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Identification of fungi species from solid waste by 16s RNA technology

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ABSTRACT

The majority of phylogenetic analyses carried out these days are based on MSAs of biological sequences. Several times the quality of the alignment we use for an analysis can have a significant influence on the quality of the results of the analysis. In the current study we have a unknown gene sequence. By 16s experimental studies isolated gene was identified as the gene from the organism *Fusarium solani*. To substantiate these studies bioinformatics approach has been done using the methods like BLAST (Basic Local Alignment Search Tool) and MSA (Multiple Sequence Analysis). This approach gave us phylogenetic information and also gave the information about conservation of gene in all the organisms taken in the study. This approach gives the application of 16s rna technology in identification of microbes. The use of 16S rRNA gene sequences to study bacterial phylogeny and taxonomy has been by far the most common method because it is presence in almost all bacteria, the function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolution); the 16S rRNA gene (1,500 bp) is large enough for informatics purposes though the bacteria exits either in a multigene family, or operons.

KEYWORDS

Phylogenetic;
BLAST;
MSA;
16s;
Multigene operons.

INTRODUCTION

'Solid waste' means any garbage, refuse, sludge from a waste treatment plant, water supply treatment plant, or air pollution control facility and other discarded material, including solid, liquid, semisolid, or contained gaseous material resulting from industrial, commercial, mining, and agricultural operations, and from community activities. Total quantity of waste generated in the country (based on weighment exercise by local bodies) is not reported. However, Ministry of Urban Development in its manual on solid waste management (year 2000) has estimated waste generation of 100,000 MT.CPCB with the assistance of NEERI has conducted survey of solid waste management in 59 cities (35 metro cities and 24 state capitals-2004-05).Quantities and waste generation rates in 59 cities is as under: In India, the amount of waste per capita generated is

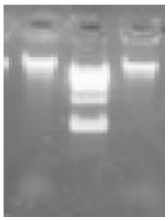

Figure 1 : Figure showing PCR amplification of rDNA fragment from fungal sample. The size of PCR amplified product is ~500 bp

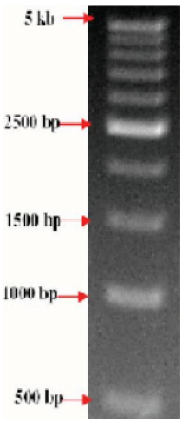

Figure 2 : Figure showing 500 bp ladder contains 10 DNA fragments of size 500 bp, 1000 bp, 1500 bp, 2000 bp, 2500 3000 bp, 3500 bp, 4000 bp, 4500 bp and 5000 bp

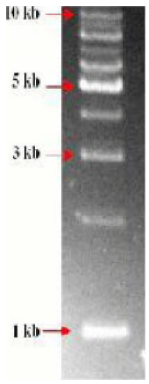

Figure 3 : Figure showing 10 kb 1 kb ladder contains 10 DNA fragments of size 1kb, 2kb, 3 kb, 4 kb, 5 kb, 6 kb, 7 kb, 8 kb, 9 kb

estimated to increase at a rate of 1%–1.33% annually[1] Organic waste is a major component of municipal solid waste. Municipal solid waste (MSW) compost contains a significant amount of humic substances[2]. Organic waste is produced wherever and whenever there is human habitation. The main forms of organic waste are household food waste, agricultural waste, human and animal waste. In industrialized countries the amount of organic waste produced is increasing dramatically each year. Although many gardening enthusiasts compost some of their kitchen and garden waste,

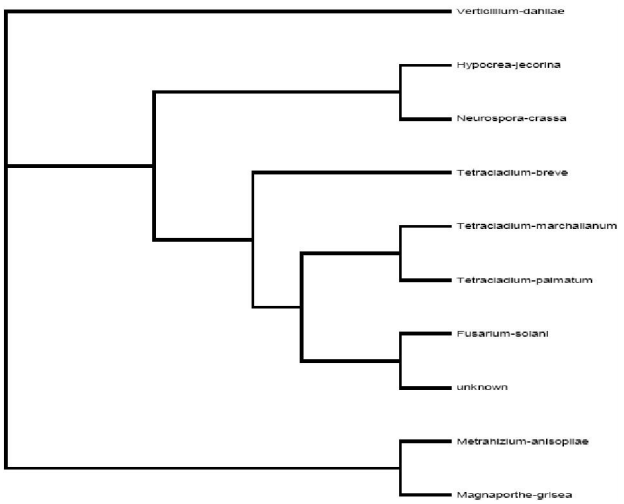

Figure 4 : Phylogram obtained from ClustalW2 after performing MSA. Unknown and Fusarium solani were observed in a single clade

much of the household waste goes into landfill sites and is often the most hazardous waste. The organic waste component of landfill is broken down by micro-organisms to form a liquid 'leachate' which contains bacteria, rotting matter and maybe chemical contaminants from the landfill. Micro organisms that dwell in solid wastes are grouped under Solid Waste Microflora (SWM). The most common organisms that are generally found in solid waste are bacteria and fungi. These micro organisms use the components of the waste as the substrate for their growth. They grow and multiply on these wastes by utilizing the various components that make up the solid waste. Further a wide variety of pathogenic microorganisms have been reported to be present in these organic wastes[3].

RESULTS AND DISCUSSION

CLUSTAL 2.0.12 Multiple sequence alignments

Sequence format is pearson

Sequence 1: Hypocrea-jecorina 5556 bp
Sequence 2: Metrahizium-anisopliae 8118 bp
Sequence 3: Tetracladium-marchalianum 5218 bp
Sequence 4: Tetracladium-breve 5236 bp
Sequence 5: Tetracladium-palmatum 5219 bp
Sequence 6: Neurospora-crassa 8847 bp
Sequence 7: Verticillium-dahliae 7216 bp
Sequence 8: Magnaporthe-grisea 8412 bp
Sequence 9: Fusarium-solani 3830 bp

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Sequence 10: unknown 1349 bp

Information of gene sequences taken for MSA, information contains the organism name from which the sequenses were retrieved and the length of the sequences (Figure 4) From MSA of all the ten sequences from *Hypocrea jecorina*, *Metrahizium anisopliae*, *Tetracladium marchalianum*, *Tetracladium breve*, *Tetracladium palmatum*, *Neurospora crassa*, *Verticillium dahliae*, *Magnaporthe grisea*, *Fusarium solani* and Unknown sequence we got the phylogram, from the phylogram analysis it was confirmed that the unknown sequence is from *Fusarium solani*. Both *Fusarium solani* and unknown sequences were observed on a same clade of phylogram. Later the alignment gave us information about the conservation level of unknown gene through genes from different organisms (Figure 5).

METHODS

Study site

Udupi (Kannada:) is a temple town located in Udupi District near Mangalore, Karnatakastate, As of the 2001 India census Udupi had a population of 113,039.The weather is fairly similar throughout the year, due to the nearby Arab sagar. Temperature ranges from 30 to 35 degrees centigrade in day time and is around 10 degrees less during night. Humidity is normally high most of the time. The rainy season is from April to September. At this time the monsoon shows its true colours in this area. The waste collected in a clean plastic container of 500gm capacity. Container closed with air tight lid and carried to the laboratory. All residential waste shall be collected at least bi-weekly. Many specific kinds of microorganisms can be obtained from organic wastes by the creation of an artificial environment for them in the laboratory which will enhance their growth over competing organisms. Characteristics of the organisms which give them special advantages over others are exploited in the formulation of culture media and the choice of incubation conditions.

Sampling

The waste collected in a clean plastic container of 500gm capacity. Container closed with air tight lid and carried to the laboratory.All residential waste shall be

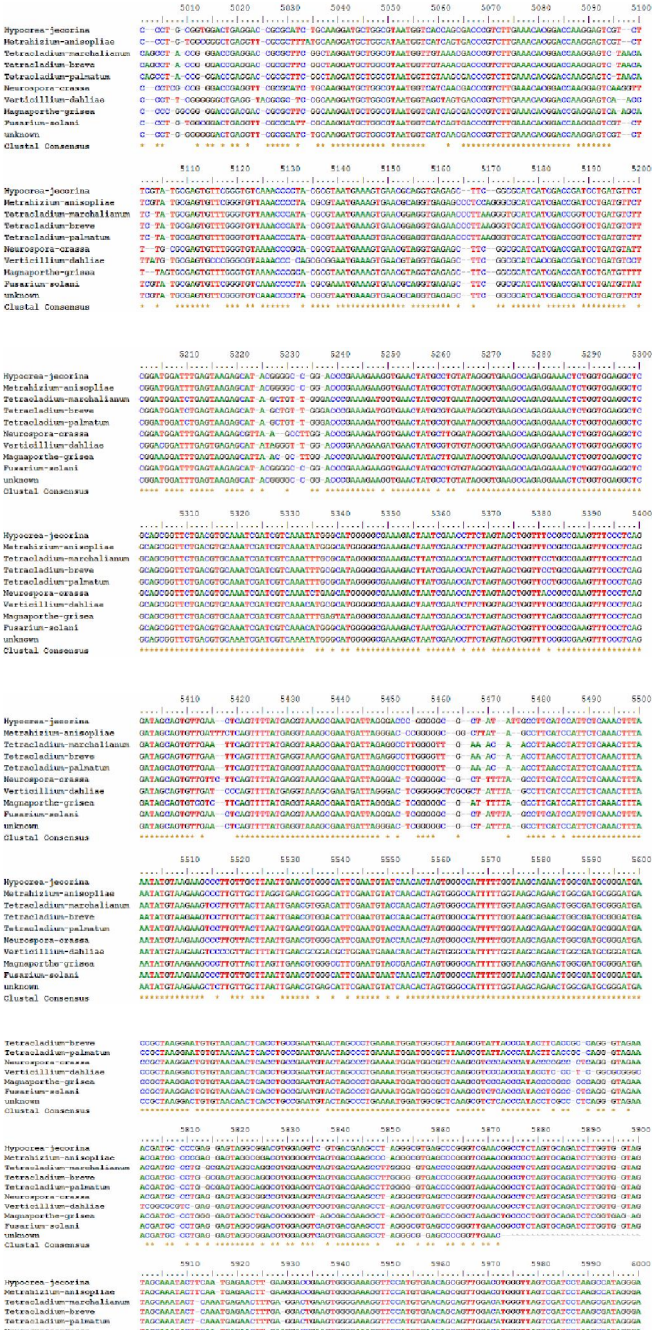

Figure 5 : Result of MSA showin the conservation of unkown gene sequence (from 4447th nucleic acid to 5782nd nucleic acid trough the sequences of all the organisms taken for study

collected at least bi-weekly. Many specific kinds of microorganisms can be obtained from organic wastes by the creation of an artificial environment for them in the laboratory which will enhance their growth over competing organisms. Characteristics of the organisms which give them special advantages over others are exploited in the formulation of culture media and the

choice of incubation conditions[4,5]. The collected materials are plated on plastic petriplate as SBM.Incubate at 28-+2°C. Observations are done every day under sterobinocular. The organisums are identified by using[6].

Extraction of genomic DNA from bacterial sample using the bacterial genomic

DNA Isolation Kit (RKT09).Phylogentic Tree Builder uses sequences aligned with System Software aligner.Phylogentic Tree Builder uses sequences aligned with System Software aligner. Adistance matrix is generated using the Jukes-Cantor corrected distance model. When generating the distance matrix, only alignment model positions are used, alignment inserts are ignored and the minimum comparable position is 200. The tree is createdusing Weighbor with alphabet size 4 and length size 1000. Weighbor Tree: Weighbor is a weighted version of Neighbor Joining that gives significantly less weight to the longer distances in the distance matrix. The weights are based on variances and covariances expected in a simple Jukes-Cantor model. Nucleotides is considered for the distance, therefore, second substitutions will not becounted and the distance will be underestimated. Jukes and Cantor createad a formula that calculates the distance taking into account more than just the individual differences.

Bootstrap

Bootstrapping is a statistical method for estimating the sampling distributionby resampling with replacement from the original sample. In making phylogenetic trees,the approach is to create a pseudoalignment by taking random positions of the original alignment. Some columns of the alignment could be selected more than once or not selected at all. The pseudoalignment will be as long as the original alignment and will be used to create a distance matrix and a tree. The process is repeated 100 times and a majority consensus tree is displayed showing the number (or percentage) of times a particular group was on each side of a bran ch without concerning the subgrouping.

The Basic Local Alignment Search Tool (BLAST)[7] finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families. BLAST was use to search for similar genes against nonredundent databases by taking unknown sequnse as a query. The most similar ortholog sequences were retrieved in FASTA format as an input for MSA. ClustalW2 is a general purpose multiple sequence alignment program for DNA or proteins. It produces biologically meaningful multiple sequence alignments of divergent sequences. It calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen. Evolutionary relationships can be seen via viewing Cladograms or Phylograms, ClustalW2 was employed for MSA and phylogenetic analysis by setting the gap opening and gap extension parameters as 1 and 0.5 respectively. The tool Tree view V1.6.6 was used to visualise the tree given by ClustalW2 in .ph format.

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