity on the earth. It is an important frontier in biology under intensive investigations. The exploration of microbial diversity has been spurred by the fact that microbes are essential for life since they perform numerous functions essential for the biosphere that include nutrient recycling and environmental detoxification. The management and exploitation of microbial diversity has an important role in sustainable development with the industrial and commercial application of microbial diversity worth millions of rupees. Given the heterogeneity of natural environments and the enormous potential of microorganisms to provide novel pharmaceuticals, fine chemicals and new technologies, the biotechnology industry has a vast, largely untapped resource for the discovery of new chemicals and novel processes. However, despite the obvious economic value of microbial diversity, microorganisms have been largely ignored in debates on the conservation and management of global diversity. There is, therefore, an urgent need to persuade policy-maker to be more concerned about the conservation, management and exploitation of microbial diversity. There are a number of reasons why the conservation of microbial diversity has not received the same attention as plants and animals. For example, microorganisms are invisible, less familiar and perceived primarily as agents of disease. With respect to the role of microorganisms in sustainable development, little is known about the potential contribution of microbial diversity to the national economy, to wealth creation and to improvements in the quality of life. An appreciation of these factors might be one way of changing government and public perception of microorganisms by showing that the sustainable use of microbial diversity has positive economic value. This would help justify the costs involved in conserving microbial diversity, but equally provide a useful indicator of the costs of inaction. In terms of the scientific rationale needed to underpin policy, quantification of microbial diversity has been limited. This makes it difficult to indicate what needs to be conserved in order to support the biotechnology industries and to understand fully the interactions between organisms responsible for maintaining a functional ecosystem.

Keywords: Plant diversity, microbial diversity, nutrient recycling, ecosystem functioning, exploration, conservation.

Introduction

The microorganisms play an integral and often unique role in the functioning of the ecosystems in maintaining a sustainable biosphere and productivity. The loss of biodiversity and their ability to provide ecological services to humans has now become a central theme in ecology. A number of major experiments have recently shown that declining plant diversity may impair such ecosystem properties as plant biomass, primary production and nutrient retention¹. Presently, the relationship between biodiversity and ecosystem functioning in ecological and environmental sciences has emerged as a central issue. However, few experiments have directly tested the consequences of changing the diversity of ecosystem components other than plants^{2,3,4}. Recently⁵, simultaneously manipulated the diversity of primary producers (algae) and decomposers (bacteria) in aquatic microorganisms and found complex interactive effects of algal and bacterial diversity on algal and bacterial biomass production. Both algal and bacterial diversity had significant effects on the number of the carbon source used by bacteria, suggesting nutrient cycling associated

with microbial exploitation of organic carbon source as the link between bacterial diversity and algal production. Several explanations are possible but the theory is sorely lacking. Because producers and decomposers are two key functional groups that form the basis of all ecosystems interactions between producer diversity and decomposer diversity might have major consequences on the functioning of ecosystems^{6,7}. Thus, it is now generally accepted that the extent of microbial diversity has not been adequately characterized and that there is an immense mismatch between the knowledge of that diversity and its importance in both ecosystem process and economic development⁸. Soil quality has been defined as the capacity of the soil to function within ecosystem boundaries to sustain biological productivity, maintained environmental quality, and promote plant and animal health^{9,10}. Nutrient immobilization by decomposers and competition for inorganic nutrients between plants and decomposers are known to occur, but at equilibrium, the two functional groups must be limited by different factors in order to allow their coexistence and ecosystem persistence.

Analysis of Microbial Diversity

The dissimilar macro-ecologists and microbial ecologists working on natural communities were faced with quite unique challenges posted by, for instance: i. the large number of individuals per samples (for example, more than 109 organisms per gram of soil^{11,12} ii. the problem of differentiating between different populations and the very high diversity at a relatively small scale (more than 104 species per gram of soil)¹³ and iii. the difficulty of defining a microbial species or some other unit that encompasses the appropriate level of diversity for biomanagement of diseases¹⁴. The immediate challenge facing microbial ecologists is how best to quantify microbial diversity in natural environments. Estimates of the microbial diversity must accommodate the spatial and temporal variability in microbial population. Scale effects, both temporal and spatial, are not only of fundamental importance in the quantification of biodiversity, but present basic questions for microbial ecology, the resolution of which could lead to development of fundamental theories and hypotheses as to how microbial communities are structured in space and time, how they respond to environmental pressures and how diversity is connected to function¹³. Spatial effects include an assessment of the relationship between community composition and scale. This is analogous to the area-species curve in macroecology but would require that appropriate measures of microbial diversity be substituted for the classical eukaryotic species. The structure of such curves would be particularly important in predicting the location of the undiscovered diversity and would provide insight into how microbial diversity changes relative to the environment. Such studies could be extended to include an evaluation of biodiversity in 'comparable' (for example, the same soil type or similar vegetation cover) but geographically isolated habitats thereby providing information on microbial dispersal, evolution and selection¹³. Chronological shifts in microbial diversity are brought about by changes in the environment of the microorganisms and may be induced by the organisms or imposed on the community from outside¹⁵. A prerequisite to the quantification of diversity in natural samples is an understanding of the magnitude and level at which such changes operate. There is a need to know which taxonomic rank is most susceptible to change, what are the implications for estimates of microbial diversity at a given site, and whether the data can be used as quantitative indices of sustainability. Advances in the analysis and quantification of microbial diversity will undoubtedly require extensive, collaborative and interdisciplinary studies. In addition to the development of new procedures, the efficacy and importance of the existing techniques will need to be re-evaluated, and protocols developed to enable extrapolative approaches to be used in sites where limited resources preclude intensive studies. Biodiversity estimates will need to be based on stable and readily analyzed properties of the microbial community. Estimates based on phenotypic, and to a lesser extent chemical techniques, are likely to reflect the physio-chemical environment and as such may be influenced by community composition and function at a

particular site at a given time. Thus, sample and collecting procedures need to be standardized temporally. Analysis of microbial communities using ribosomal ribonucleic acid (rRNA) sequences is likely to be less sensitive to variability in the physio-chemical environment provided the methodology can be improved and the basis in nucleic acid extraction and implication procedures removed¹⁶. Although significant steps have been taken over the last few years, the required technology is still in its infancy and is as yet unable to provide the tools necessary to quantify microbial diversity in anything but the simplest of natural habitats. Nevertheless, with an awareness of the limitations of the existing methods and human resources, a reassessment of the 'species' as the basic unit of microbial diversity, and the introduction of new methodologist, significant progress in the quantification of microbial diversity can be expected. Positive effects of plants species diversity on ecosystem process have been attributed to two classes of mechanism: functional niche complementarity and selection of extreme trait values. In both cases, biodiversity provide a range of phenotypic trait variation. The same two classes of mechanism operate with microbial diversity. Greater productivity of systems containing a greater diversity of both producers and decomposers is predicted by the increased likelihood that each functional group contains a species that is highly efficient in using resources. The importance of microbial diversity in sustainable development was shown by the serendipitous discovery of penicillium by Alexander Fleming and subsequent research efforts led to development of several other bioactives which has revolutionized the treatment of infectious diseases. Another emphasis of discovery of *Thermus aquaticus* by Thomus Brock, the source of thermostable deoxyribonucleic acid (DNA) polymerase used in polymerase chain reaction (PCR) had changed the face of modern biology. In the area of plant microbe interaction and sustainable plant development, the rhizosphere competent bacteria most probably resulted from along co evolution with plants, and the soil acted as a reservoir of these microbes. The signaling compounds produced by plants may act either as attractants or repellants of bacterial populations. The greenhouse and field experiments showed that VAM inoculation controlled the incidence of plant diseases¹⁷.

Conservation of Microbial Diversity

The problem of biodiversity is essentially one of conflict resolution between the human kind on one side and living organisms inhabiting different environment on other side. The UNCED (United Nations Conference on Environment and Development) process has helped place the loss of biodiversity and it's conservation on global agenda. The Convention on Biological Diversity (CBD) that emerged from the UNCED or Earth Summit at Rio de Janerio in June 1992 is now a treaty. According to the World Conservation Monitoring Center, 1,604,000 species have been described at the global level. India accounts for 8% of global biodiversity existing in only 2.4% land area of the world. Microbial diversity conservation requires

certain specialized techniques for applications in reclaiming a degraded habitat. Both ex situ and in situ techniques can be employed to preserve the biodiversity.

Ex-situ Preservation: The most effective and efficient mechanism for conserving biodiversity is to prevent the destruction or degradation of the habitat. Because of the uncertainties associated with in-situ conservation of microorganisms, ex-situ preservation plays a major role in microbiology and include the gene banks, culture collections and microbial resource centers forming the repository for microbial isolates and do away with need for costly and time consuming re-isolation protocols. The CBD encourages adoption of measures for ex-situ conservation of biodiversity, preferably in the country of origin. Application of this approach is supported by the World Federation for Culture Collection (WFCC) and Directory of Collection of Cultures of Microorganisms. Moreover four other associations that directed towards this effort are Oceanic and Atmospheric Administration for marine microbial diversity, National Institute of Health for deciphering the emerging microbial pathogen diversity, American Society for Microbiology and American Phytopathological Society. In India this work has been carried out by Ministry of Environment and Forestry and the Ministry of Science and Technology that includes various departments such as the Department of Agriculture Research and Education, Indian Council of Forestry Research and Education, Department of Biotechnology. The level of the Microbial Type Culture Collection section of IMTECH, Chandigarh has now been upgraded to an International Depository Authority (IDA) and it involves the culture collection and maintenance as well as distribution of pure cultures internationally. The ex-situ collections of microorganisms form the key repositories of biodiversity and an essential resource for the future as these could be linked to the research programs and developmental aspects of the country that owns it by integrating the microbiological aspects, molecular evolution, systematics and microbial chemistry with genome science. Enhanced funding of stock centers and greater emphasis on education and research in microbial systematics will amplify the broad base of research into microbial diversity.

In-situ Preservation: Basically, in-situ preservation involves on site conservation of the microbial flora involving the conservation of the ecosystems and natural habitats and the maintenance as well as recovery of viable populations of species in their natural surroundings and in case of the domesticated or cultivated species, in surroundings where they have developed their distinctive properties. Conservation of all subsets of life existing in interplaying networks will lead to preservation of microbes as well. Avoiding deforestation and planting trees (Aforestation) will not led the surface soil to be washed out by torrential rains, which contains diverse microflora. Further, avoiding pollution of water bodies such as oceans, river or lakes will preserve phytoplanktons, zooplanktons (rotiferans, microalgae, diatoms, dinoflagellates) and other floating

microbes such as *Vibrio parahaemolyticus*, *Bacillus* sp., *Spirillum* sp., *Aquaspirillum* sp. and others. Certain countries such as Italy, Canada, Brazil, Mexico, Chile, Argentina, are facing new kind of natural conservation on account of widespread jungle fires. Microbial diversity in forest soils is a key factor in ecosystem function. Staddon have described the role of fire and its impact on conservation of microbial diversity of forest soil. Large-scale endemic fires in Andes Mountain ranges increased the carbon content besides considerable increase in phosphorus, calcium, zinc and other trace elements. This contributed towards increase in number and variety of microorganisms in soil after second or third rains. National Biodiversity Conservation Board has taken interest in microbial diversity and its preservation. Twelve countries in the developing world have issued a declaration on 18th Feb 2002 requesting an international agreement to ensure fair and equitable sharing of benefits derived from world's biodiversity. Among the countries China, Mexico, India and Brazil claim to house nearly three quarters of the Earth's biodiversity. The declaration was also signed by Indonesia, Costa Rica, Colombia, Kenya, Ecuador, Peru, Venezuela and South Africa and declaration regarding potential need for sustainable utilization of biodiversity and promotion of conservation of various subsets of biodiversity as the wet-land ecosystems (Third Joint Work Plan or The Ramsar Convention) was done while the Convention on Biological Diversity in Hague in April and the United Nations World Summit on the Sustainable Development in Johannesburg, South Africa, in August emphasized on biodiversity and sustainable utilization by equitable protection and sharing of biodiversity and targeting to stop its loss to a halt by 2010. The corporate-led globalization and economic models imposed by WTO were also discussed and were found to be the main driving force and underlying cause of biodiversity loss. Forest conservations were reviewed with exclusion on large-scale monoculture tree plantations and time-bound action plants for stopping the convention of natural forests. The Convention also issued statement that a tough and clear stance on the spread of GM crops and genetic pollution, invasion by alien species that threaten the ecosystem as well as ban on terminator technologies is needed. An integral component of Conservation biology will be proper economic valuation. The direct value of microbes rests in their utilization in biotechnology, as single cell protein products, as biofertilizers, while indirect value involve their role as decomposers and involvement in recycling of plant and animal matter, as indicators of environmental pollution, as bioremediation agents and in other subtle functions of human life.

Utility of Microbial Diversity

Microbial diversity existing in natural ecosystems has the following major applications;

Biogeochemical role in cycling of matter: Soil acts as the source of nutrition for the growth of a spectrum of microorganisms which have remarkable ability to degrade a vast variety of complex organic compounds due to their metabolic

versatility. Microbes carry out recycling of phosphorus, oxygen, carbon, nitrogen and sulphur elements and replenish the environment with these by degrading substrates obtained from dead and decaying plant and animal remains. Haack have analyzed various factors that affected the accuracy, reproducibility and interpretation of carbon source utilization patterns of microbial communities. The replenishment is essential on account of reuse of vital elements and hence microbes work as efficient decomposers and scavengers to clean up the biosphere.

Sustainable land use: Microorganisms play a key role in soil fertility including its maintenance. They can act as indicators for assessment of sustainable land use in space and time. A combination and improvement of several concepts of sustainability may help obtain an integrated signal of functional ecophysiology of microbiota. Human encroachments and disturbances such as addition of pesticides affect the microbial components of an ecological niche and thus a simultaneous effect is observed on biotransformation reaction occurring in soil. Major processes as sulphur oxidation, nitrification and nitrogen fixation are inhibited by pesticide application. Requena has discussed the role of the mycorrhizal symbiosis in restoration of degraded semiarid and other ecosystems. Gadd reviewed the metal tolerance of VAM fungi at heavy metal contaminated sites. Large effects of mycorrhizae as in increasing accumulation of Cu, Ni, Pb, and Zn in grass *Ehrharta calycina* have been found, especially at low soil pH. Arsenate tolerance in plants appears to depend on modifications to the P transport system and the root absorbing capacity. The tolerant genotypes of plant were found to depend on VAM fungi for P uptake and consequent success. Moreover mycorrhizal fungi connect individuals of same or different plant species, enabling direct interactions between the members of a grassland or forest community and consequently mycorrhizal fungal diversity determines plant diversity, ecosystem variability and productivity.

Microbial products for agriculture purposes: Modern agriculture depends upon heavy input of chemical fertilizers that are produced by using non-renewable and constantly depleting petroleum based feed stocks. Thus use of biofertilizers that involve a spectrum of microbes including members of family Rhizobiaceae, certain actinomycetes as *Frankia*, cyanobacteria, free-living or loosely associated bacteria as *Azotobacter*, *Azospirillum* and certain, phosphate solubilizing fungi help in bioameliorating the important nutrients as nitrogen, phosphate to soil that in turn increases growth and yield of crop plants. Shen has given selective utilization of existing diversity of microbial species and their function, which present opportunities to improve agricultural production. Shen and colleagues have given two successful cases of its application in China; one is use of yield increasing bacteria (YIB), which are a mixture of several wild type bacteria as *Bacillus* sp. isolates from natural ecosystem using specific methods. YIB were found to promote crop growth and suppress some diseases. Even

mycorrhizal fungi increase levels of several resistances inducing inhibitory chemical (polyphenols and terpenes) production in plants. Further, mycorrhizae provide amino acids and hormones, which are secreted in high amounts thus increasing longevity of plants. The diversity of soil microbial communities can be key to the capacity of soils to suppress soil-borne plant diseases. The health of soil can be defined in terms of its microbiological capacity to suppress the activity of plant pathogenic microbes which could be general owing to aseptic activities of a myriad of undefined organisms or specific suppressiveness due to antagonism. Raaijmakers and Weller reported the concomitant increase in the population of fluorescent pseudomonads that produce antifungal antibiotic 2,4-diacetyl phloroglucinol (DAPG) in wheat monocropping system and thus specific, suppressiveness of take-all disease in wheat that increased the yield. Recently in India, DAPG production by *P. fluorescens* was reported to suppress rice bacterial blight. Kumar has reported *in vitro* antibiosis of three fluorescent pseudomonads isolated from rhizosphere/rhizoplane of crop plants against 7 fungal and 2 bacterial plant pathogens and thus exhibited the role in disease suppression and crop improvement. Hence, rhizosphere bacteria could be used as biocontrol agents for plant diseases. Kennedy isolated specific rhizobacteria and reported the management of weeds by these soil bacteria.

Biodegradation of Xenobiotics: Human kind is increasingly using pesticides as BHC, DDT, 2,4-D,2,4,5-T for getting rid of unwanted weeds, insect pests or pathogenic microorganisms. Removing chemical from the environment can be achieved by ease and in environment-friendly manner by biological methods that involve use of microbes and plants to degrade a xenobiotic compound and thus decontamination of the polluted site (Bioremediation) or purification of hazardous wastes in water (Biotreatment). Biological treatments are more effective as these methods convert toxic chemicals to less toxic ones and possess a significant degree of self-regulation. Microorganisms have diverse capacities to biotransform and, in some cases, to completely destroy toxic chemicals in our environment. Since these transformations alter the chemistry of the hazardous chemical, they may also alter toxicity, environmental fate, and bioaccumulation potential. Several halogenated chemicals such as the chlorinated aromatic compounds, which are major contaminants, nitroaromatics and other conjugated hydrocarbons-polluted contaminated sites could be reclaimed by use of the vanguard organisms isolated from contaminated sites by enrichment cultures. *Spingomonas paucimobilis* BPSI-3 that was isolated from PCB contaminated soil was observed to degrade halogenated PAHs and biphenyls. Head and Swannell reported bioremediation of petroleum hydrocarbon contaminants in marine habitats by anaerobic hydrocarbon metabolism via bioaugmentation and stressed to reject the approach of nutrient amendment as it can potentially exert as oxygen demand due to biological ammonia oxidation. Samanta have detailed the role of *Ralstonia* sp for the polycyclic aromatic hydrocarbon degradation. Pointing explained the feasibility of bioremediation by white-rot fungi. In nature majority reactions result in mineralization of the

contaminant but sometime recalcitrant formed during the process act as potent toxic compound than the original xenobiotic chemical. *Ps. putida* and *Burkholderia cepacia* have even been genetically engineered to cover a wider range of contaminants though pseudomonads possess metabolic plasmids too. Lajoie stressed the use of recombinant field application vectors for PCB and non-ionic surfactant degradation, as single microbe hardly possesses all the enzymes for mineralization of a xenobiotic. The specificity of the pollutant and the microbe degrading it depends upon the enzymes involved in the selective chemotaxis of the microbe towards the contaminant. The second phenomenon is being of great interest as it increases the bioavailability of a pollutant to bacteria. Recently, have described the selective chemotaxis of the microbe towards the site of contamination. As heavy metals are common contaminants worldwide and are a threat to soil quality and sustainability of natural soil resource, rescuing of the heavy metal contaminated soils by microbes (in situ bioremediation) is a low cost and effective tool to minimize environment pollution is in use today. Evdokimova have shown that though microbial diversity decreases in copper, nickel, cobalt and sulphur compound contaminated sites in Kola Peninsula, still the spectrum of fungi, bacteria and actinomycetes were found to bioconcentrate these heavy metals by volatilization or accumulation in cell capsules etc. *Ps. fluorescens* AF39 accumulated heavy metals such as nickel and others and the whole process was observed to be rapid and pH dependent. Biomarkers or technically biosensors are available now to obtain the presence of specific contaminant at a particular site.

Applications in Industry: Biotechnology has a practical definition that “it is application of power of microbe’s biosynthetic capabilities to manufacture high-value products that are too complex for chemical synthesis by economically viable processes, resulting in value addition and financial gains”. Soil microorganisms have been the most valuable source of natural products, providing industrially important antibiotics and biocatalysts. The development of novel cultivation-dependent and molecular cultivation-independent approaches has paved the way for a new era of product recovery from soil microorganisms. In particular, gene-mining based on the construction and screening of complex libraries derived from the soil metagenome provides opportunities to fully explore and exploit the enormous genetic and metabolic diversity of soil microorganisms which has resulted in isolation of novel biocatalysts and bioactive molecules including biocatalysts of industrial interest such as amylases, lipases, proteases, dehydratases, oxidoreductases and agarases. Richardson described a high performance amylase for starch liquefaction that was generated by combining environmental discovery evolution for the identification and optimization of the enzyme. Many microbes are extracted from soil and used in industrial production of various kinds as food processing and production, development of biocides, biocontrol agents, medicines and other natural products and pharmaceutical companies spend millions of dollars annually screening soil and litter for useful

microorganisms. The microbe itself could be a naturally occurring or even it could be genetically engineered through human ingenuity.

Microbial enzyme technology has in recent years grown to a multimillion dollar industry and exploration of microbial strain for discovering enzymes with novel properties as well as in discovery and production of other pharmaceutical products. Discovery of several novel genes isolated from new microbes provided phylogenetic similarities in divergent groups of microorganisms. Thus such information opens new ways to analyze and classify microbes on basis of new advanced methods. Determination of actual genes involved in causing disease by gene function studies and discovery of novel therapeutic products targeting these genes would be better nested approach rather than to know the whole genome of a pathogen. The ten most clinically important human pathogenic bacteria include approximately 1,500 to 6,000 genes. Thus deciphering the essential genes (genes that produce proteins required for growth and survival of bacteria) would elicit the process of production of new antimicrobial compounds inhibiting the growth process. A new programme ViaGene has been devised that predicts the essentiality of bacterial genes using a combination of DNA sequence descriptors and cutting edge pattern recognition methods. Watve have devised a mathematical model to estimate the number of antibiotics produced by genus *Streptomyces* of the order of 100,000 and suggested the decline in drug production from *Streptomyces* due to decline in screening efforts rather than the exhaustion of compounds. The oligophilic bacteria could be used as tools to monitor asepsis in pharmaceutical production units. A new area of interest and rediscovery is phage therapy that involves the use of bacteriophages for therapeutic measures i.e. for control of pathogenic bacteria in several countries of Eastern Europe and former Soviet Union. Biotechnologically phages could be utilized for control of environmentally problematic introduced genetically engineered microbes.

The microbiology of graves differs a lot at different stages of degradation of corpse. Hopkins et al have studied the microbiology of graves by experimental burial of seven pigs in hornbeam (*Carpinus betulus*) woodland in a forensic experiment in United Kingdom. Selected microbial characteristics of soils from 0-15 and 15-30 cm depths of graves of 3 pigs and control soil have been determined 430 days after burial. The grave soil showed more total carbon, microbial biomass carbon and total nitrogen and increased rates of respiration as well as increased nitrogen mineralization compared to control soil. Large alkali soluble sulphur concentration of soils from graves indicated reducing conditions in grave. The microflora also became selective for the aerobic and then anaerobic conditions prevailing in the graves. Several kinds of keratin-degrading fungi as *Trichophyton*, *Microsporum* etc appeared first, followed by bacterial infections of buried bodies. So, by knowing the changed microflora we can predict the death time (vaguely) of the organism and the depth of buried body.

Conclusion

The over industrialization in the world has forced the existing biota and abiotic factors to undergo drastic changes that have culminated to the extinction of diverse forms of flora and fauna. The role of microorganisms in maintaining the dynamic equilibrium and integrity of the biosphere is important because the existence of life is dependent upon the sustained, microbial-mediated transformation of matter in both terrestrial and aquatic environments. Presently, simple ecosystem plants and microbes are linked through recycling of different material. The ecosystem considers a diversity of plant organic compounds and a diversity of microbial species. Nutrient recycling efficiency from organic compounds to decomposers is then the key parameter that controls the ecosystems processes such as primary and secondary productivity and producer and decomposer biomass. The ecosystem predicts that microbial diversity has a positive effect on nutrient recycling efficiency and the ecosystem processes through either greater intensity of microbial exploitation of organic compounds of functional niche complementary, much like in plants. Microbial niche breadth and overlap should not affect ecosystem processes unless they increase the number of organic compounds that are decomposed. The present era of information big bang thus demands the better management and utilization of these bioresources and the enforcement of the information lacunae or the gaps regarding the critical analysis of the effective measures proposed to be taken to produce the biosphere. Microorganisms are significant gene pools and so must be regarded as cultural heritage and should be transferred to next generation in a normal and healthy condition.

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