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ORIGINAL ARTICLE

Recent advancement in Biotechnological and Molecular approaches of Actinomycetes: A review

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ABSTRACT

Actinomycetes are a diverse group of medically, industrially and ecologically important bacteria, studied as much for the diseases they cause as for the cures they hold. Along with also as a source of industrial important product which are helpful for mankind. However, many of the rare genera of actinomycetes have been neither explored nor manipulated for their biotechnological and industrial potential. With the help of molecular approaches and recent advances in genomics and sequencing technologies, microbial community analyses using culture-independent molecular techniques have initiated a new era of actinomycetes ecology. This review summarizes recent progress in the area of molecular microbial ecology with an emphasis on novel techniques and approaches that offer new insights into the phylogenetic and functional diversity of actinomycetes assemblages for better production of qualitative and quantitative industrial and pharmaceutical products .

Keywords- Actinomycetes, diversity, Molecular approach, compounds, RAPD, RFLP, and metagenomics.

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INTRODUCTION

Actinomycetes: Biotechnological aspects

Actinobacteria belong to the subdivision Actinomycetales of the Prokaryotae.They form a distinct phylogenetic line in the 16S rDNA tree and have been of major scientific interest in the past decades, with the discovery a large number of metabolites produced by its diverse genera. Actinobacterial metabolites have a major biotechnological contribution from antibiotics to enzyme inhibitors and anti-cancer agents to various alkaloids. Actinobacteria are ubiquitously distributed in terrestrial, freshwater and marine environments and are involved in the breakdown of organic matter and xenobiotic compounds. [1]. Actinobacteria are Gram positive, filamentous organisms inhabiting the soil. They are wide spread in distribution[2,3]. They produce a vast array of secondary metabolites such as enzyme[4] immuno-modulators, antibiotics, antihelminthic and anticancer agents[5-7]. Among them Streptomyces, the well-known saprophytes of the soil are the major producer of antibiotics[8]. About 75-80% of the drugs available commercially are being derived from Streptomyces [5,6]. Reports reveal that the continuous screening of actinobacteria, especially Streptomyces, will lead to the discovery of 100 000 new compounds with diverse applications[9]. It presence is diverse and it found in soil, water, air, rhizosphere region. Most of the pharmaceutical and industrial compound were isolated from marine actinomycetes [10,11].

Actinomycetes identification and characterization: Molecular approach

For identification and characterization of any valuable biological organism (prokaryotic or eukaryotic), nucleic acid based molecular approach is considered to be the most powerful approach providing a considerable ranges information about the organism and its true relatedness with other. Classification and identification of organisms related to molecular systematic was totally based on nucleic acid hybridization studies in the past decades, but the introduction of nucleic acid sequencing techniques has been proved to be its best status till date [12]. Systematics of actinomycetes and other bacteria is totally based on 16S rDNA giving an increasing demand for phylogenetic studies in a significant way (Yokota, 1997). The primitive part of analysis of the evolution of actinomycetes and its identification based on bacterial sequences of 16S ribosomal DNA has been initiated by isolating DNA of 16S rDNA [13] and implying the polymerase chain reaction (PCR) strategy to amplify the 16S rRNA coding gene followed by direct sequencing of purified DNA fragments [14]. For sequencing reactions, DNA sequencer is used to determine the order of base arrangement within the length of sample [15]. The generated sequences are further analyzed by computer software to find out the identity of query sequence with genetic information available in global database followed by phylogenetic analysis which gives a general overview of evolution of actinomycetes and the identification of the organisms upto the genus level only. With the advancement of PCR technology, polyacrylamide matrix based denaturing gradient gel electrophoresis technique recently introduced in microbial ecology for better separation of the PCR products [16] . Recently urea and formamide based denaturing gradient gel electrophoresis (DGGE)[17]]and temperature gradient gel electrophoresis (TGGE) [18] based on temperature gradient across the gel are quite promising techniques for getting a sequencing based DNA fingerprint of microbial communities. However the normal PCR based detection technique does not allow the visibility of amplified DNA fragment of relatively less abundant but very important microbial species. To overcome this problem, two strategies are now adopted in the field of microbial biotechnology that permits the analysis of selected populations within complex microbial communities and these are depicted here. First of all the PCR strategy is simplified in the way to design a forward primer for selectively amplification of 16S rDNA of actinomycetes where variable combinations of different reverse primers are tested with the forward one. The second approach is related to TGGE or DGGE where the fragments generated from actinomycete specific PCR is directly analyzed under UV ray exposure. In addition to these two approaches another indirect approach is also followed now a day where a DNA amplicon specific to an actinomycete was generated in PCR was further used as a template to amplify and analyze fragments in gradient gel for a second bacterium specific PCR.

Actinomycetes identification and characterization: Utility of advanced technologies

The admission of recent advanced technologies has given a good opportunity for exploring the untapped and novel microbial isolates with novel properties, where most of the technologies are well adopted for characterization of actinomycetes in all aspects as we can consider its importance as natural reservoirs of excellent enzymes. The most prominent and promising technique for enzymatic characterization of any microbial population is High Throughput Screening (HTS) methods but this method is extended very little for actinomycetes. The HTS method is mainly used in drug discovery related field of biology and chemistry and some of the important applications of HTS methods in microbial technology are also discussed here. Fluorescence activated cell sorting (FACS) is a technology that can quickly separate the cells in a suspension based on size and the color of their fluorescence. It is basically governed with some fluorescent substrates that are very specific for a particular enzyme is used in the experiment. The positive fluorescence designates a definite biocatalytic activity of the clone and the technology is successfully engaged for desired clones sorting from a genomic DNA library [19]. A successful application of microfluidic FACS (mFACS) chips in prokaryotic system was published in a reputed journal on 1999. *Escherichia coli* cells expressing green fluorescent protein were separated from a background of non-fluorescent *E. coli* cells which facilitated a substantial enrichment of micron-sized fluorescent bead populations with different colors. This separation was also confirmed the viability of the bacteria after extraction from the sorting device. This device can be performed as functional for Actinomycetes cell isolation from a complex microbial population where the device shall be worked as stand-alone devices or as components of an integrated microanalytical chip [20]. Another efficient enzyme-fluorescence technology is Gel MicroDrop technology which is basically based on detection of positive clones specific to particular enzymes by capturing the fluorescence emitted as a result of catalytic breakdown of biotinylated substrate by the clone [21]. Another old but interesting strategy called Metagenomic approach mainly deals with the preparation of a clonal library from the metagenome obtained from extreme habitats (ocean beds, arid regions, stratosphere, hot stream areas and other) taking into consideration of inability of growing of actual microbes under laboratory conditions. Though this technique also ensured the rapid screening methods by exploring the bioactive potential for unculturable

microbes but it limits the freedom of common advantage mainly exhibiting the low or no expression of desired gene(s) [22]. Other most promising and pioneering approaches can be explored for Actinomycetes characterization are metagenomic DNA shuffling, substrate-induced gene expression screening (SIGEX) and pre-amplification inverse-PCR (PAIPCR) that introduce a deeper knowledge of functional metagenomics for a particular microflora [23]. The prokaryotic operon based lux technology is basically deals with transformation of environmental bacteria governing genes of the lux operon from the marine bacteria that was successfully employed for *Vibrio fischeri* and *V. harveyi* microbial strains [24]. Most of the studies related to population dynamics, metabolic activity and spatial distribution of actinomycetes in microfloral samples are transformed strains can be encouraged by using lux technology. Another parallel research related to the influence of soil physical and chemical factors on both root colonization and the expression of characters governing antagonism in the rhizosphere is equally important.

Now a days, another most relevant and potential approach for evolutionary study is "directed evolution" approach and this is mostly consist of construction of library of genetic variants and simultaneous screening of mutants governing desired enzymatic properties. The most prominent variants are screened and reorganized by creating and repeating several rounds of library to get the best variants showing an identical process of natural evolution but limited in a particular pace and time [25]. Recent trend in HTS method also ensure the advancement of technology with the space of rapidity, parallel execution and economic to the screening protocol.It is consisting of drop based microfluid platform carries a complex system governing a small foot print chip with an array of insoluble substrates specific for the enzyme of interest [26]. Reporter gene technology is another aspect of advancement of screening methods which deals with simplicity and sensitivity of reporter enzyme ie, Green fluorescent protein (GFP) have made easier detection of genes in host systems [27].
Beside these all types of well adopted HTS based method; proteomics approach is currently documenting its well acceptance in the way of discovering the microbial worlds. One of the proteomics based technique is MALDI-LTQ-Orbitrap which deals with screening of target proteins from complex matrices and suspensions. This technique is working in coordination of both ion trap and MALDI system and is functional based on the principal of both liquid and gas chromatography separations [28]. Other promising ionization technique is electrospray ionization mass spectrometry (ESI-MS) based on measurement of femtomole quantities of proteins in a system [29]. Other *in silico* techniques are quite promising to characterize potent enzymes from database and some of them are Predictive 3DQSAR CoMFA and CoMSIA has the ability to predict superior enzymes finely related to its structural properties and microenvironment developed during *in silico* reaction with substrate. Though prediction of these models finally require the functional validation in laboratory to correlate its property and function scientifically [30].

Actinomycetes identification and characterization: Whole genome sequencing approach

Although molecular and advanced technologies have great role to bloom up the knowledge by exploring the Actinomycetes and across the microbial world, but there are some ambiguities still remain related to genomics controlling the whole mechanics of microbe's activity. In such cases whole genome sequencing (WGS) approach out from single cells has made a scientific breakthrough, which unlocks the entire molecular and biochemical potential of uncultured microbe from a complex environment. The complete genome sequencing of microbes revealed the chemistry of biosynthetic gene clusters that sometimes are present but not well known for synthesizing any secondary metabolites (cryptic clusters) [31]. In this issue, WGS is much more capable of classifying biosynthetic gene clusters in comparative way as well as giving a strong hand to systematically catalog the level of natural product diversity, is an important first step towards a full exploitation of secondary metabolites in bacteria.
From the past 10 years, advancement in nucleic acid sequencing technology that is basically comprising the improvement of designing of noble vectors used in library construction and sophistication of shotgun sequencing techniques, helps in mesmerizing the eye of knowledge near to gene finding and annotation (both known and noble) to frame up the whole genome of an organism.
The major breakthrough in microbial and prokaryotic genome sequencing initiative was successfully achieved by some research centers where the names usually come first are The Institute for Genomic Research (TIGR), the Pathogen Sequencing Unit at the Sanger Centre and Department of Energy in association with Joint Genome Institute which made a big examples in the area of microbial genomic sequencing program [32]. Currently, a total number of six actinomycete genera namely Mycobacterium, Corynebacterium, Rhodococcus, Arthrobacter, Frankia, and Streptomyces have potential amount of

completed genomes information to improvise the core analysis of gene potential and secondary metabolic diversity. [33].

A retrospective analysis of 17 microbial genome sequences completed at TIGR during the past few years also revealed that when these genome projects entered closure, the extent of genome completion and the accuracy of assembly varied significantly (I. Paulsen, unpublished data). For example, at eightfold sequence coverage, the *Thermotoga maritima* genome was represented by 98 contigs (>1 kb in size) and was missing only 26 genes (~1.5% of the total) in the final annotation [7]. This contrasts with the *Streptococcus pneumoniae* genome of similar size, whose initial assembly contained 265 contigs and was missing 115 genes (~6% of the total) [11]. This difference likely reflects the fact that the genomes of some microbes (gram-positive organisms, for example) are not well represented in random DNA libraries. Some of the most interesting biology may be encoded in the missing genes of each organism. Due to the larger percentage of repetitive DNA in the *S. pneumoniae* genome, many of the initial contigs contained misassemblies that were revealed only during genome closure. Currently there is no method for assigning quality values to genome assemblies as there is for DNA sequence reads, and this makes it difficult for anybody wishing to make use of preliminary data to know how reliable they really are. As a result, considerable additional work may be required to make full use of draft sequence data. Any such ad hoc attempts to improve draft sequences by additional sequencing or to close gaps in a draft project are inefficient and expensive and rapidly negate any initial cost savings. While we agree that a draft sequence can be of tremendous benefit to interested investigators and acknowledge that every completed project generates draft sequence data as part of the process, there are several reasons why we believe that complete genome sequence should be the standard whenever possible, particularly with microbial genome projects.

CONCLUSION

Various types of physical and chemical pretreatment methodologies have been devised for isolating desirable rare actinomycete genera. The use of these genus oriented methods in industrial screening programmes has provided a significant impetus towards the discovery of new bioactive compounds. A large number of antimicrobial compounds have been isolated from actinomycetes sp. while rare and novel species of this genus are expected to contain as yet undiscovered bioactive metabolites. Different molecular approaches such as genetic fingerprinting, metagenomics, metaproteomics, 16S r RNA, genus specific primers, RAPD, RFLP, Proteomics and bioinformatics tools are vital for discovering and characterizing the vast actinomycetes diversity These techniques useful to circumvent the problem of recharacterization of known bioactive molecules and to help in screening novel compounds. Use of the above mentioned strategies should make feasible the discovery of novel pharmaceutical and industrial important product.

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