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REVIEW ARTICLE

Carl Woese: from Biophysics to Evolutionary Microbiology

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Abstract This article is a tribute to Carl R. Woese, a biophysicist turned evolutionary microbiologist who passed away on December 30, 2012. We focus on his life, achievements, the discovery of *Archaea* and contributions to the development of molecular phylogeny. Further, the authors share their views and the lessons learnt from Woese's life with the microbiologists in India. We also emphasize the need for interdisciplinary collaboration and interaction for the progress and betterment of science.

Keywords Woese · Molecular phylogeny · *Archaea* · rRNA

Introduction

"Microbiology today has a new-found wealth far greater than any it possessed before. The source of that wealth is the universal phylogenetic tree—the framework essential for understanding organismal relationships. The power that flows from phylogenetic ordering permeates the field." These words of Carl Woese from 'The Prokaryotes' [56] exemplify the foresight and vision of this astute scientist, who was not a microbiologist by training.

Some of the greatest pioneers in microbiology were not microbiologists by training, but by interest, e.g. Antony van Leeuwenhoek and Louis Pasteur. Similarly, Prof. Carl Richard Woese (popularly known as Woese) was a biophysicist by training, but the fascination for microbes and his passionate desire to explore these hidden heroes of nature made him a pioneer in evolutionary microbiology [53, 57, 58]. Woese was born on 15th July 1928 in Syracuse, New York, USA. He obtained his BS degree in physics and mathematics from Amherst College. He received his doctorate in biophysics from Yale University where he further continued his postdoctoral training in the same field. He continued working as a biophysicist for two years at the General Electric Research Laboratory in Schenectady, NY (http://www.igb.illinois.edu/news/carl-r-woese-1928-%E2%80%93-2012). In 1964, he joined as a faculty in the Department of Microbiology at University of Illinois, Urbana, USA and spent his entire scientific carrier in the same department. Woese died of pancreatic cancer on 30th December 2012 at the age of 84 (http://www.igb.illinois.edu/news/carl-r-woese-1928-%E2%80%93-2012).

At University of Illinois, Woese focused his attention on bacteria and archaebacteria and extensively studied the protein making machinery, the ribosomes and the rRNA genes of these organisms. Woese, described for the first time that bacteria evolve and show phylogenetic relationships [22, 54, 55, 57]. Based on his studies, Woese established a system for calculating the phylogenetic distance between the organisms [57]. Prior to his work, microbiologists merely had the knowledge of taxonomy and followed the traditional method of studying phenetic traits to differentiate the species. The information related to bacterial phylogeny was completely lacking then. In 1990, based on the reconstruction of rRNA phylogenetic tree, he proposed a separate domain for Archaeabacteria and introduced the concept of three domains of life: *Bacteria*, *Archaea* and *Eucarya* [50–53, 58]. The small subunit (SSU) rRNA based tool of Woese became so powerful that it revolutionized the world of microbial evolution and phylogeny and opened avenues for the study of complex microbial communities using metagenomics [26]. Woese

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was so impressed with microbes that in his interview with New York Times in 1996, he said that if all multicellular organisms are wiped off from the earth then it would barely affect the microbial community, whereas the destruction of microbial community would lead to instant death of all life forms on earth (http://www.nytimes.com/2013/01/01/science/carl-woese-dies-discovered-lifes-third-domain.html?_r=0).

Woese earned many honours during his life for his path breaking findings. Along with several other honours and awards, he received the Leeuwenhoek Medal from Royal Netherlands Academy of Arts and Science in 1992 and National Medal of Science in 2000. (http://www.news-gazette.com/news/university-illinois/2012-12-30/visionary-ui-biologist-carl-woese-84-dies.html).

If it was not for Woese's enormous contribution and dedication towards microbial phylogeny and ecology, microbial evolution would not be understood experimentally. The group of Microbial Culture Collection, Pune is obliged to share his findings with Indian microbiologists and students by means of this obituary. Woese's life is a lesson for any individual embarking on interdisciplinary collaboration and interaction with an aim to better the future of microbiology.

Woese's Contribution to Microbiology

Woese's Early Work

As mentioned earlier, Woese was trained as a biophysicist. He first entered into microbiology during his postdoctoral period at Yale University in mid-1950. Initially he studied the effect of radiation (X-ray) on inactivation and survival of *Bacillus* spores and also worked on nucleic acid metabolism during the germination of *Bacillus* endospores [47, 48]. He reported that *Bacillus* spores were inactivated in both single or multi-hit exposure experiments [48] and that metabolism of nucleic acid differs in both germinating spores and vegetative cells [47]. Later, he diverted his attention on ribosomal development and inclined his study towards evolution of genetic code [49].Woese was interested in the evolutionary history of nucleic acids and for that he selected ribosome as a target. Because from his earlier work he knew that ribosomes are most conserved elements in all organisms, it would work as an ideal molecular chronometer [21]. He initiated this work in 1966 using oligonucleotide cataloguing, a labour intensive method for nucleotide sequencing. In this method, RNA fragments appear as fuzzy spot on x-ray film and reading these spots was a very cumbersome job in those days. Worldwide, only 2–3 scientists were aware with this method and Woese was one of them. While Woese was able to read his first catalog in 1 year [21], it took him a decade of hard work to catalog 60-bacteria from different groups. Finally in 1977, based on his findings he published the tripartite distribution of life and announced that bacteria are different from archaeabacteria [57].

Proposal of the Domain *Archaea*

Woese introduced the term 'Archaea' (singular: Archaean) as 'archaebacteria' was misleading and not precise. Later on, 'Archaea' was well-received and widely accepted in the textbooks of microbiology and evolutionary biology.

Archaea have special features not detected in eukaryotes and bacteria, such as the presence of pseudomurein, ether-linked lipids and flagellin proteins. Initially it was considered that these groups of microorganisms only inhabit extreme habitats like hot springs (thermophiles), brine lakes (halophiles), and habitats with extremely low and high pH (acidophiles and alkaliphiles respectively). Later developments in culture-independent approaches proved that *Archaea* are widespread in different kinds of habitats including the arctic cold water, thermal vents, rice fields and other terrestrial and fresh water habitats and constitute a major part of the microbial life on Earth [4, 18, 27]. Along with the ubiquitous distribution, *Archaea* are also a part of carbon and nitrogen cycling, produce industrially important enzymes, help in anaerobic digestion, biogas production and metal leaching, etc.

Like many exceptional discoveries, the proposal of *Archaea* did not receive wide acceptance at the beginning. He, along with George E. Fox reconstructed the tree of life in 1977 and proposed that Archaeabacteria are different than eubacteria and originated separately [57] during the course of evolution. But he faced much criticism from Nobel laureate Salvador Luria and well known environmental biologist Ernst Mayr. Even then, Woese continued his work on the same aspect without being distracted by the scientific hostility [http://home.planet.nl/~gkorthof/korthof88.htm]. In 1990, he proposed the concept of three domains of life (*Bacteria*, *Archaea* and *Eucarya*) and shortened the term Archaeabacteria to Archaea. Apart from scientific hostility, the criticism faced by Woese was based on several other factors. First, his bold conclusions were based on a single and short gene sequence (SSU rRNA) which was presumed to lack enough phylogenetic information. Second, it was a challenge to the well-established prokaryote–eukaryote dichotomy. Later, in 1990, the term Archaea and the concept of the three domains were widely accepted after the publication of the whole genome sequence of *Methanococcus jannaschii* and confirmation of Woese's finding by using alternative methods [5].

Small Subunit (SSU) rRNA as a Tool for the Study of Microbial Evolution

In 1965, Zukarkandl and Pauling [60] showed that molecules are the documents of evolutionary history of organisms and can be used for inferring phylogenetic information. However, until late 1970s, the field of microbial evolution and phylogeny was overlooked. Most of the microbiological textbooks in that era were dealing with phenotypic and biochemical-based taxonomy (chemotaxonomy) and were lacking phylogenetic data. With Woese's revelation that bacteria can evolve and show phylogenetic relationships based on the use of SSU rRNA as a molecular marker (molecular chronometer) for the study of microbial evolution revolutionized the area of microbial phylogeny and ecology. Since this first publication on the use of rRNA for microbial phylogeny in 1977, a number of other good articles have been published on different aspects of rRNA including its structure, role in depicting the microbial phylogeny as an ideal marker [22, 23, 38, 46]. Until 1980, microbiologists disregarded SSU rRNA gene sequencing, but later it emerged and got established as an essential tool for microbiology. Further, the discovery by Norman Pace (a collaborator of Woese) that cultivation of microorganisms for the study of phylogeny was no more essential [24], merely cloning and sequencing of SSU rRNA gene or other molecular markers from total community DNA was sufficient to get phylogenetic information about the community. These studies of Woese and Pace revolutionized the area of microbial phylogeny and ecology, respectively, which were largely neglected before. The aforementioned findings provided an opportunity to apply SSU rRNA as a universal tool for the study of microbial community in natural habitats, which in turn gave rise to the concept of metagenomics.

Contribution to Microbial Ecology and Diversity

Woese's method of using rRNA as a molecular chronometer may be regarded as the greatest invention that has immensely contributed in the development of microbial ecology and diversity and where it stands today. Although Beijerinck and Winogradasky also contributed to microbial ecology by establishing the role of microorganisms in biogeochemical cycling, the drawbacks of the cultivable approach proved as a limiting factor in developing a complete understanding of microbial ecology. Apart from contributing to microbial systematics and phylogeny, SSU rRNA gene based study of community structure, function and dynamics has extensively revolutionized this field. The work of Woese and Pace was instrumental in the development of culture independent approach as a tool to study community structure, function and dynamics. Since the past two decades, the use of culture independent approach has become a mainstay in the field of microbiology which was crushed since the time of Winogradasky. Today, almost all methods of microbial community profiling, including next generation sequencing are dependent on rRNA gene analysis [2, 14, 19, 34, 40]. It gives better insights not only into community structure (richness) and distribution (evenness), but also permits examination of consequences of external factors (pollutants and climatic perturbations) on the survival and dynamics of microbial communities in different ecological niches. In brief, one can say that Woese's work opened avenues for applying existing ecological theories to the field microbiology, thus establishing microbial ecology as a full-fledged discipline.

Apart from providing the valuable inputs in community structure and dynamics (spatial and temporal variations), SSU rRNA based approaches are now widely used to study *in situ* functionality of microorganisms even in highly complex ecological habitats (human gut, thermal vents, polar environment, etc). Development of SSU rRNA based group specific primers and probes has enabled quantification and *in situ* detection of specific functional groups using quantitative real time PCR and TaqMan® chemistry [17]. In addition, its combination with stable isotope probing methods have aided in extracting information on substrate utilization potential of microbes in their natural environment [44]. Its use has not only advanced our understanding in soil ecology and biodiversity but has added valuable knowledge towards rapid diagnosis of disease and study of host-microbe interactions [10]. It is now understood that conclusions drawn from the gut-microbiome studies are likely to provide valuable clues towards solving problems related to human health and disease [20, 26].

The use of SSU rRNA has also supplemented the efforts to cultivate novel organisms whose existence was previously unknown due to technical limitations. Before the use of rRNA gene based culture independent approach, microbiologists had only limited knowledge about microbial diversity. Due to limitation and biases of cultivation based approaches, they were able to mostly cultivate the fast growers but lacked awareness about ecologically significant slow growing microbes [32]. The discovery and use of SSU rRNA gene has revealed the 'black-box' of microbial diversity and corroborated the pre-existing evidence of great plate count anomaly and dormancy related to existent microbial seed bank in nature [31]. It not only widens the window of microbial diversity but allows for answering questions like 'who is there and dominating?' and 'what types of habitats are suitable for growth of which groups of organisms?' thus giving information unique to the niche under study [32]. Upon obtaining information about existence, dominance and ecological importance of particular group of microorganism using culture independent approach, it has now become possible to cultivate key organism using modified media and culture conditions [32]. Several strategies have been developed to cultivate the novel groups of microorganism in order to study them closely under controlled laboratory conditions to facilitate research and industrial exploitation [1, 11, 12, 20, 59]. Cultivation of abundantly distributed marine bacterioplankton of SAR-11 clade and *Acidobacteria* are a testament to such exploitation [15, 36]. This has resulted in accumulation of vast amounts of data pertaining to microbial diversity of diverse niches resulting in discovery of several novel groups of microorganisms due to insights gathered from systematic and organised culture independent approaches. Literature on cultivation and diversity suggests an increase in number of new taxa reflected in various culture repositories around the world and by their sequences being deposited in sequence databases (http://rdp.cme.msu.edu/).

In brief, Microbial Ecologist and Taxonomists should be highly indebted to Woese for his findings, which immensely contributed to microbial ecology and diversity. These modern approaches are a direct culmination of Woese's discovery and use of SSU rRNA gene sequence as an ideal biomarker for study of evolutionary history of microorganisms. This has renewed the zeal in detailed study of microbial taxonomy post Winogradsky and Beijernkk era thus returning microbial ecology to the forefront of many microbiological exploration initiatives.

Metagenomics, Microbial Taxonomy and Ecology Research in India

Advances in science at international level have also had their impact on science in India and Woese's work is no exception to that. Once 16S rRNA gene sequencing became a mandatory requirement for the description of taxa, several investigators from India used it in their description of new taxa. As discussed earlier that cultivation and taxonomical characterization is essential part of microbiological research even in the current era of genomics and proteomics [32]. In past 10–15 years Indian research in microbial cultivation and taxonomy has made good progress and till date we have described more than 300 novel taxa in International Journal of Systematic and Evolutionary Microbiology from different niches (http://www.bacterio.cict.fr) [8, 9, 29, 30, 37]. In the past few years there have been studies on microbial diversity of diverse habitats including the Western Ghats [45], psychrophilic habitat of Pangong Lake, Antarctic and Arctic soil and sediments [6, 7, 42], effluent treatment plant and pesticide contaminated dumpsite [3, 35, 39, 41] marine sediment [43] and human gut [9, 20, 25]. Some laboratories have also focused their attention on SSU rRNA gene sequences and developed a phylogenetic framework, detected species specific signature sequences and designed improved PCR-primers using bioinformatic tools to improve the taxonomic resolution and environmental detection of related microorganisms [13, 16, 28, 33]. All these studies are a proof that research in microbial cultivation, taxonomy and ecology have substantially progressed in India over the past decade and Woese's contribution is undeniable.

Conclusion

Woese's journey from biophysics to evolutionary microbiology itself indicates that there is no interdisciplinary limitation to be successful in any given area. Even knowledge of a different discipline can augment scientists' ability to harness the complete potential of a new discipline. Apart from Woese, there are many eminent scholars, such as Nobel Laureate Venkatraman Ramakrishnan (physicist) who used the interdisciplinary approach in order to reach the summit of their areas of interest, in this case, solving the crystal structure of bacterial SSU rRNA. It is evident that no discipline is self-sufficient and needs collaboration and interaction with other disciplines for gaining a broader perspective of the problem. For example, in order to carry out a full-fledged study in microbial ecology (study of community structure and dynamics), there is a need of expertise in geochemistry, microbiology, bioinformatics and biostatistics. Rarely can a single scientist do the entire job in order to arrive at a logical conclusion on the acquired data. In our view, due to inculcation of mathematics and physics in his early career, Carl Woese was able to understand and interpret the data to develop a metric system using SSU rRNA for the calculation of phylogenetic distance, which otherwise was a very difficult job for a pure microbiologist without any mathematical knowledge. Woese's journey highlights the need to promote interdisciplinary collaboration between scientists from different fields to explore any discipline in its fullest form. It is very intriguing that the situation in Indian science is different from the scientific scenario in other parts of the world. Even today, we are following the old tradition and have restricted ourselves in a very strict boundary of distinct disciplines like 'botany', 'zoology', 'biotechnology', 'microbiology', 'biochemistry', 'environmental science', etc. Interactions between collegiate academicians and scientists are rare, and may be ascribed to internal resistance and/or the feeling of superiority of a certain discipline over others. Areas of microbiology like microbial ecology, fermentation technology and population genetics need essential inputs from mathematicians and statisticians. In conclusion, there is an imminent need to cross the boundaries of discipline and promote interdisciplinary collaboration in order to expand and explore the microorganisms in a broader context, as was achieved by Woese.

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