International Journal of Inclusive Development
Citation IJID: **8**(01): 105-112, June 2022
DOI: 10.30954/2454-4132.1.2022.23
Peer-Reviewed Journal

As Study on Plant Pathology Perspective of Fungal Genome Sequencing in Manipur

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Received: 15 Mar., 2022 **Revised:** 29 May, 2022 **Accepted:** 02 June, 2022

Abstract

Plant Pathology perspective of Fungal Genome Sequencing in Manipur is a first glimpse into the genomic basis of the biological diversity of filamentous fungi and yeast. The genome sequence of the budding yeast, with a small genome size, unicellular growth, and rich history of genetic and molecular analyses was a milestone of early genomics. The subsequent completion of fission yeast, and genetic model, *Neurospora crassa* initiated a revolution in the genomics of the fungal kingdom. In due course of time, a substantial number of fungal genomes have been sequenced and publicly released, representing the widest sampling of genomes from any eukaryotic kingdom,

Keywords: Fungal, Genome, Genetic, Growth, Ecology

Plant Pathology perspective of Fungal Genome Sequencing in Manipur is a first glimpse into the genomic basis of the biological diversity of filamentous fungi and yeast. The genome sequence of the budding yeast, with a small genome size, unicellular growth, and rich history of genetic and molecular analyses was a milestone of early genomics. The subsequent completion of fission yeast, and genetic model, *Neurospora crassa* initiated a revolution in the genomics of the fungal kingdom. In due course of time, a substantial number of fungal genomes have been sequenced and publicly released, representing the widest sampling of genomes from any eukaryotic kingdom. An ambitious genome-sequencing program provides a wealth of data on metabolic diversity within the fungal kingdom, thereby enhancing research into medical science, agriculture science, ecology, bioremediation, beanery, and the biotechnology industry. Fungal genomics have higher potential to positively affect human health, environmental health, and the plant's stored energy. With a significant increase in sequenced fungal genomes, the known diversity of genes encoding organic acids, antibiotics, enzymes, and their pathways has increased exponentially, Currently, over a hundred fungal genome sequences are publicly available; however, no inclusive review has been published. This review is an initiative to address the significance of the fungal genome-sequencing program and provides the road map for basic and applied on genome complexity, sequencing data volume, read length and quality. We benchmarked the most requested assemblers. Monopole polishers on our sequencing data. The assembly performed with canu and polished with Medaka was considered the most full and accurate.

How to cite this article: Devi, L.B. (2022). As Study on Plant Pathology Perspective of Fungal Genome Sequencing in Manipur. *Int. J. of Inclusive Develop.*, **8**(01): 105-112.

Source of Support: None; **Conflict of Interest:** None

After further elimination of redundant consigns using Purge Haplotigs, we achieved a high-quality genome of total length completeness. We also obtained a complete circular mitochondrial genome with a length of 38.7 kb. The achieved assembly expands studies on *F. oxysporum* and plant-pathogen interaction in flax (Banerjee, 2001).

In the present work, a highly pathogenic isolate of Fungal Genome sequencing which is the most harmful pathogen affecting flax was sequenced for the first time. To achieve a high-quality genome assembly, we used the combination of two sequencing platforms-Oxford Nanopore Technologies with long noisy reads and Illumine with short accurate reads. Given the quality is crucial for Nanopore sequencing, we developed the protocol for extraction of pure high-molecular-weight DNA from fungi. Sequencing of NDA extracted using this protocol allowed us to obtain about genome coverage with reads and coverage reads. Several tools; were developed for genome assembly; however, they provide different results depending widely used for the production of seeds and fiber. Flax seeds in healthy alpha-linolenic acid, lignans, and soluble dietary fibers. They are of great medicinal and nutraceutical value and are potential functional animal feed for improving reproductive capacity and quality of meat, eggs, and milk. Linseed oil is used in the production of paints, enamels, and resins while flax fiber is used in textile and composite industries Fungal Widespread diseases of flax-fusarium wilt, which leads to estimated yields losses of 20% and in some cases up to 100%. The pathogen is soil-borne, and infection occurs mainly through the roots. Then it spreads inside the vascular tissues, causing water and nutrient blocking, and eventually leading to wilting, yellowing and browning of top parts of the plants, and finally death, molecular genetic studies are the basis for revealing the origins Manipur, Imphal, whole Genome Sequencing spanning different ethnic, linguistic and socio-cultural sections of the northeastern states will be carried out as part of the Genome India Project, launched in the region on Manipur.

Plant Pathology to interact with one another without the need for third parties. Financial transactions or legal communication are examples of interactions that traditionally rely on third parties to complete the work. In this regard, are advantageous because, rather than relying on the competence and trustworthiness of third parties, they enforce contracts and agreements using solid code (Anand, 2003).

Fungal genome sequences' are a group of heterotrophic eukaryotic organisms which are highly versatile and occupy almost all the natural habitats. Less than known fungi species are strict saprophytes and are able to colonize plants. Many economically important crops are prone to diseases by a small fraction of these saprophytes. Fungal phytopathogens have become a serious factor in the economy of crops as they are responsible for causing wide spread and devastating epidemics and also significantly affect the annual rop yields. It is because of these reasons, they have attracted the attention of farmers as well as plant breeders and scientists (Vishwakarma *et al.* 2013).

Fusarium is a devastating phytopathogenic fungi belonging to Ascomycota. This filamentous fungus cans strike any crop since it has a broad host range which includes rice, wheat, almost all horticultural crops, ornamentals and almost all other agricultural commodities. The Fusarium species have a wide host range including banana, cabbage and tulip. Many economically important diseases are caused by Fusarium species such as Fusarium rot on apples by Fusarium species, sugarcane wilt in sugarcane (Celler, 2005).

Fungal Genome Sequencing Fusarium species are present in soil as well as on above-ground and subterranean plant parts, plant debris, and other organic substrates. They are commonly present in tropical and temperate regions and are also found in extreme climatic conditions like deserts, alpine and arctic regions. Fertile and cultivated land souls show the presence of many Fusarium species in comparison to forest uncultivated soil where it is less prevalent. Mostly Fusarium species are abundantly present in soil thus regarded as soil borne fungi and are associated with roots in the form of parasites and saprophytes. In the aerial plant parts, the species have active and passive means of dispersal and hence are able to cause many diseases of economic impact. Airborne Fusarium species are rarely found in the cultures obtained from soil or the roots of plants. Fusarium species can grow on

a variety of substrates and have efficient dispersal mechanisms owing to their worldwide distribution Fungal Genome Sequencing in Manipur.

From a taxonomic point of view, Fusarium species produce three types of a sexual spores namely macroconidia, microconidia and chlamydospores. Conidia are produced on monophialides and in sporodochia. Microconidia are predominantly uninucleate and germinate poorly and variably, with efficiency of germination ranging from 1-20%. On the other hand, macroconidia are multinucleate, produced abundantly and have the ability to germinate rapidly and hence, help in the efficient reproduction the fungus. Chlamydospores are viable, accessory spores are produced mitotically. They are produced due to structural modifications of vegetative hyphen segments or conidial cells and mainly have newly synthesized cell wall material stored in them. Their main function is to help the fungus survive in the soil. A sexual reproduction in *Fusarium* sp. is accomplished by macro conidia and micro conidia, while sexual state of the fungus has not been observed (Li, 2007).

Fungal Genome Sequencing Yellowing and wilting of the lower leaves of the plant is the main symptom of infection. Many other symptoms are seen including root browning or purpling of the vascular tissue, spots on the leaves and a dry and crumbly decay. If the fungus is observed, its color appears to be whitish, reddish, yellow or dirty tan. The most difficult part about fungal infection is that by the time the infection is detected, the plants are about to die and cannot be rescued neither by applying fungicides nor by biological controls. The identification of infection also doesn't prevent the spread of the fungus to other fields Fungal Genome Sequencing.

Till date, effective and eco-friendly methods have not been devised for the control of this devastating pathogen. Therefore, utilizing a biological method is a tactful choice. Butt *et al.* (2006) stated three biological methods for degenerating the entophytic fungi or to control the fungi-by transposable elements, by mycovirus and by swtiching off or silencing off the genes by DNA methylation. A novel biocontrol attempt using the hypovirulence factor against root diseases of fruit trees has been proposed. In the field, there are many strains within a species of fungi exists. Some of them are virulent whereas others are hypo virulent. The hypo virulent isolates are of two kinds, some of them are genetically predisposed to by hypo virulent while others became hypo virulent because of infection by one or more myco viruses. From a phyto-pathological perspective, both of these can be developed as biocontrol agents (Kazian, 2004).

Mycoviruses are the viruses that infect fungi. Many mycoviruses lead secret lives and can also reduce the virulence of the fungi by a phenomenon known as hypo virulence. Since the fungal diseases hold a lot of significance in agriculture and strategies for their control and prevention are very limited, mycovirus induced hypo virulence has gained a lot of attention worldwide. Ecologically and intellectually, the concept of using one pathogen to control another pathogen is quite interesting. A based infectious reverse genetics system has been developed for the microbial members of Hypoviridae family. This has led the scientists to analyze the basis aspects of this virus-fungus-plant interaction and also the interactions between virus and its hosts. Most of the mycoviral infections do not produce any pathogenic symptoms and are hence, latent. But certain mycoviruses interfere with expression of genes involved in biological functions. This leads to modifications in the phenotype and growth rate of the fungal host. Fungus infected by a mycovirus becomes hypo virulent and cannot cause disease in the host. It also exhibits a debilitating phenotype when it is cultured. This is characterized by abnormal colony formation and a reduction in growth rate. Many significant changes in morphology and physiology are caused by virual infections such as altercations in cytological characters and novel attenuated phenotypes related to virulence. Importance of viral infections in environmental health research is due to their effect on the levels of production of toxins and metabolites Fungal Genome Sequencing in Manipur (Sanders, Mark Frederick, 2009).

Hypo virulence caused by mycoviruses is a biological phenomenon thereby the disease causing ability of the fungal pathogenesis reduced or completely lost. Hypo virulence is considered to play an important role in counter-balancing the plant diseases in nature. Hypo virulent or incapacitated strains of plant-pathogenic fungi that carry these transmissible viruses have attracted

a lot of attention because they can be potentially employed as biological agents for the control of fungi and can be used a saprobes for deciphering the mechanisms of fungal diseases. Myco viruses are cryptic in nature, this is because in most of the cases, the fungi which have been infected with myco viruses show no observable symptoms and are identical in phenotype to non-infected fungi of the same species. Because of this peculiar reason, the only way to diagnose an infected fungus is to detect viral genome, most likely a double stranded RNA. Double-stranded RNA is an imperative non-specific indicator of the presence of RNA viruses in bacteria, fungi and plants. It represents the genome of an RNA virus or its explicative form. An accurate measurement of the size can provide vital information pertaining to the virus-like particle infecting a host. If it is isolated and cloned, it can be used a specific diagnostic probe without the need for purifying the virus itself. Analysis of the sequence of these clones can deliver significant information for the taxonomic positioning of these virus-like particles associated and can also help greatly in the identification of the virus. If microbial infection is found to adversely affect the pathogen city of a fungus, then they an be used as potent means of biological control against fungal diseases.

Basically present in four phyla of the true fungi i.e. Chytridiomycota, and Basidiomycota. Fungi are frequently infected by two or more non-related viruses and also with defective and satellite Ghabrial and Suzuki, 2009). Some viruses use fungi as vectors and are distinct from mycoviruses because they are not able to reproduce in the fungal cytoplasm. It is generally thought that the natural host range of mycoviruses is confined to only closely related vegetative compatibility groups that allow cytoplasmic fusions. However, some mycoviruses can replicate in taxonomically different fungal hosts also. Mitoviruses found in the *Fusarium coeruleum* and *Fusarium globosum* and other two fungal species *Ophiostoma novoulmi* and *Sclerotiniahumoeocarpa* are the best examples of such viruses. Mycoviruses are commonly found throughout the important taxonomic groups of filamentous fungi and most mycoviruses having double stranded RNA are classified into eight families base their physiochemical properties – Birnaviridae, Partitiviridae, and Reoviridae. The mycoviruses present in Fusarium species till now have been placed in four families based on phylogenetic analyses. Even though mycoviral have been reported to be present. The association of these viruses with hypovirulent traints in the host organism has been suggested only for a few isolates which include *F. graminearum* strain Fungal Genome Sequencing in Manipur.

Plant Pathology mycovirus associated hypovirulence is not well characterized in genus Fusarium therefore present investigation was aimed to the isolate and characterized hypovirulence associated mycovirus from Fusarium species.

Specific objectives

1. Isolation and molecular characterization of Fusarium species.
2. Genetic diversity analysis of isolated fungal strains using random amplified polymorphic DNA.
3. Isolation and molecular characterization of double stranded RNA mycovirus from Fusarium species.
4. Assessment of the pathogenesis of hypo virulent and cured virulent strains of Fusarium species on different fruits.

The national institute of the Department of Botany and it will be actively collaborating with the Genome India initiative undertaken by the Centre Research at the Indian Institute of Science.

The project will also lead to a new understanding of the different ethnic groups of India – a country with the highest diversity of ethnic groups, and which puts India in a unique position for the mapping of the human genome.

The objectives of this initiative are to systematically document the genetic information from whole genome sequencing for thousands of Indian individuals belonging to different geographical locations and diverse population groups across the country, to facilitate genome wide association studies at a cheaper cost in India for any genetic disease or trait.

For instance, when you buy a house, you end up paying hefty sums to brokers and concerned parties. When real-estate transfer moves to dapps, it will only involve the seller and the buyer, obviating the

need for a middleman and their commissions. That's the promise being offered by dapps or decentralized apps. But are just platforms and have native tokens incentivizing the developers. And you can take a closer look at some tokens with crypto exchange platforms like little money for household expenses. In addition to that, old age related health issues are an additional burden," said Dinabandhu Sahoo, Director of Manipur, Imphal-based Institute of Botanical and Sustainable Development.

"This burden can be reduced by personalized medicine which can be achieved by use of the genetic information," said Sahoo while launching the project for the northeast. Genomics of the fungal kingdom: insights into eukaryotic botany Fungal Genome Sequencing.

The help in identification of the genetic disease burden of specific subsets of populations, which will have "far-reaching implications" in improving the healthcare landscape of this region, scientists involved.

"The people of Manipur, Imphal have many things which are unique and the genetic diversity of the people is not well understood. The genome, or genetic material, of an organism, bacteria, virus, fungi, or human is made up of DNA. Each organism has a unique DNA sequence, which is composed of bases. If you known the sequence of the bases in an organism, you have identified its unique DNA fingerprint, or pattern. Determining the order of bases is called sequencing. Whole genome sequencing is a laboratory procedure that determines the order of bases in the genome of an organism in one process.

The use of whole genome sequencing produces large amounts of data. These data can be stored in which is a publicly accessible database. As more fungal genomes are analyzed and their data become publicly available, they can serve as reference genomes during other investigations. Public access provides the scientific community the most up to date and comprehensive DNA sequence information to aid outbreak investigations.

Despite the importance and utility of fungi, until quite recently what was known about their genomes was primarily derived from the sequence of the yeast *Saccharomyces cerevisiae*. But there has been an explosion in fungal genomics that has greatly expanded our view of the genetic and physiological diversity of these organisms. We provide here an overview of available fungal genomes and highlight some of the biological insights that have been derived through their analysis. We also discuss insights into the fundamental cellular biology shared between fungi and other eukaryotic organisms. These highlights are not intended to be comprehensive. Specifically, we focus on results derived from whole-genome analysis of fungi other than yeasts, as the genomics of *S. cerevisiae* and related organisms is covered elsewhere in this issue.

The revolution in fungal genomis has been driven by the evolution of genome sequencing technology. Current whole genome shotgun sequencing and assembly technologies produce fungal genome sequences with unparalleled accuracy and long-range contiguity at ever-reduced cost. These methods represent an advance over the clone-by-clone approaches used to sequence the first eukaryotic genomes. The clone-by-clone approach relied on labor-intensive clone-restriction mapping to pick sequencing templates, and required separate shotgun libraries for each clone to be prepared, tested, sequenced, and assembled. Ultimately, these maps were not sufficient to protect against both unnecessary overlap and errors originating both with the maps and sequencing. The adoption of more efficient high-throughput sequencing methods coupled with the simplicity of WGS strategies has greatly accelerated the pace of genome sequencing while dramatically reducing costs. Advances in assembly algorithms the inclusion of end sequences from large insert clones routinely yield assemblies with high-sequence quality and continuity.

A special case of repeated sequences are diploid genomes. In diploids, the extent of heterozygosity can vary dramatically across chromosomal regions. Regions of low polymorphism will be incorrectly merged during assembly, while highly polymorphic regions are separated. Consequently, alletlic differences are difficult to distinguish from distinct prologs. When possible, these complications have been avoided by sequencing a haploid form of the organism, or minimized by sequencing a loosely related haploid as an aid. But in many cases, such as with Candida abicans, sequencing a diploid is unavoidable.

New assembly algorithms are being developed to more accurately assemble whole-genome sequence data from diploid data sources. Ongoing advances in sequencing technology also promise to further revolutionize fungal genomes. Although much work is still needed to optimize and fully validate these new approaches, their value is already apparent. For example, pyrosequencing methods implemented Life Sciences, have successfully generated sequence from N. crass that could not be acquired through conventional sequencing methods. These sequences were found to be rich, which likely precluded efficient cloning in bacterial libraries. New instruments also provide the ability to inexpensively produce amounts of data, albeit consisting of short reads – tens to hundreds of base pairs per read compared for conventional Sanger. The potential cost reduction enables more strains or species to be sequenced for the current cost of producing a single genome. While early efforts have focused on producing high-quality reference sequences for individual strains or species, these new technologies will propel us to more fully describe the molecular diversity within related strains.

The subsequent sequencing of additional fungal genomes provided the opportunity to study intron dynamics over a wider evolutionary distance. With these additional data, Stajich and colleagues studied the patterns of intron gain and loss across fungi spanning nearly the fungal kingdom. With Homo sapiens and *Arabidopsis thaliana* as out groups, the authors developed a maximum likelihood approach to estimate loss and gain events and thereby calculate densities at various nodes in the fungal tree. Based on a set of more than orthologous protein coding genes, the authors found numerous positions shared among plants, animal, and fungi, and they concluded both that these were common and present at the origin of the eukaryotic crown. Since the fungal last common ancestor, nearly all lineages were predicted to have suffered substantial intron loss, with particularly significant loss occurring at deeper branches and at the outset of the Hemiascomycete lineage. Interestingly, these authors also find gain to be as significant as loss in several recent lineages including the Euascomycetes consistent with and the lineage leading.

First, the sequences would be of value of the research community, serving to accelerate efforts to understand gene function by using model systems. Second, the experience gained would inform approaches to sequencing the human genome and other similarly sized genomes. Third, functional relationships between sequences of different organisms would be revealed as a consequence of cross-species sequence similarity. Ultimately, with the involvement of more than one thousand scientists worldwide, two human genome sequences were published in 2001. With this development came established methods and analysis

One noteworthy observation coming from the comparison of multiple genome sequences is how divergent fungi are at the genome level, despite apparent morphological and physiological similarities. For example, comparisons of the genomes of *Magnaporthe grisea* and *N. crassa*, related ascomycetes thought to have shared a common ancestor as recently as 200 million years ago ; revealed an average amino acid identity of only 47% and virtually no conserved synteny. Only 113 regions were identified containing four or more genes in conserved colinearity. More generally, analyses of available complete fungal geomes reveal a rapid breakdown of conserved synteny over a relatively shot evolutionary time span (data not shown). Even members of the same genus can display a remarkable divergence at the genomic level. A comparison of three species of *Aspergillus* – revealed only 68% average amino acid identity between any pair of species, an evolutionary distance comparable to that between human and fish. At this distance, roughly of A. indulines could be mapped to a systemic block with either more than one thousand scientists worldwide, two human genome sequences. With this development came established methods and analytic standards that were used to sequence other large genomes.

A major challenge for de nova sequencing, in which sequences are assembled for the very first time (such as with the is the production of individual DNA reads that are of sufficient length and quality to span common repetitive elements, which are a general property of complex genome sequences and a source of ambiguity for sequence assembly. In many of the early de novo whole genome sequencing projects, emphasis was placed on the

production of so-called reference sequences, which were of enduring high quality and would serve as the foundation for future experimentation.

Despite these advances, a number of challenges remain. Repetitive sequences present the single biggest difficulty in assembling sequence data. The modest level of repetitive sequence ameliorates this problem in most fungi. However, the high identity repeats associated with telomeres, centromeres, arrays remain difficult. Often these regions are not cloned in bacterial libraries, while in other cases these regions are cloned and sequenced but not correctly assembled. Although follow up analyses can accurately reconstruct telomeres, more robust automated methods are needed, as are independent mapping methods for assessing the size and position of these difficult to sequence regions.

A special case of repeated sequences are diploid genomes. In diploids, the extent of heterozygosis can vary dramatically across chromosomal regions. Regions of low polymorphism will be incorrectly merged during assembly, while highly polymorphic regions are separated. Consequently, allelic differences are difficult to distinguish from distinct paralogs. When possible, these complications have been avoided by sequencing a haploid form of the organism, or minimized by sequencing a closely sequencing in which sequences are assembled for the very first time is the production of individual DNA reads that are of sufficient length and quality to span common repetitive elements, which are a general property of complex genome sequences and a source of ambiguity for sequence assembly. In many of the early de novo whole genome sequencing projects, emphasis was placed on the production of so-called references sequences, which were of enduring high quality and would serve as the foundation for future experimentation.

Whether using physical maps or the whole genome shotgun sequencing approach, the sequencing exercise involved randomly fragmenting either cloned or native genomic DNA into very short segments that could then be inserted into bacterial cells as plasmids for amplification, producing many copies of the segments, prior to nucleic acid purification and sequence analysis. In a process known as assembly, computer programs were then used to stitch the sequences back together to reconstruct the original DNA sequencing target. Assembly of whole genome shotgun sequencing data was difficult and required sophisticated computer programs known as a assembly, computer programs were then used to stitch the sequences back together to reconstruct the original DNA sequencing target. Assembly of whole genome shotguns sequencing data was difficult and required sophisticated computer programs and powerful supercomputers and, even in the years following the completion of the HGP, whole genome shotgun sequence assembly remained a significant challenge for whole genome sequencing projects.

Although the first whole genome sequences were in themselves technological and scientific feats of significance, the scientific opportunities and the host of technologies those projects spawned have had even greater impacts. Among the most significant technological developments have been in the area of next-generation DNA sequencing technologies for human genome analysis. Certain of those technologies originally were designed to re-sequence genomes (as opposed to do novo sequencing). In re-sequencing, short sequences are produced and aligned computationally to existing reference genome sequences computationally to existing reference genome sequences generated, at least initially, using the older do novo sequencing methods. Next-generation sequencing approaches are characterized generally by the massively parallel production of short sequences, in which multiple DNA fragments are generated simultaneously and in sufficient quantity to redundantly represent every base in the target genome. Although such technologies propelled whole genome sequencing into the mainstream of biology, innovation persisted as companies and a academic laboratories strived to reach the mapping of an individual human.

In total, over fungal genomes sequences are currently publicly available with over 40 additional projects underway. These genomes represent important human pathogens, plantpathogens, saprophytes, and model organisms. They also encompass fungi that grow as yeasts, from mycelium or pseudo-hyphae, or are capable of dimorphic growth. In addition, they include representatives of all four major fungal groups, i.e., as competes, basidiomycetes, zygomycetes and chytrids. Importantly, the majority of available fungalgenomes fall into clusters of related genomes that enable comparative analysis

across a range of evolutionary distances. These clusters also included related organisms that differ in terms of specific physiological traits pathogenicity), thus allowing the setraits to be explored propelled whole genome sequencing into the mainstream of biology, innovation persisted as companies and academic laboratories strived to reach the mapping of an individual human genome for less than was anticipated.

The last decade has witnessed a revolution in the genomics of the fungal kingdom. Since the sequencing of the first fungus of available fungal genome sequences has increased by an order of magnitude. Over complete fungal genomes have been publicly released with an equal number currently being sequenced – representing the widest sampling of genomes from any eukaryotic kingdom. Moreover, many of these sequenced species form clusters of related organisms designed to enable comparative studies. These data provide an unparalleled opportunity to study the biology and evolution of this medically, industrially, and environmentally important kingdom. In addition, fungi also serve as model organisms for all eukaryotes. The available fungal genomic resource, coupled with the experimental tractability of the fungi, is accelerating research into the fundamental aspects of eukaryotic biology. We provide here an overview of available fungal genomes and highlight some of the biological insights that have been derived through their analysis.

Much of the increase in bioengineering activity can be credited to electrical engineers. In the bioengineering meetings were dominated by sessions devoted to medical electronics. Medical instrumentation and medical electronics continue to be major areas of interest, but biological modeling, blood-flow dynamics, prosthetics, biomechanics dynamics of body motion and strength of materials, biological heat transfer, biomaterials, and other areas are now included in conference programs.

Today there are many more examples of interaction between biology and engineering, particularly in the medical and life-support fields. In addition to an increased awareness of the need for communication between the engineer and the associate in the life sciences, there is an increasing recognition of the role the engineer can play in several of the biological fields, including human medicine, and, likewise, an awareness of the contributions biological science can make toward the solution of engineering problems.

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