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ORIGINAL ARTICLE

Isolation Of Bacteria From Personal Computer Hardware And Sequence Analysis Of Hypervariable Region V6 For Molecular Identification

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Abstract:

The usage of computers is increasing day by day. Computers just like microbes are ubiquitous and they continue to have an increased presence in almost every aspect of our occupational, recreational, and residential environment. This research seeks to investigate what kinds of microorganisms, especially bacteria that actually can contaminate computer hardware. Bacterial 16S ribosomal RNA genes generally contain nine "hypervariable regions" (V1 – V9) that demonstrate considerable sequence diversity among different bacterial species and can be used for species identification. We identified the unknown bacteria isolated from computer hardware by molecular detection and sequence analysis of 16S ribosomal RNA hypervariable region V6.The gene sequence obtained was studied in the region from V3-V6. Sequence similarity dendogram were created for hypervariable regions V3-V6. After complete analysis and using bioinformatics tools our investigation demonstrated that the V6 (nucleotide 986-1043) contains diverse character & maximum distinguish power for the Bacillus subtilis species analyzed.

KEYWORD:

16S rRNA, hypervariable region (V6), gene segments, phylogenetic study.

INTRODUCTION

Recent articles have indicated that computer hardware may act as a reservoir for microorganisms and may contribute in the transfer of microbes including pathogens (D.N.A Tagoe, Ansah et al., 2011). Given that computers are not routinely disinfected, the opportunity for the transmission of microorganism is potentially great. Therefore, it is very important to identify the microflora on computer hardware and analyze for its pathogenecity, if any. Thus, this research seeks to investigate what kinds of microorganisms, especially bacteria that actually can contaminate computer hardware. In our topic we focused on identification of isolated bacteria from computer hardware by sequence analysis of 16S r RNA and focused on V6 hypervariable region. Sequence analysis of the 16S rRNA gene has been widely used to identify bacterial species and perform taxonomic studies (Choi et al., 1996; Clarridge, 2004; Munson et al., 2004; Petti et al., 2005; Schmalenberger et al., 2001).

HYPERVARIABLE REGION-

Bacterial 16S rRNA genes generally contain nine "hypervariable regions" (V1 – V9) that demonstrate considerable sequence diversity among different bacterial species and can be used for species identification (Van de Peer et al., 1996). Hypervariable regions are flanked by conserved stretches in most bacteria, enabling PCR amplification of target sequences using universal primers (Baker et al., 2003; Lu et

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al., 2000). The primary structure of 16S rRNA is highly conserved. It is 1,542 nucleotides in length. Bacterial 16S ribosomal RNA (rRNA) is differentiated into conserved region and hyper variable region. Various bioinformatics tools like FASTA, BLAST, MEGA were used for analysis and bacterial identification.

The objectives of this study were to establish presence of microbial agent in mostly used hardware in daily live. Once identification is complete, specific instructions can be made for any adverse effect on either human health or even health of computer in the sense: maintenance of computer and its peripherals. Some reports already established presence of bacteria on surfaces of daily used computer peripheral such as key board and mouse. If proves presence of bacteria on hardware surface, this will be the first report of presence of computer bacteria inside the CPU cabinet. This may provide initiation step to study microenvironment of a computer.

MATERIALS AND METHODS

Materials

The following materials were used during the dissertation work.

Plasticware- Micropipettes - 0-20 µl, 20-200 µl, and 100-1000 µl, Sterile tips for micropipettes PCR component- PCR reaction mix, Thermocycler (Biorad Thermal Cycler Gradient), Instrument- Vortex and Spinix shaker, Microcentrifuge, 10,000rpm max speed, 37°C Shaker Incubator.

Method

Sample collection and Isolation of bacteria on Nutrient Agar

The hardware RAM from two personal computers which was in use for about six years was collected in sterile conditions at normal room temperature. It was inoculated in Nutrient agar of pH 7 for bacterial growth. After 24 hrs incubation at 37°C, the best one showing maximum growth was subcultured for obtaining pure culture.

Bacterial DNA extraction by (Rapid geneO-Spin Column based) method and PCR amplification-

The bacterial DNA was extracted by rapid spin column method and stored at 2-8°C. The DNA thus obtained was subjected to PCR for further amplification of the DNA product. Both Gradient PCR and Scale up PCR was performed. The most common universal primer pair was used for gene amplification referred to as 20F and 1540R, and internal primers 800F and 900R were used.

20F- 5′-AGTTTGATCATGGCTCA- 3′, 1540R- 5′ AAGGAGGTGATCCAACCGCA- 3′
800F-5′-GTAGTCCACGCCGTAAACGA-3′,900R-5′-CGCCTGGGGAGTACGGCCGCA- 3′

The gradient PCR was done in Biorad thermalcycler to obtain DNA product 0f 1.5 kb size. A tube was loaded with 100ul final volume of a solution containing Nuclease free water , 10X buffer, 50mM MgCl2, 10mM dNTP mix, 20F, 1540R, Taq Polymerase enzyme. Master mix was then aliquoted in 7 PCR tubes as 14ul in each 200 µl capacity PCR tube. Vortex, spin and placed on thermal cycler block and started thermalcycler. The cycling conditions were as follows: Initial denaturation at 94°C for 5 mins, Denaturation at 94°C for 1 min, Primer annealing(gradient) 48°C/58°C for 0.50 sec, Primer extension at 72°C for 1.30 min, Repeat step 2 for 29 times, Final extension at 72°C for 5 mins then store at 4°C. PCR amplification was carried out under same cycling conditions obtained by gradient PCR annealing temperature at 56°C. The PCR products thus obtained in both PCR were visualized under UV light after agarose gel electrophoresis (1% gel) .

DNA Sequencing and Analysis-

After visualizing a single bright band, 50 µl of the 1.5kbp scale up PCR product and 100 picomoles of each forward and reverse sequencing primers : 800F and 900R was outsourced to “Merck Specialists Pvt Ltd; Bangalore” for DNA sequencing. PCR product was purified by Sanger Chain method, followed by cycle sequencing which was carried out by Sanger's modified method of DNA sequencing and after post cycle sequencing purification, sequenced products were read by ABI 3730 Genetic Analyzer.

After cycle sequencing and post cycle sequencing purification both the products were run for generating chromatogram.

Applied Bioinformatics-

The received data after DNA sequencing was studied for clean sequences by bioinformatics analysis for interpretation. The DNA sequence was received and read using BioEdit. Reverse complement was used to convert the reverse sequence into the forward orientation. The raw DNA sequence was compared with the existing sequences in the GenBank database using the BLAST tool from NCBI for identification of the unknown bacteria isolate. The reverse sequences were subjected to BLAST2 to locate the region exhibiting sequence identity. Sequence was selected from BLAST result and clustalW was performed. Output file was saved in form of alignment as well as FASTA. Alignment file was used for showing multiple sequence alignment in colour and FASTA format file saved. MEGA 5.05 was used for creating a phylogeny tree using neighbour joining bootstrap method. Finally, a blast was conducted amongst Bacillus group of bacteria.

RESULTS AND DISCUSSION

Culture of isolated bacteria-

The bacteria was isolated and grown in nutrient agar media and subcultured to obtain pure Culture.

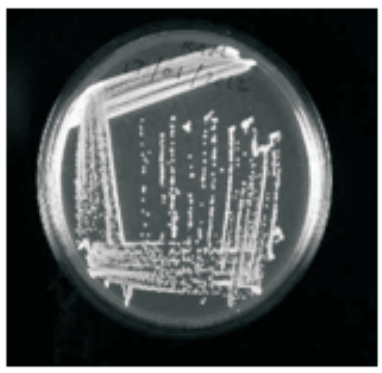


Fig 1:- Pure culture of bacteria

DNA extraction by spin column method and PCR amplification-

DNA was extracted from the nutrient broth by Spin column kit based method.The purified genomic DNA thus extracted was stored at 2-8° C.After extraction gradient PCR was done and gradient temperature was 56°C/48°C and annealing temperature was found to be 56°C.

16S rRNA Gradient PCR for standardization of annealing temperature

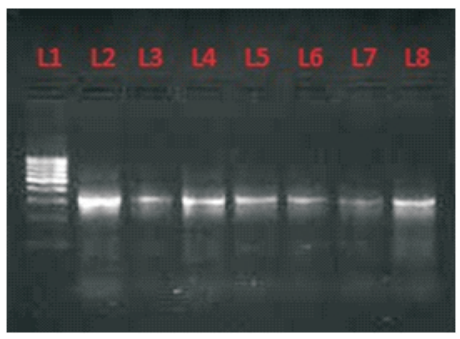


Fig 2:- Gradient PCR: Lane 1: Molecular wt. marker 500 bp ladder; Gradient Temperature- Lane 2: 56°C;

Lane 3: 54.2°C; Lane 4: 52.0°C; Lane 5: 50.1°C; Lane 6: 48.8°C; Lane7: 48°C; Lane 8(DNAzol method)

Scale up PCR for amplification of 1.5kbp DNA product

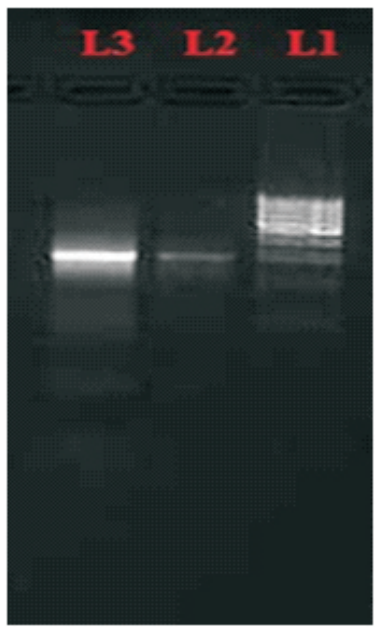
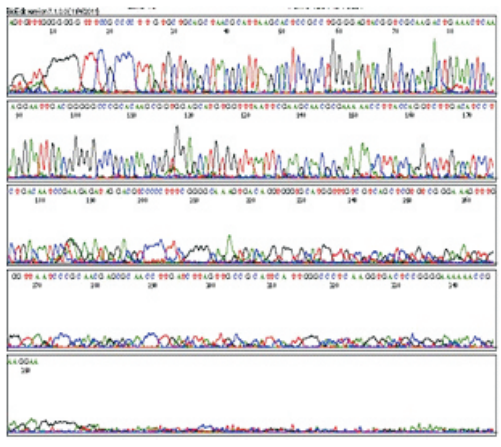


Fig 3:- Scale up PCR: Lane 1: Molecular weight marker 500 bp ladder; Lane 2: 5µl of PCR product; Lane 3: 25 µl of PCR product both amplified at 56° C. An intense band was observed in Lane 3 corresponding to 1.5 kbp product

DNA sequencing and analysis-

After scale up PCR the amplified DNA was outsourced to Merck Specialists Pvt Ltd; Bangalore. Cycle sequencing was carried out by Sanger's Chain Termination method. The chromatogram of the obtained sequenced is as follows.

The chromatogram of the sequence generated by 800F



The forward sequence was subjected to BLAST, after BLAST analysis it was confirmed that the unknown bacteria was Bacillus subtilis with 99% identity.

BLAST2 –

BLAST2 of obtained bacteria Bacillus subtilis was performed with reference sequence E.coli and V6 hypervariable region was obtained. Then BLAST2 of different Bacillus subtilis species was performed and their respective V6 hypervariable regions were obtained

BLAST outputs for 800F

Subject:J01859.1

Query:>B_subtilis_ATGLAB

>lcl|23485, Length=1541, Score = 241 bits (130), Expect = 1e-67, Identities = 234/281 (83%), Gaps = 20/281 (7%), Strand=Plus/Plus

Query 7 AGCTAACGCATTAAGCAC

-TCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGA 65

Sbjct 859 AGCTAACGCGTTAAG

-TCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGA 917

Query 66

ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAAAA 125

Sbjct 918

ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAA 977

Query 126 CCTTACCAGGTCTTGACATCCTCTGACAATCCGAAGAGAT

-AGGACGTCCCCCTTTCGGG 184

Sbjct 978 CCTTACCTGGTCTTGACATCCACGGA-AGTTTTCAGAGATGAGAATGTGCC--TT-CGGG 1033

Query 185 GCAAA-GTGA--CAGGTGGGTGCATGGTTTGTCGTCAGCTCGTGTCGGGAAA-GTTTGGG 240

Sbjct 1034 --AACCGTGAGACAGGTGC-TGCATGGCT-GTCGTCAGCTCGTGTTGTGAAATGTT-GGG 1088

Query 241 TTAA-TCCCGCAACGAGCGCAACC-TTGATC-TTAGTTGCC 278

Sbjct 1089 TTAAGTCCCGCAACGAGCGCAACCCTT-ATCCTTTGTTGCC 1128

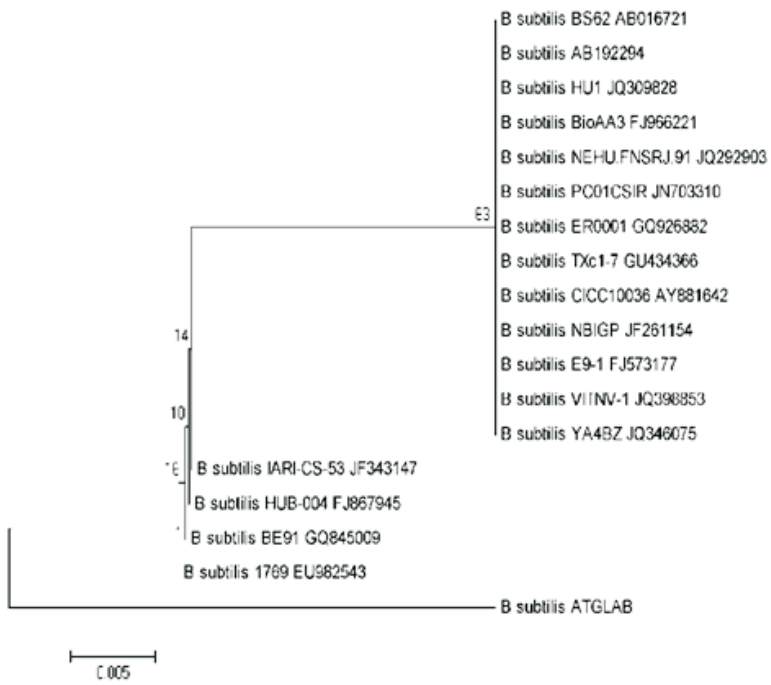
V6 hypervariable region

>B_subtilis_ATGLAB

GGTCTTGACATCCTCTGACAATCCGAAGAGATAGGACGTCCCCCTTTCGGGGCAAAGTGA

MEGA structure-(Molecular Evolution Genetic Analysis)-

Phylogenetic Analysis of hypervariable region V6 by MEGA 5.05 (Neighbour Joining Bootstrap method)



Phylogenetic analysis by MEGA 5: Phylogenetic trees showing the relationship among 16S rDNA gene sequences of Bacillus subtilis. Construction of phylogenetic trees was carried out by the neighbour-joining bootstrap method. The values indicate the percentage of occurrence in 1000 bootstrapped trees and the scale bar represents 0.005 nucleotide substitutions.

DISCUSSIONS

The isolation and microbial analysis of personal computer RAM showed different types of bacterial species. This confirms work by (Eltablawy, S.Y. and Elhifnawi, H.N. 2009) who found between 99%-100% contamination on computer keyboard and mice in a research centre. Pure culture was derived from mixed culture of bacteria to obtain single isolated colonies. This method is based on the principle that each viable microorganism will develop into a single colony. For molecular identification of microorganisms by 16S rRNA gene PCR was done. Both gradient PCR and Scale up PCR was done for gene amplification to obtain 1.5 kbp product. These gene amplicon was then subjected to BLAST were it was identified that the unknown bacteria was Bacillus subtilis. Then BLAST2 was performed, and E.coli was taken as subject and V6 hypervariable region was determined. HVR is that region which is diversified in each and every individual bacteria (Chakravorty et al., 2007) and suitable for distinguishing all 110 pathogenic bacterial species.Then unknown sequence and sequences of 17 other Bacillus subtilis species were used to construct phylogenetic tree by ClustalW and MEGA 5.05. In this topic it is determined that V6

hypervariable region is the best region for constructing phylogeny of different species of Bacillus subtilis species. Our investigation demonstrated that the V6 (nucleotide 986-1043) contains diverse character & maximum distinguish power for the Bacillus subtilis species analyzed. V6 region can be used for further studies like designing universal primer and probes for 17 other Bacillus subtilis species in the study.

CONCLUSION

The presence of computers in our daily lives is increasing day by day. Microorganisms are closely associated with the health and welfare of society some are beneficial while others are detrimental. Therefore, it is very important to identify the microflora on computer hardware and analyze for its pathogenecity, if any, so that some precautionary measures can be taken. The traditional identification of bacteria on the basis of phenotypic characteristics is generally not as accurate as identification based on genotypic methods. Comparison of the bacterial 16S rRNA gene sequence has emerged as a preferred genetic technique. 16S rRNA gene sequence analysis can better identify poorly described, rarely isolated, or phenotypically aberrant strains (Jill E. Clarridge, III 2004). This study has focused on hypervariable region of 16S rRNA and specially on V6 region and how about its analysis we can identify an unknown bacteria and create relationship and make phylogeny among closely related bacterial species and it is observed that V6 region is the best hypervariable region to differentiate among Bacillus species. The present finding suggests that the bacteria Bacillus subtilis is present on the personal computer component surface, RAM. Many explorations can be derived from this finding including carbon and energy source for bacteria, degradation of particular component, which is otherwise pose problem due to e-waste. Future studies can be extended in biodegradation of toxic components in personal computer.

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