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Seclusion, Categorization and Molecular Documentation of Microorganisms from Intestine of Gut from Fish by Using 16s R DNA Sequencing

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Abstract: ***The genomic DNA sequence of an organism specifies the features of its vendor, but knowing this string of letters by no means tells you the exact Mother Nature of the conforming organism. This present study deals with to Isolation, characterization and molecular identification of Microorganisms from Intestine of Gut from fish by using 16DNA Sequencing. Bacterial strain was isolated and characterized using various biochemical tests and confirmed through molecular approach. Bacterial 16S r DNA gene was amplified using suitable primers. The amplified 16S r DNA gene sequence was compared with the sequence in NCBI sequence database and Phylogenetic and molecular evolutionary analyses were conducted using 16S r DNA sequencing.***

There are different organisms were isolated and identified the three of its bacillus and remaining one is Pseudomonas sp. Bacillus sp. is a gram positive, oval shaped bacteria which forms white raised irregular colony. The Pseudomonas is a gram-negative aerobic gammaproteobacterium. The sequence, when submitted to NCBI gene bank database using BLAST, showed 99 – 100% maximum identity and query coverage.

Keywords: DNA Sequence, NCBI, Bacillus sp, Pseudomonas sp.

INTRODUCTION

In reality, a long and difficult road divides the resolve of the last base of a genome and the understanding of how the organism in question evolved, grows, propagates, interacts with its environment and, ultimately, dies. This empathetic will be the Holy Grail of generations of geneticists to come. The term genomics was first introduced in 1986 by T. H. Roderick to describe the study of complete genomes, refers to the kind of research that is used to get answers to the above questions. However diverse the clarification of this term in the scientific communal may be, the following descriptions more or less cover the subject. There is two vital classifications of the genomics. There are Structural genomics and functional genomics. In structural genomics the scientific discipline of mapping, sequencing and analyzing genomes, while functional genomics refers to the analysis of genome function. The structural genomics phase has a clear end-point with the completion, annotation, and publication of a genome sequence. The fundamental approach of functional genomics is mounting the scope of research from studying single genes or proteins to studying all genes or proteins concurrently in a systematic manner, in order to obtain a panoramic view of the organisms' genetic potentials (Hieter&Boguski, 1997). DNA Sequencing, first devised in 1975, has become a powerful technique in molecular biology, analysis of genes at the nucleotide level. It is the determination of all or part of the nucleotide sequence of a specific deoxyribonucleic acid (DNA) molecule. Another important use of DNA sequence is identifying restriction sites in plasmids. The fundamental reason for which the DNA molecule is sequenced is to make predictions about its function and to facilitate manipulation of the molecule.Griffin, H. G. & Griffin, A. M. (1993). The basic technology for this process, DNA sequencing, is fairly rapid and inexpensive, highly reproducible and readily obtainable to effectively any research group through specified sequencing centers. Databases of gene sequences and computer applications to compare them are, likewise, freely available. For these reasons, DNA sequence analysis has taken an increasingly significant role in taxonomic studies in recent years (Stackebrandt et al., 2002). The dawn of genome sequencing projects has been utterly dependent on recent improvements in

sequencing techniques; a span ago such efforts would not have been practically viable endeavors. Therefore, a short indication will be given on expansions from the (Maxam& Gilbert., 1977) chemical sequencing method to present-day, fully automated systems based on the dideoxy chain-termination –or enzymatic- method of (Sanger *et al.,* 1977). Both methods produce nested sets of (radioactively) labeled polynucleotides, from 1 to 500 bases long, that begin at a fixed point and terminate at points that depend on the location of a certain base in the unique DNA strand. The polynucleotides are then separated by polyacrylamide gel electrophoresis (PAGE), and the order of nucleotides in the novel DNA can be read directly from an autoradiograph, or a fluorogram in the case of fluorescent labeling (Griffin & Griffin, 1993).Today, sequencing systems exist that are fully automated through almost all stages of the process from tooth picking colonies off a plate, culturing cells, extracting DNA, and Sequencing of the template, to the computer-assisted assembly of the raw sequence data(Burland 1993). Once a DNA sequence has been completed, the annotation phase begins. The aim of this phase, which involves of a logical order of analyses, is to detect as many as possible primary structural features within the DNA that have been sequenced. This includes identification of open reading frames (ORFs) and verification of codon usage, identification of the start (start codon + ribosomal binding site) and stops sites of ORFs, and analysis of conceivable terminator structures and promoters. All the analyses deliberated below are accomplished with the aid of computer programs designed for that persistence (Kunst*et al,* 1998). In this chapter, an overview will be given of strategies and methods that are employed in genome sequencing. The present work carries out the identification of the most efficient bacterial strains and studies on the sequence of different isolates from the gut of fish intestine. And also done the complete genome sequence and phylogenetic analysis of the different organisms of 16S rDNA is presented.

Experimental Methods

The studies were carried out in Department of Zoology, Rani Anna Government College for Women, Gandhinagar, Tirunelveli.

Isolation of Bacteria:

The fish samples were collected from the aquarium of the local area of Nagercoil. The samples were processed sterile condition allow for bacteriological analysis. Bacterial isolates were screened on Nutrient Agar (NA) plates by the standard pour plate method. Plates were incubated at 37°C/24h and a total of one hundred and forty-four isolates were obtained, from that one isolate was selected and used for further studies. The isolated bacteria were identified based on colony characteristics, Gram staining methods and by various biochemical tests as given by Bergey's (1984) Manual of Determinative Bacteriology.

'Biochemical Characterization of the bacteria:

Selected strain was grown in nutrient broth culture medium containing2.5% peptone, 1.0% yeast extract, and 0.5% beef extract. Cultures (50 ml in 250-mlconical flasks) were inoculated with 5% (v/v) inoculums and incubated at 37 °C with vigorous orbital shaking at 120-150 rpm. To make a solid medium, 1.5% agar was added to the broth (Himedia, India). The shape and color of the colonies were examined under the microscope after Gram staining. Isolates were biochemically analyzed for the activities of Oxidase, Catalase, MR-VP test, Urease test, Motility, Indole production (Table 1) and Citrate utilization (Table 2). The tests were used to identify the isolates according to Bergey's Manual of Determinative Bacteriology.

Bacteria DNA isolation

Bacterial genomic DNA was isolated as per the standard protocol (Hoffman and Winston, 1987). A single colony was inoculated in nutrient broth and grown for overnight at 37ºC.Cells were harvested from 5 mL of the culture and to this 100µL of lysozyme was added and incubated at RT for 30 min, followed by the addition of 700 µL of cell lysis buffer(Guanidiumisothiocyanate, SDS, Tris- EDTA). The contents were mixed by inverting the vial for 5 min with gentle mixing till the suspension looked transparent. 700 µL of isopropanol was added on top of the solution. The two layers were mixed gently till white strands of DNA were seen. The DNA extracted from the aqueous layer was ethanol precipitated. The DNA pellet was dried and dissolved in 50µLof 1X TE buffer. The quality of the DNA was checked by running on 0.8% agarose gel stained with ethidium bromide (0.5 µg/µL). A single intense band with slight smearing was noted. The extracted genomic DNA was used as template DNA for amplification of the 16S rDNA gene.

DNA sequencing of 16S rDNA gene fragment

The 16S rDNA purified PCR product (100ng concentration) was subjected to the sequencing using ABI DNA 3730 XL sequencer (Applied Biosystem Inc). Sequencing of the 16S rDNA gene of the bacterial isolate was done from both the directions. The sequence obtained was subjected to BLAST search and the bacterial species were determined. The percentages of sequence matching were also analyzed and the sequences were submitted to NCBI-Gen Bank and obtained accession numbers.

Computational analysis (BLAST) and Identification of Bacterial Species

BLAST (Basic Local Alignment Search Tool) is a web-based program that is able to align the search sequence to thousands of different sequences in a database and show the list of top matches. This program can search through a database of thousands of entries in a minute. BLAST (Altschul et.al 1990) performs its alignment by matching up each position of search sequence to each position of the sequences in the database. For each position, BLAST gives a positive score if the nucleotides match, it can also insert gaps when performing the alignment. Each gap inserted has a negative effect on the alignment score, but if enough nucleotides align as a result of the gap, this negative effect is overcome and the gap is accepted in the alignment. These scores are then used to calculate the alignment score, in "bits" which is converted to the statistical E- value. The lower the E-value, the more similar the sequence found in the database is to query sequence. The most similar sequence is the first result listed.

RESULTS

16 s rDNA Sequencing and phylogenetic tree analysis were made and assigned by means of the Bioserve accelerating discovery by top ten sequencing producing significant alignments and seventh hence the SM1, ninth for SM2, fifth for SM3 were sequenced and identified as *Bacillus sp* and blasted on NCBI with JX025164, JX02565, JX02566, JX02567. The sequence alignment of eighth is SM5 were sequenced and identified as Pseudomonas sp.

16S rDNA Sequence of Sample SM1

In the present sample, the bacterial identification the SM1 is identified as *Bacillus sp*. The reported identity of the sample is bound by limitations of a sequence of base pairs up to the range of 1- 1441.When compared to the top ten sequencing producing alignment described *Bacillus thuringite NSIS* 16SrDNA and 16S-23SIGS, strain CMBLB had the accession no of AM29232.1. And this have the minimum and maximum score value of 2697, and the query average is 99% and the bacterial species is identified and confirmed as *Bacillus sp* for maximum 100%.And the result from NCBI of the phylogenetic tree also confirmed as the SM1 is the *bacillus sp*.

16S rDNA Sequence of Sample SM2

In the present sample, the bacterial identification the SM2 is identified as *Bacillus sp*. The reported identity of the sample is bound by limitations of a sequence of base pairs up to the range of 1- 1501.When compared to the top ten sequencing producing alignment described *Bacillus licheniform is a* gene for 16SrDNA strain M1-1, had the accession no of AB039328.1. And this have the minimum and maximum score value of 2724, and the query average is 99% and the bacterial species is identified and confirmed as *Bacillus sp* for maximum 99%.And the result from NCBI of the phylogenetic tree also confirmed as the SM2 is the *bacillus sp*.

16S rDNA Sequence of Sample SM3

In the present sample, the bacterial identification the SM3 is identified as *Bacillus sp*The reported the identity of the sample is bound by limitations of a sequence of base pairs up to the range of 1- 1441.When compared to the top ten sequencing producing alignment described 16SrDNA isolate AB1A, had the accession no of AM062677.1. And this have the minimum and maximum score value of 2661, and the querry average is 99% and the bacterial species is identified and confirmed as *bacillus sp* for maximum 99%.And the result from NCBI of the phylogenetic tree also conformed as the SM3 is the *bacillus sp*.

16S rDNA Sequence of Sample SM5

In the present sample, the bacterial identification the SM5 is identified as Pseudomonas sp. The reported identity of the sample is bound by limitations of a sequence of base pairs up to the range of 1- 1021.When compared to the top ten sequencing producing alignment described Uncultured bacterium clone 62232932, had the accession no of EU804954.1. And this have the minimum and maximum score value of 1846, and the querry average is 100% and the bacterial species is identified and confirmed as *bacillus sp* for maximum 99%.And the result from NCBI of the phylogenetic tree also conformed as the SM5 is the *pseudomonas sp*.

DISCUSSION

The traditional identification of bacteria on the basis of phenotypic characteristics is generally not as accurate as Comparison of the bacterial 16S rDNA gene sequence has emerged as a preferred genetic technique. The sequence of the 16S rDNA gene has been widely used as a molecular clock to estimate relationships among bacteria (phylogeny), but more recently it has also become important as a means to identify an unknown bacterium to the genus or species level (Sacchi*et al.*, 2002). The use of 16S rDNA gene sequences to study bacterial phylogeny (Amann*et al.*, 1995) The present investigation discussed about the isolated strains were sequenced and analyzed with the blast all the strain were identified as three of them are *Bacillus sp*and one is *Pseudomonas sp* With the use of 16S rDNA gene sequence to characterize the bacterial isolate from the gut region of fish intestine were found. When compared to the assession these four strains are closely related to them. So thus the genotyping method using 16S rDNA gene sequence is the simple and effective in strain identification.

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