



E-ISSN: 2278-4136  
P-ISSN: 2349-8234  
JPP 2017; 6(5): 2120-2122  
Received: 10-07-2017  
Accepted: 11-08-2017

**Jamilah Ali Ahmed Zaed**  
Department of Molecular and  
Cellular Engineering Jacob  
Institute of Biotechnology &  
Bioengineering Sam  
Higginbottom University of  
Agriculture Technology and  
Sciences, Allahabad, UP, India

**Poonam Singh**  
Department of Molecular and  
Cellular Engineering Jacob  
Institute of Biotechnology &  
Bioengineering Sam  
Higginbottom University of  
Agriculture Technology and  
Sciences, Allahabad, UP, India

## Study of microbial diversity in great Man-Made river water

**Jamilah Ali Ahmed Zaed and Poonam Singh**

### Abstract

In the recent years the development of genomics and introduced new techniques and greater possibilities in the study of microbial diversity in the environment. The present research was done on bacterial diversity in water samples from Great man-made river in Libya by using Metagenomics, new technology in the world of genetic engineering field. DNA sequences or phylogenetic analysis depended on a single gene, the 16S small subunit ribosomal RNA (rRNA) gene. DNA nucleotide sequence were analyzed with 16s r RNA by BLAST and phylogenetic tree to obtained the relation between types of bacterial sequence get in the present study. The first sequence CG20160324A shows similarity to *Staphylococcus saprophyticus*., Second sequence CG20160324B shows similarity to *Vibrio vulnificus* strain NBRC 15645, Third sequence CG20160324C shows similarity to *Streptococcus pyogenes* strain-1-273 and Four sequence CG20160324AD shows similarity to *Aeromonas salmonicida*. This diversity study proves that All the species identified shows toxic characters.

**Keywords:** genomics, bacterial diversity, phylogenetic analysis, toxic.

### Introduction

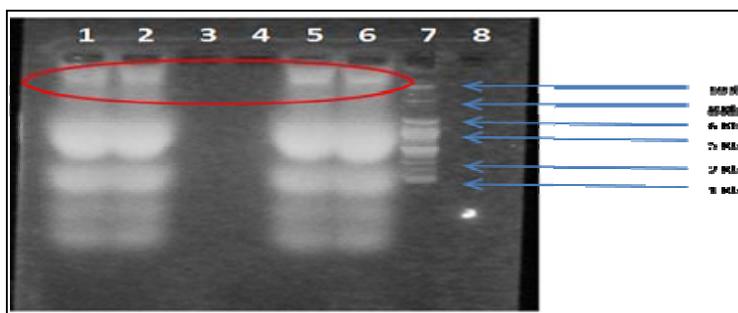
Bacteria are as important agents in biogeochemical processes in all watery ecosystems. It is fully known that heterotrophic prokaryotes play prominent role in the structure and dynamics of web networks and in the remineralization of organic matter (Azam *et al.*, 1983 [2]; Azam and Long, 2001) [1]. Metagenomics provides access to the functional gene installation of microbial communities and thus gives a much broader description than phylogenetic surveys, which are often based only on the diversity of one gene, for example 16S rRNA gene. It can also be supplemented with metatranscriptomic or metaproteomic approaches to characterize expressed activities (Wilmes and Bond 2006 [4]; Gilbert *et al.*, 2008 [3]).

### Materials and Methods

Four surface water sample was collected from Great man-made river at Tripoli sate in Libya. Isolation of DNA was done by Metagenomics technique from Water Samples of Great man-made river. Quantification of isolated DNA was done by using UV-Vis Double Beam Spectrophotometer. Amplification of DNA was performed in a total volume of 30  $\mu$ l. with 16s r RNA. Sequencing analysis was done by using bioinformatics tools (BLAST and CLUSTAL OMEGA tool).

### Results and Discussions

Isolated DNA were run for gel electrophoresis having molecular wifor samples A, B, C and D showing (Figure 1).

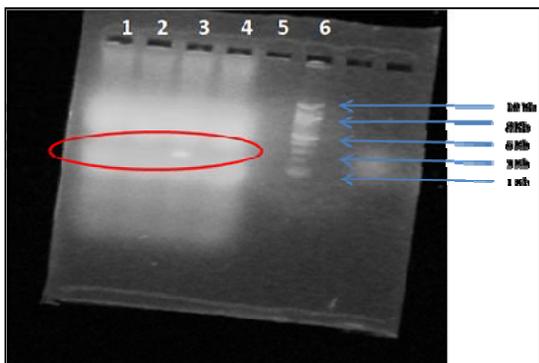


**Fig 1:** Show the electrophoresis genomic DNA isolated from Great man-made river of samples A, B, C and D at Libya.

**Correspondence**  
**Poonam Singh**  
Department of Molecular and  
Cellular Engineering Jacob  
Institute of Biotechnology &  
Bioengineering Sam  
Higginbottom University of  
Agriculture Technology and  
Sciences, Allahabad, UP, India

Lane 7: DNA Ladder Size: 1kb  
 Lane 1: Sample A (Genomic DNA) Size: More than 10 Kb  
 Lane 2: Sample B (Genomic DNA) Size: More than 10Kb  
 Lane 5: Sample C (Genomic DNA) Size: More than 10 K  
 Lane 6: Sample D (Genomic DNA) Size: More than 10 Kb  
 Isolated DNA were amplified by PCR with 16s rRNA primer running it in gel electrophoresis, (Figure 2).

Lane 1: Sample A (PCR product) Size: 1.2-1.3 Kb  
 Lane 2: Sample B (PCR product) Size: 1.2-1.3 Kb  
 Lane 3: Sample C (PCR product) Size: 1.3-1.4 Kb  
 Lane 4: Sample D (PCR product) Size: 1.3-1.4 Kb  
 The genome of Bacteria with 16s r RNA gene were amplified by PCR. and sequencing was done by sanger's method. The sequences code result are shown in Table 2.



**Fig 2:** Agarose gel electrophoresis of amplified DNA product by PCR from Great man -med river of water samples A, B, C and D.

**Table 2:** The sequence code of Bacterial genomic DNA of Great man-made river water sample

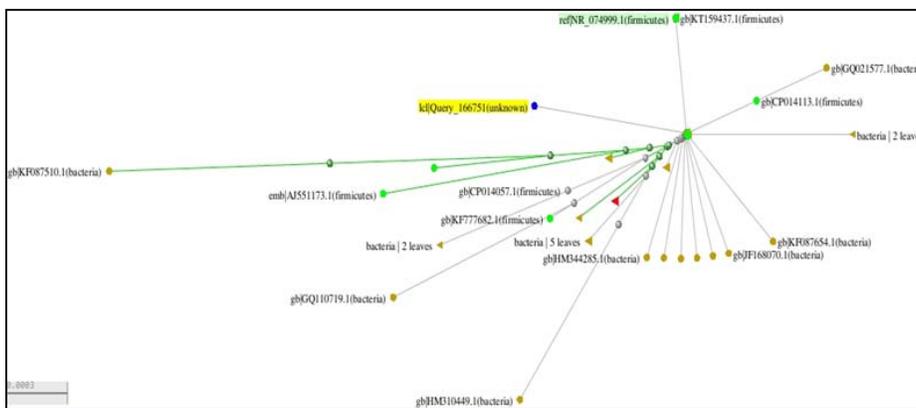
Sample No.	Sample ID	Sample description	Code sequence
1	A	Great man-made river	CG20160324A
2	B	Great man-made river	CG20160324B
3	C	Great man-made river	CG20160324C
4	D	Great man-made river	CG20160324D

**Sequencing analysis by bioinformatics tools**

**(a) The first sequence was CG20160324A for sample A of Great man- made river.**

The BLAST result show percentage of similarity of sample A sequence CG20160324A with another sequence. It was observed that the First sequence is new and not show 100 % similarity, the largest percentage of similarity was 96 % with *Staphylococcus saprophyticus*. (Figure 3).

Lane 6: DNA Ladder Size: 1kb



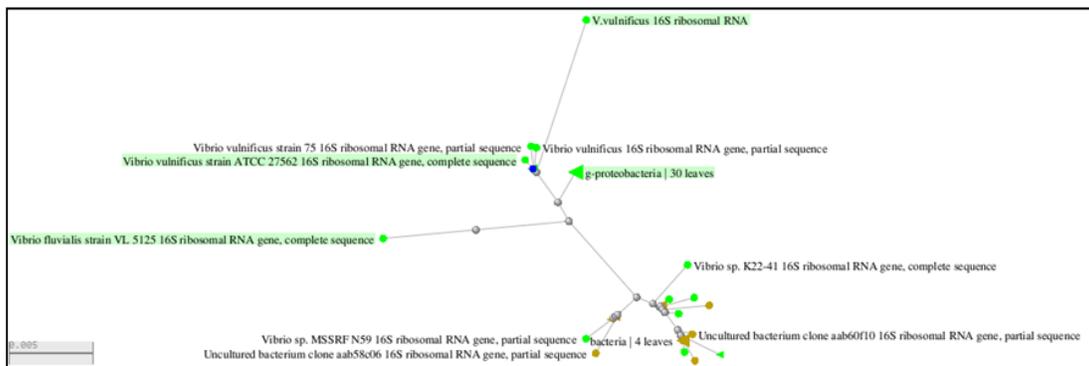
**Fig 3:** Phylogenetic tree of *Staphylococcus saprophyticus* strain.

After analys it was observed that first sequence with NCBI and BLAST result of Query to 166751 (unknown) and closest to NR-o74999.1 (firmicutes) gb1 KT159437.1 (firmicutes) and related to big tree, which are Gram-positive, coagulase-negative facultative bacterium belongs to Micrococcaceae family.

**sample from Great man- made river.**

The BLAST result show percentage of similarity The second sequence CG20160324B with another sequence, it was observed that the second sequence is new and not show 100% similarity, the largest percentage of similarity was 99% with *Vibrio vulnificus* strain NBRC 15645. The design of phylogenetic tree of *Vibrio vulnificus* strain NBRC 15645 and relation with another bacterial in (Figure 4).

**(b) The sequence was CG20160324B for sample B water**

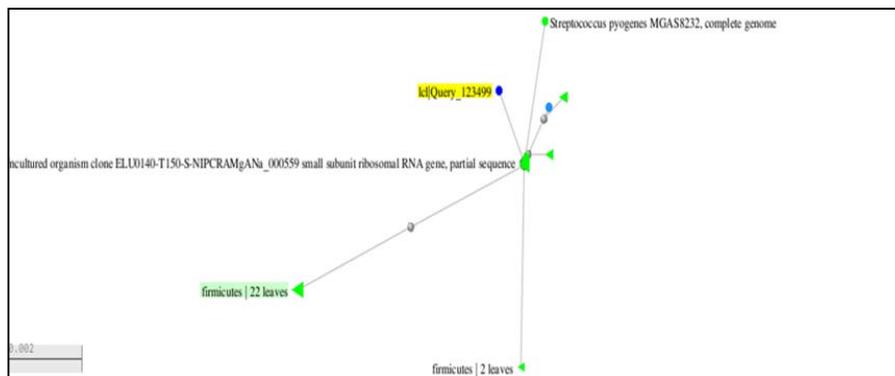


**Fig 4:** show the phylogenetic tree of *Vibrio vulnificus* strain NBRC 15645.

After analysis second sequence from phylogenetic tree, the result shows similarity to *Vibrio vulnificus* strain NBRC 15645 and closest with *Vvulnificus* 16s ribosomal RNA, and *Vvulnificus* strain VL 5125 is considered as the father was *g*-protobacteria. This type of bacteria is considered as highly toxic.

**(c) The Third sequence was CG20160324C for sample C water from Great man med river.**

The BLAST result show percentage of similarity The third sequence CG20160324C with another sequence, observed for the third sequence from Great man- med river is new and not show 100 % similarity, The largest percentage of similarity was 97 % with *Streptococcus pyogenes* strain-1-273. (Figure 5).



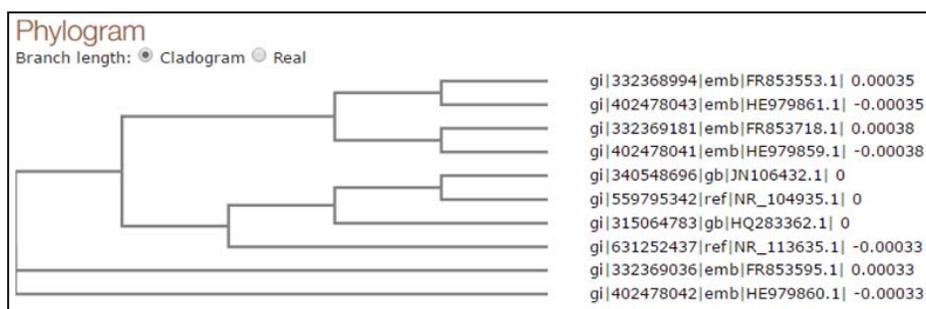
**Fig 5:** Phylogenetic tree of *Streptococcus pyogenes* strain-1-273

After analysis third sequence from phylogenetic tree the result was similarity to, *Streptococcus pyogenes* strain-1-273.that have following and Query-123499 and closest to *streptococcus pyogenes* MGAS8232, complete genome.

**(d)The fourth sequence wasCG20160324D for sample D from Great man-made river**

The BLAST result show percentage of similarity The fourth

sequence CG20160324D with another sequence, observed for the fourth sequence from Grate man -med river is new and not show 100 % similarity, the largest percentage of similarity was 98 % with *Aeromonas salmonicidasub* phylogenetic tree of *Aeromona ssalmonicidasub* a chromogenes. and relation with another bacteria in (Figure 6).



**Fig 6:** Phylogenetic tree of *Staphylococcus saprophyticus* strain

After analysis fourth sequence, the result of phylogenetic tree was similarity to *Staphylococcus saprophyticus* strain, The genus *Aeromonas*, is toxic type and comprises a collection of Gram-negative bacteria.

**Nucleotide sequence accession numbers**

All Nucleotide sequence data from this study were submitted to the DDBJ Sequence under accession numbers for 4 samples.

Sample No.	Sample I.D.	Sample Source	Code Sequence	Accession No.
1	A	Great man-Made river	CG20160324A	LC140943
2	B	Great man-Made river	CG20160324B	LC140944
3	C	Great man-Made river	CG20160324C	LC140945
4	D	Great man-Made river	CG20160324D	LC140946

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