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Phylogenetic Relationship between Microbial Communities in Waste Water

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Abstract: *Waste generation and its control have taken an important role in our environment, since most of the wastes are simply dumped on disposal yards. Therefore the greatest challenge to the environmentalists is the eco-friendly management of this waste and application of microorganisms in effective waste management.In order to design an efficient biological waste water treatment it is important to know the micro biota composition of the wastewater and their phylogenetic relationship. Patterns in the spatial distribution of organisms provide important information on the biodiversity and the complexity of ecosystems. The present study was carried out to isolate the most frequently occurring microorganism from waste water, sludge and effluent samples and to determine their Phylogenetic relationship by Blast analysis.*

Keywords: *Phylogeny, waste water, biodiversity.*

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I. Introduction:

Wastewater with high organic load causes many ecological problems. It shows adverse effects on both flora and fauna; its discharge to the land alters physical and chemical properties of the soil, thus reducing the fertility of land for crop production and its discharge to the water bodies may results in eutrophication, affecting the aquatic life and making water unfit for drinking (Manu *et al* .,2011). Hence, the challenge for the safe disposal of the wastewater cannot be ignored. Environmentalists and Government are looking for cheap, efficient, effective and long lasting solutions for wastewater treatment and recycling (Vishakha *et al* ., 2013). The greatest challenge to the environmentalists is the eco-friendly management of this waste and application of microorganisms in this context has got an age over other available technologies (Amrita Saha *et al*., 2014). In order to design an efficient biological waste water treatment it is important to know the micro biota composition of the wastewater. In recent years, a number of studies have been conducted to investigate biogeographic patterns of microorganisms, including bacteria, Archaea, fungi, and other microbial eukaryotes. Today, several studies have demonstrated that there are biogeographic patterns for microbes in natural habitats such as soil, freshwater, and the ocean (Xiaohui Wang *et al*.,2016). A growing body of research has shown that microorganisms, exhibited phylogenetic relationship patterns in different habitats at various taxonomic resolutions. The shaping mechanisms of phylogenetic relationship in microbial communities can be explained by contemporary environmental heterogeneity and historical events (Martiny JBH *et al*., 2006). Bacteria are generally identified by 16S rRNA sequencing. The rRNA is the most conserved (least variable) gene in all cells. Portions of the rRNA sequence from distantly-related organisms are remarkably similar (P. Sujatha *et al*., 2012).

II. Aim And Objectives:

Aim

To determine the microbial communities in wastewater and to study their phylogenetic relationship in order to design an efficient treatment wastewater module

Objectives

1. Isolation and Identification of bacterial isolates
2. Extraction of DNA from theseisolates
3. PCR Amplification
4. Gene sequencing
5. Determination of phylogeny by BLAST analysis

III. Materials And Methods

Area of Study:
The study was conducted at Madras Christian College, Chennai, Tamilnadu. The study was carried out using wastewater samples collected from different sectors.

Collection of Samples:
Different waste water samples from various sectors were collected in sterile containers.

- Sewage water from the Farm of Madras Christian College.
- Effluent from the General and Industrial Leathers (P) Ltd, Chrompet.
- Wastewater sample from Koovam,Adayar River.
- Municipality wastewater sample from West Tambaram, Chennai.
- Food Wastewater sample from a catering unit at Mudichur.

The samples were then transported immediately to the Microbiology laboratory for analysis.

Characterization of Isolates:

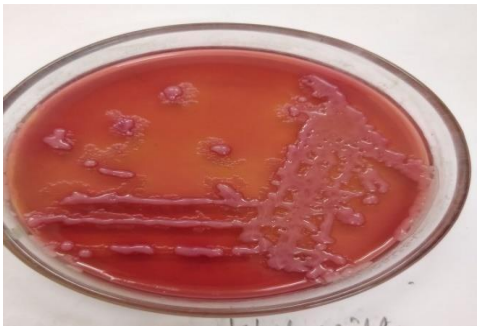
- Morphological characterization of the isolates was done by observing the size, color, elevation, margin of the colonies on basal and selective media.
- Preliminary tests like Gram's staining, motility and biochemical tests were done for the identification and characterization of bacteria. (According to Bergey's manual of Systematic bacteriology, 9thedition, 1994).
- ***DNA Extraction:***
  Extraction of DNA from the bacterial isolates was done as per the protocol (Xiaohui Wang *et al*., 2017).
- ***PCR Amplification:***
  The Polymerase chain reaction (PCR) amplification of partial 16s rRNA gene was carried out with the bacterial primer set 16F 27(5'-CCAGAGTTTGATCMTGGCTCAG-3') and 16R 1525X (5'-TTCTGCAGTCTAGAAGGAGGTGWTCCAGGC-3'). PCR was performed in an automated gene amplification PCR system 9700 thermal cycler. The template DNA was amplified via PCR reaction with the following conditions:
  Initial denaturation was done at 94^0C for 2minutes followed by 35 amplification cycles at 94^0C for 1minute; annealing temperature of primers was 55^0C for 1 minute (Soni,*et al*., 2009). The amplified product was subjected to electrophoresis.
- ***Agararose Gel Electrophoresis***
  The quality and intactness of the extracted DNA was examined by running
  on 1% agarose gel which contain 0.3µl ethidium bromide as well as on 0.8% agarose gel.(Pei Yun Lee,*et al*.,2012).
- ***Gene Sequencing***
  For bacterial classification generally sequencing of 16 S rRNA gene was used as an important identification tool (Clerck*et al*.,2004).Phylogenetic dendrograms were constructed to know the genetic relationship between the bacterial isolates.
- ***Determination Of Phylogeny By Blast***
  The basic local alignment tool (BLAST) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. Using heuristic method, BLAST finds similar sequences not by comparing either sequence in its entirety, but rather by locating short matches between two sequences(Scott McGinnis *et al*., 2004)

IV. Result

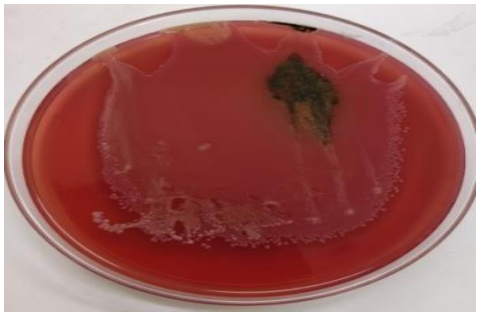
Characterization of Bacterial isolates:
A total of ten bacterial isolates were isolated out of which six isolates were characterized by Gram's staining, motility and biochemical tests, followed by their growth characteristics on selective media.
Figure 1 lists the growth of the bacterial isolates on Selective media

Sample: Koovam waste water
Organism: *Shigella flexneri*
Plate: Maconkey Agar
Colony: Non lactose Fermenting

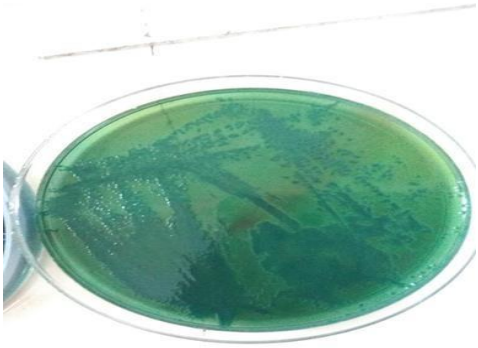
Sample:Sewage
Organism: *Escherichia coli*
Plate:Eosin methylene blue agar
Colony: Metallic sheen



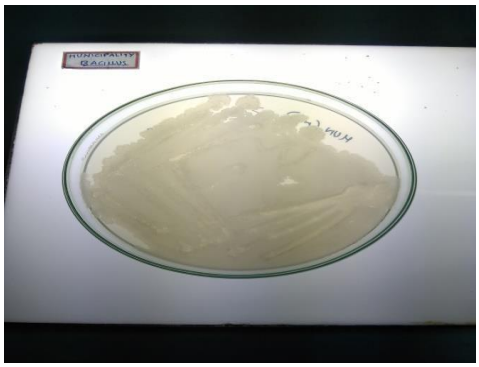
Sample: Koovam waste Water
Organism: *Klebsiella*
Plate: Maconkey Agar
Colony: Pink mucoid colonies



Sample: Effluent
Organism: *Salmonella typhimurium*
Plate: Salmonella-Shigella Agar
Colony: Black colour colony

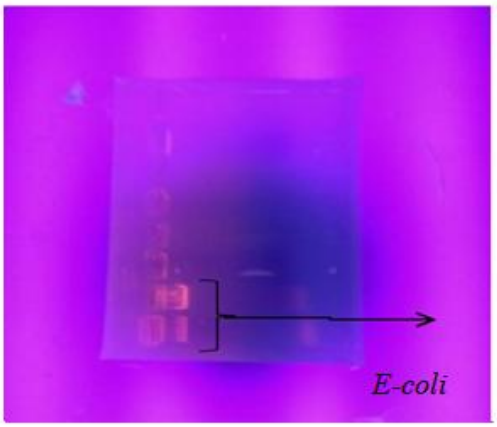


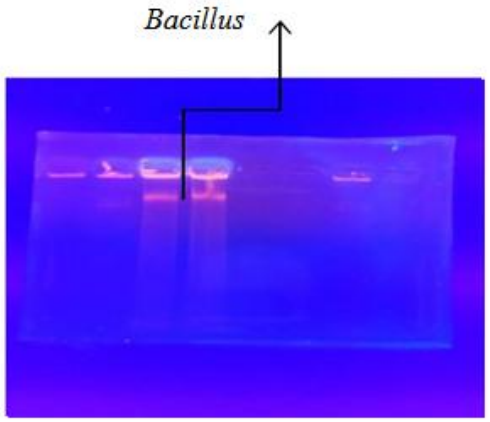
Sample: Food wastewater
Organism: *Vibrio parahemolyticus*
Plate: Thiosulphate-citrate-bile salt sucrose agar.
Colony: Green colonies



Sample: Municipality wastewater **Organism:** *Bacillus licheniformis*
Plate: Nutrient agar
Colony: Dried White Colonies

Identification of Bacterial Isolates by Molecular characterization:
The quality and intactness of the extracted DNA from the selected bacterial isolate were examined by running on 1% agarose gel as represented in Fig .2

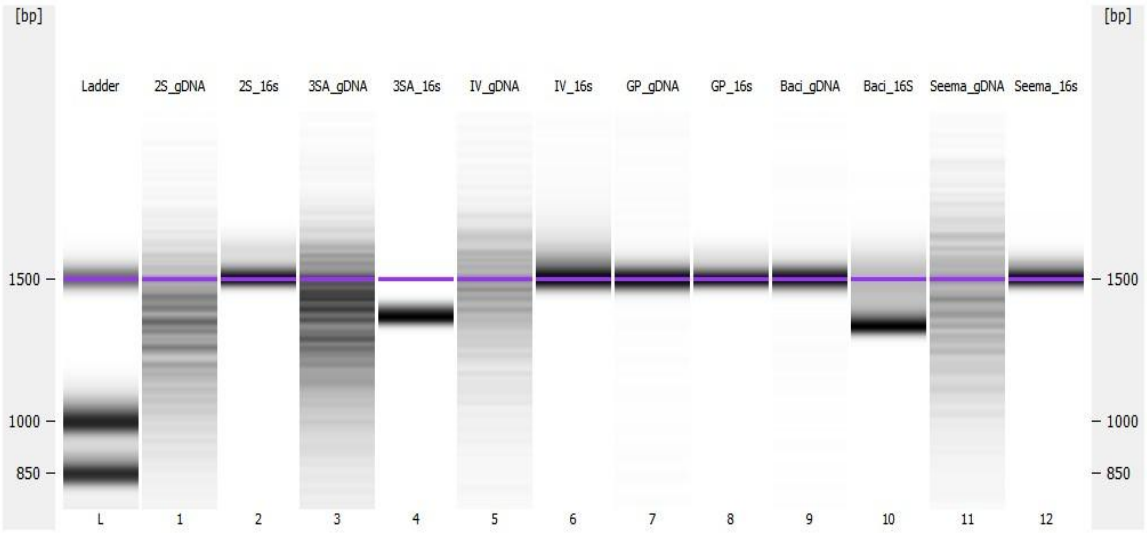




PCR Amplification

The amplified PCR product obtained were carried out for agarose gel electrophoresis. .The intense single bands were observed on 2% agarose gel stained with ethidium bromide. The DNA samples of three bacterial isolates (*Bacillus licheniformis, Shigella flexneri, Escherichia coli and Salmonella paratyphi A*) were run on the agarose gel and the bands were visualized when observed under the Gel doc (Fig.3)

Fig .3 PCR products of extracted DNA on agarose gel



Gene Sequencing

The sequencing of the 16S rRNA gene was done. The 16S Reverse sequence data of three bacterial isolates (*Bacillus licheniformis, Shigella flexneri and Escherichia coli*) were shown in Figure 4, 5 and 6.
Fig.4: *Shigella flexneri*

>16S Reverse Sequence Data

TTTACCCCTATTAAAGTGAAGGTCCTATAAAATTGTAGCGCCCTCCGAGGTTAAGCT
ACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCG
GGAACGTATTCACCGTGGCATTCTGATCCACGATTACTAGCGATTCCGACTTCATGG
AGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTATGAGGTCCGCTTGCTC
TCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGCACGTGTGTAGCCCTGGTCGTAGGGCCATG
ATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCT
CCTTTGAGTTCCCGGCCGGACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAAC
CCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCCTCACCGTTCCCGAAAGCAC
AATTCTCATCTCTGAAAACTTCGTGAATGTCAAGACCCGGTAAGGTTCTTCGCGTTGCATCCGAAT
AAACCAATTGTCCACCGCTGGGGCGGGCCCCCGTCATTCATTTAATTTTACCTTGCGGCCGACCCC

CAAGGCGGCGACTTAACCGTTAACTCCGAAGCCACACCCAAGGACAACCCCCAAGTACACTGTTT
AGCGTGAACACCAGGTATCTATCCGGTTGGCCCACGCTTTCTCACCTGAGCGTATCTTCTCAGGGC
CCCTTTCACCCGAATCTCCAACTCACATTCCGCTACCCGGAATACCCTCTAAAACAGTGCGATACA
TAGTCCAGTGAGCGGATTACTTATTAAACCCTCGGGGTTACCAGATTCATACTGTCTCGGTACCCC
GTTGACCAGATACGATCTCGGCACTGTAGAGAAAAAAATTATC TCCTGAAAAAT

Fig. 5:*Bacillus licheniformis*

>16S Reverse Sequence Data

GGAAACGGGGAATAATGACTAGTCTGGCCACTTCAGCGGCTGGCTCAAAGGGTACC
TCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGGC
CGGGAACGTATTCACCGCGGCATGGTGATCCGCGATTACTAGCGATTCCAGCTTCAC
GCAGTCGAGTTGCAGACTGGGATCCGAACTGAGAACAGATTTGTGGGATTGGCTTA
ACCTCGCGGCTTCGCTGCCCTTTGTTCTGCCCATTGGAGCACGTGTGTAGCCCAGGT
CATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCA
GTCACCTTAAAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTT
GCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTG
TCACTCTGCCCCCGAAGGGGAAGCCCTATCTCTAGGGATGTCAGAAGGATGTCAAG
AACCTGGTAAGGTTCTTCGCCGTGCTTCGAATTAAAACCACATGCTCCCACCGCCTT
GGTGCCGGGCCCCCGTCAATTCTTTTGAGTTTCAAGTCTTGCCAACCGGTAATTCCCA
AGGCGGAGTGCCTTAATTGCGGTTAAGCTGGCAGCACCTAAAGGGCGGGAAACCCC
TCTTAACAACTTAAGCCACTCATTCGTTTACGGCGTGGGAACTACCCAGGGGATCTC
TAATCCTTGTGCGCTCCCCACGCCTTTTCGCGGCCTCACGCTCGGTTACGGGACCAG
AAGATGCCCTCGCGCACTTGTGTTCTCCAATCCCTCACGATTCACGGCTTACAGTGG
AATCCACTTTTCCCCTCCGCCACTCAGGTCCCGAGTTCAAAAG

Fig. 6: *Esherichia.coli*

>16S Reverse Sequence Data

ACTTGCCATATTGTTTAAAATACAAAACATTATAGCGGGGCCCGAAGGTAAGCTCACCACTTCTTT
TGGAACACACTCCCATGGTGTGACGGGCGGTGTGGACAAGGGCCGGGAACGTATTCACCGGGGCA
TTGTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGGAGACTCCAATCCGGACTA
GAACGCAGTTTATGAAGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGGAACA
CGTGGGGAGCCCTGGTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTTCAGTTTATC
ACTGGGAGTCTCCTTTGAGTTGCCGGCCGGACGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGC
GGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACTGCTCC
CGAAGGCACATTCTCATCTCTGAAACGTCCGAGGATGTCAAGACCAGGGTAAGGTTCTTCGCGTTG
CATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCCGTCAATTCATTTGAGTTTAACTTG
CGGCGGTACTTCCCAGGCGGACGACTAATCGCGTTAGCTTCGGAAGTCAACGCTCTGAAGCCACA
ACCTCCTAGTACAACATCGTTTACGCGTGGACTTACCAGGGTATCTATTCCGGTGGTCCCAAGCTTT
GCACCTGAGGTCAATCTTGGACAAGAAGTCCCCCCTCGCCACAGATTCCTCGATTCAAGGAAATAC
CGTCAACTGGATTCCCACCCCCCCCCTAAAAGAAATCAAGGGGGTGGCGGGGG

BLAST Analysis

The 16srRNA sequence was compared using NCBI BLAST similarity search tool. The 16S forward and reverse sequence data were run on BLAST. According to the sequences producing significant alignment,identity shown by *Bacillus* was 91%, whereas *Shigella* and *E.coli* showed 93%and 91% similarity respectively. Based on the 16srRNA sequences, phylogenetic dendrograms were constructed to know the genetic relationship between the bacterial isolates and the phylogenetic tree showing close homologs to strains were represented in Fig 7,8and 9.

Fig. 7: Phylogenetic tree showing close homologs to *Shigella flexneri* strain.

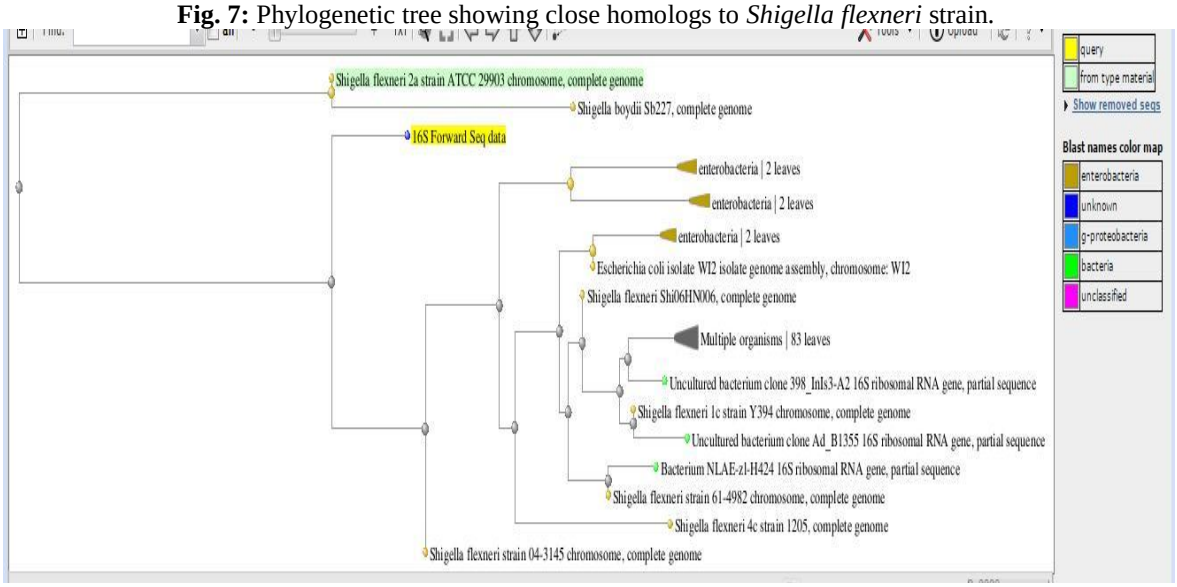


Fig. 8: Phylogenetic tree showing close homologs to *Bacillus licheniformis* strain

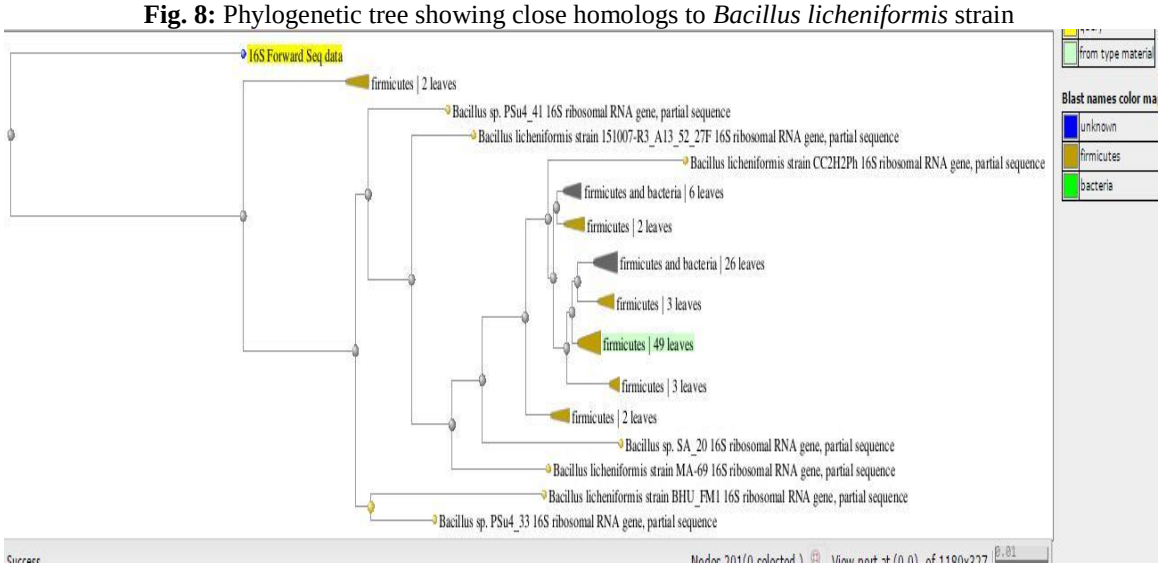
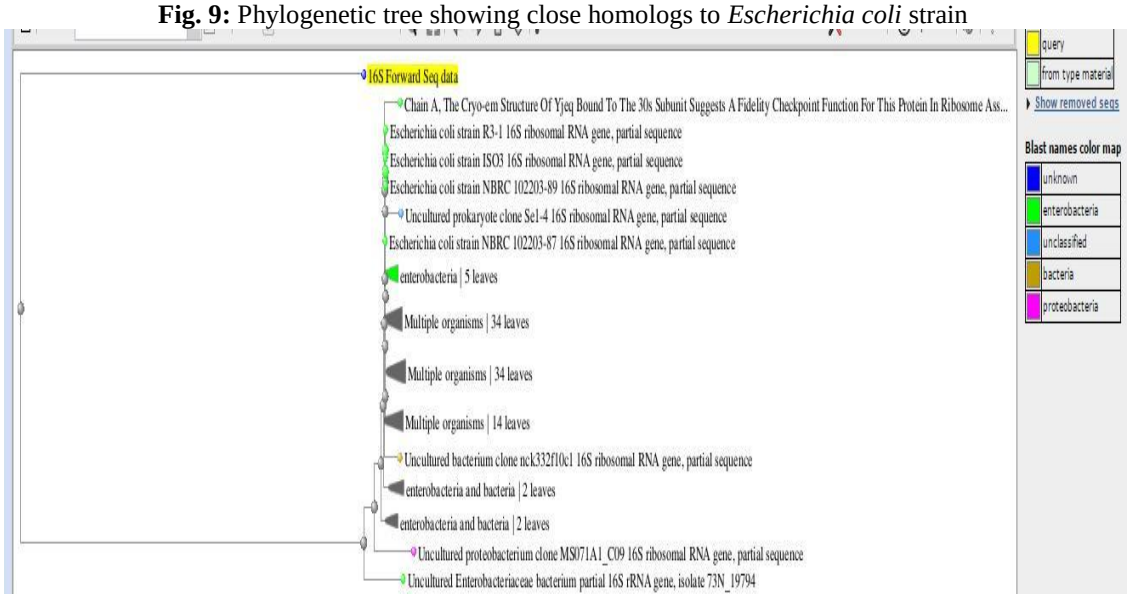
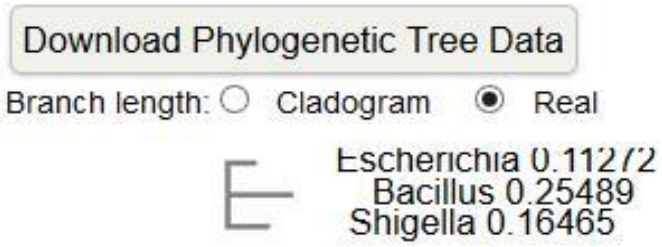


Fig. 9: Phylogenetic tree showing close homologs to *Escherichia coli* strain



Clustal Omega program was used to show closely related phylogeny between *Bacillus sp* and *Shigella sp* as represented in Fig. 10

Fig. 10: Phylogenetic dendograms



V. Discussion

The aim of this study was to isolate most frequently occurring and optimally performing microbial isolates from the various sectors of wastewater. A total of 10 bacterial isolates were obtained out of which six isolates were characterized namely *Shigella flexneri,Escherichia coli,Klebsiella, Salmonella typhimurium, Vibrio parahemolyticus and Bacillus licheniformis*. In the previous study microorganisms isolated from effluent included *Proteus sp, Staphylococcus aureus, Escherichia coli, Klebsiella sp Pseudomonas sp, Aspergillus niger, A. flavus, Fusarium sp and Penicillium sp* (T. A. Ogunnusi *et al*., 2014).Genomic DNA of the microbial community was extracted using direct extraction technique, followed by PCR targeting the 16S rDNA region. Distinct fragments of approximately 1100 bp in sizes were successfully amplified using PCR and cloned onto Escherichia coli XL-1 Blue. (Christy Chan Sien *et al*., 2015). In the current study molecular analysis of extraction of DNA showed similar bands of molecular weight. The quality and intactness of the extracted DNA was examined by running on 1% agarose gel which contained 1 µg/ml ethidium bromide.The 16 S rRNA sequence was compared using NCBI blast similarity search tool. (R.C. Edgar *et al*., 2004). In the present study the two strains *Bacillus licheniformis* and Shigella flexneri were found to be closely related.

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