Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
JPP 2017; 6(5): 2116-2119
Received: 07-07-2017
Accepted: 08-08-2017

**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

**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

# Study of Microbial diversity in Ganga river water sample by the use of Metagenomics technique

**Jamilah Ali Ahmed Zaed and Poonam Singh**

**Abstract**
Microbes are diverse and ubiquitous organisms that play a key role as drivers for ecosystem processes and effected on all living including fish, plant, animal and human also, few of the microorganisms present in nature have been cultured and describe. It is generally accepted that less than 1% of bacteria and fungi, present in most homeland have been cultivated for study in pure culture. In the recent years the development of genomics and introduced new techniques and greater possibilities in the study of microbial diversity in the environment, and those techniques metagenome. This research was done on bacterial diversity in water samples of Ganga river in India by using Metagenomics, new technology in the world of genetic engineering field. This study was based on DNA sequences classification or phylogenetically depended on a single gene, 16S RNA (rRNA) gene. The sequence of nucleotides DNA aligned with Bioinformatics tool. It was observed in this study that metagenomics is a good technique for isolation of microbial DNA from water sample by crossing culture process. which show good amount of DNA concentration. That amount of DNA concentration was amplified and sequenced with 16s r RNA. BLAST and phylogenetic tree analysis was done to obtain the relation between types of bacterial sequence analyze in the research. It was observed that The first sequence CG20160320A shows similarity with *Bacillus sp.c-2-33*,Second sequence CG20160320B shows similarity with *Bacillus anthracis* strain BHR1P1B3, Third sequence CG20160320C shows similarity with *Pseudomonas aeruginosa* strain AT cc and Four sequence CG2016032OD shows similarity to *Escherichia coli* strain ZH193. First type strain is not toxic and second, third and fourth types were toxic especially *Bacillus anthracis* strain BHR1P1B3.

**Keywords:** Ubiquitous, metagenome, phylogenetically, *Bacillus anthracis*

## Introduction
The Rivers are the chief source of fresh and renewable water for humans and freshwater ecosystems (Vorosmarty *et al*., 2010) [10] microbial diversity in flowing freshwater (lotic) is not as much of studied rather than in marine or lake ecosystems (Zinger *et al*., 2012) [11]. Their diversity and structure are determined by the temporal and spatial variability of physicochemical and biotic parameters (Hale *et al*., 2015) [3]. Recently, terminal restriction fragment length polymorphism (T-RFLP) and Sanger sequencing were used to describe the microbial community structure and composition (Mark *et al.*, 2016) [6]. Most studies of microbial communities in systems ranging from the open ocean to soil to the human gut have depended on a single gene, the 16S small subunit ribosomal RNA (rRNA) gene (Costello *et al*, 2011) [1].

## Materials and Methods
Surface water samples were collected from Ganga River at site in Allahabad UP state India. Isolation of DNA was done by Metagenomics technique.Sequence, analysis was done by using bioinformatics tools (BLAST and CLUSTAL OMEGA).

## Results
By using metagenomics technique DNA of 10 kb were extracted directly from water samples from Ganga river, India. (Figure 1)

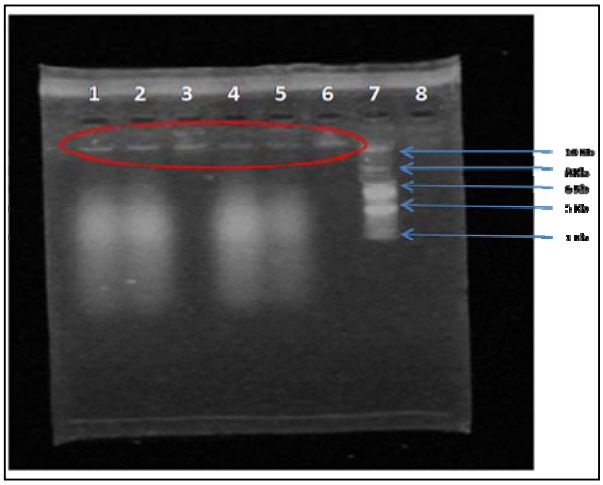


**Fig 1:** Show the electrophoresis of genomic DNA isolated from Ganga river, India in water sample A, B, C and D

Lane 7: DNA Ladder Size: 10 kb
Lane 1: Sample A (Genomic DNA) Size: More than 10 Kb
Lane 2: Sample B (Genomic DNA) Size: More than 10 Kb
Lane 4: Sample C (Genomic DNA) Size: More than 10 Kb
Lane 5: Sample D (Genomic DNA) Size: More than 10 Kb

Amplification of genomic DNA of Microbes was done by PCR with 16s r RNA primer and gel electrophoresis was performed, lane 1,2,5 and 6 shows bands of 10 kb,8 kb,6 kb and 2 kb respectively of sample A,B,C and D and in lane 8 DNA ladder of 10 kb was used.

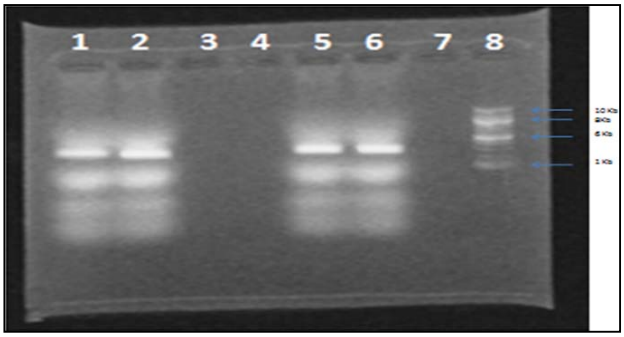


**Fig 2:** Agarose gel electrophoresis of amplified product from colony PCR for Ganga river of water samples A, B, C and D.

Lane 8: DNA Ladder Size: 1kb
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 5: Sample C (PCR product) Size: 1.3-1.4 Kb
Lane 6: Sample D (PCR product) Size: 1.3-1.4 Kb

The Bacterial genome 16s r RNA gene amplified by PCR. and sequenced by The sanger's method. the sequences code result for the Ganga river water samples India are showen in

**Table 2:** Result for sequence code of Bacterial genome of Ganga river water sample

| Sample No | Sample ID | Sample description | Code sequence |
|---|---|---|---|
| 1 | A | Ganga river | CG20160320A |
| 2 | B | Ganga river | CG20160320B |
| 3 | C | Ganga river | CG20160320C |
| 4 | D | Ganga river | CG20160320D |

**Sequence Analysis by Bioinformatics tools**

**(a) Sequence analysis for sample A of Ganga river, India:** The BLAST result show percentage of similarity with another sequence. It was observed that the First sequence is new and it do not show 100 % percentage of similarity and the largest percentage of similarity was observed with *Bacillus sp*.c-2-33. In Figure 3 The design of phylogenetic tree of *Bacillus* sp.c-2-33 and relation with another bacterial sp.

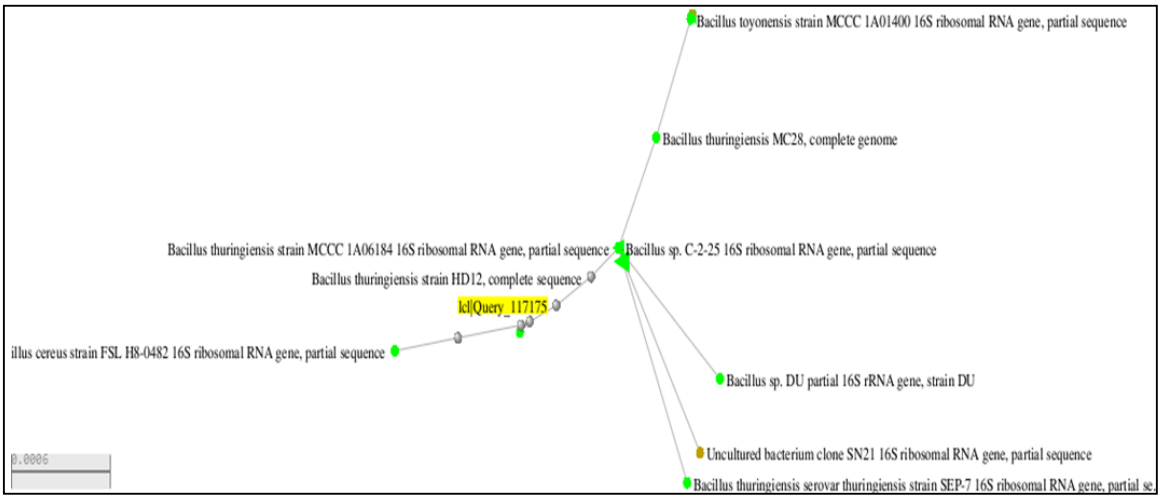


**Fig 3:** Show the phylogenetic tree of *Bacillus* sp.c-2-33

From phylogenetic tree it can find distance value, The result of sample A sequence (CG20160320A) was query to ICI/Query-117175 and make closest with it and shows similarity to *Bacillus thuringiensis* strain HD12,complete sequence and the *Bacillus thuringiensis* strain MCCC IA06I84 16s ribosomal RAN gene partial sequence represent the father for it which also proof that sample A don't have any toxic impact on ganga river India. They are gram positive, aerobic, rod-shaped and endospore forming.

**(b) Sequence analysis for sample B of Ganga river, India:** The BLAST result show percentage of similarity of the second sample CG20160320B with another sequence, the second sequence from Ganga river water is new because it did not show 100 % similarity with any sequence, The large percentage of similarity was observed with *Bacillus anthracis* strain BHR1P1B3.The understanding of evolutionary relationships is a fundamental aspect of modern biology, with the phylogenetic tree being a primary tool for describing these associations The design of phylogenetic tree of *Bacillus anthracis* strain BHR1P1B3 and relation with another bacteria shown in Figure 4.

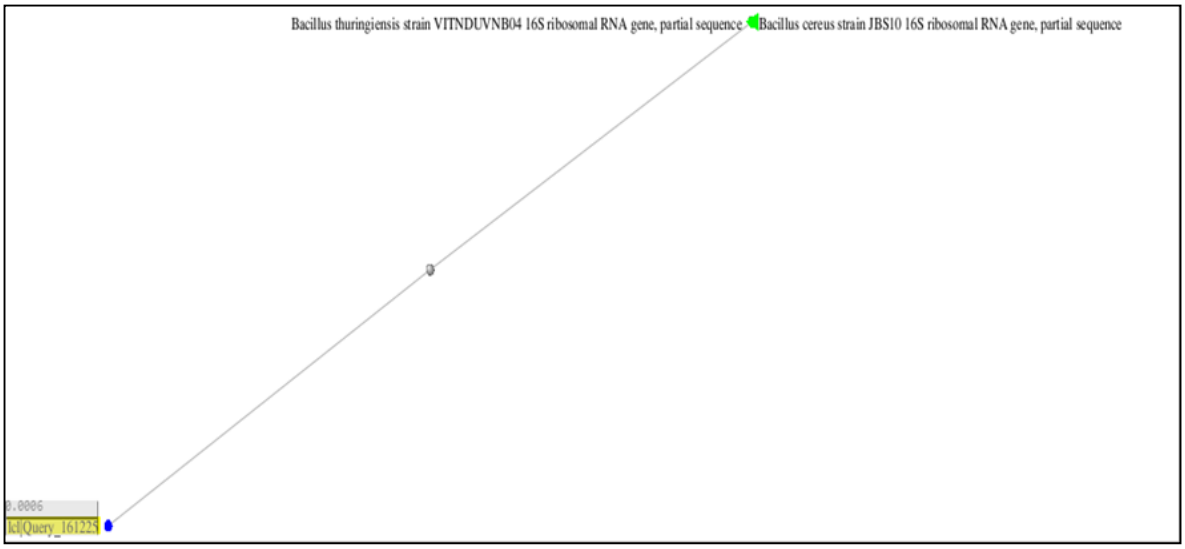


**Fig 4:** Show the phylogenetic tree of bacillus anthracis strain BHR1P1B3.

From phylogenetic tree can get distance value, The second sample from Ganga river sequence CG20160320B and was query to ICI/Query-161225 and make closest with it and similarity to it unknown and the father was *Bacillus anthracis* strain VITNDUVNB04 16s ribosomal RAN gene partial sequence and *Bacillus anthracis* strain JBS10 16s ribosomal1 RNA gene partial sequence which considered toxicity of sample B bacterial strain. They are Gram-positive, rod-shaped bacterium.

**(C) Sequence analysis for sample C of Ganga river, India**
The BLAST result show percentage of similarity observed for the sample C from Ganga river is new and it did not showing 100 % similarity, the largest percentage of similarity was 97% with *Pseudomonas aeruginosa* strain ATcc. The design of the phylogenetic tree of *pseudomonas aeruginosa* strain ATCC and relation with another bacteria shows in (Figure 5). From phylogenetic tree the distance value for the sample C from Ganga river result sequence CG20160320C was query to *pseudomonas aeruginosa* strain ATCC 101451, and considered independent type, in big tree and are toxicity, Gram

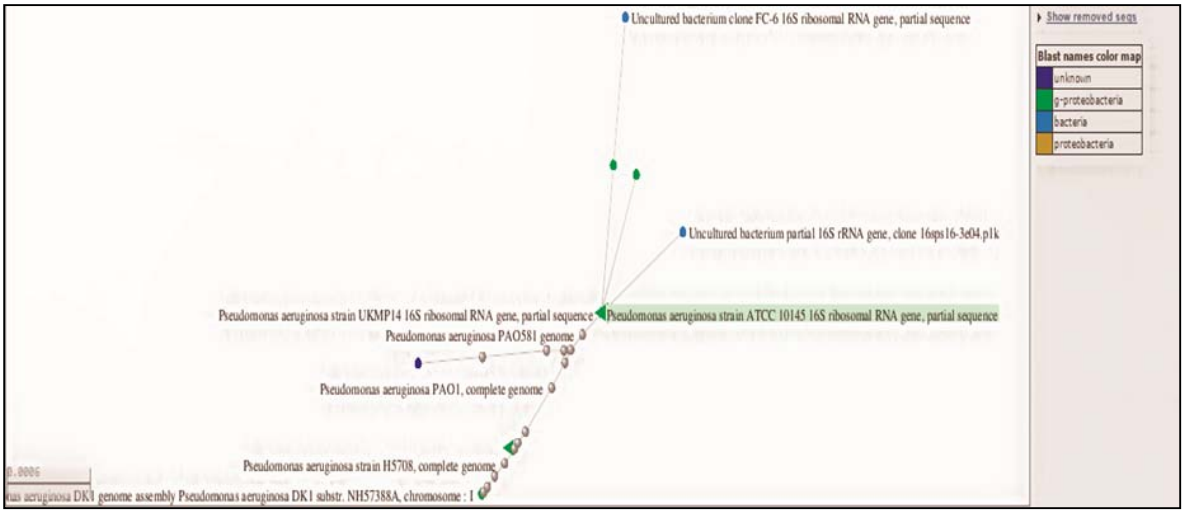


**Fig 5:** Phylogenetic tree of *pseudomonas aeruginosa strain* ATCC.

**(D) Sequence analysis for sample D of Ganga river, India**
The BLAST result show percentage of similarity for fourth sequence CG20160320D with another sequence. It was observed that sample D of Ganga river is new and not show 100 % similarity, The largest percentage of similarity was 98 % with *Escherichia coli strain ZH* 193. The design of phylogenetic tree of *Escherichia coli* strain ZH193 and relation with another types of bacteria (Figure 6).

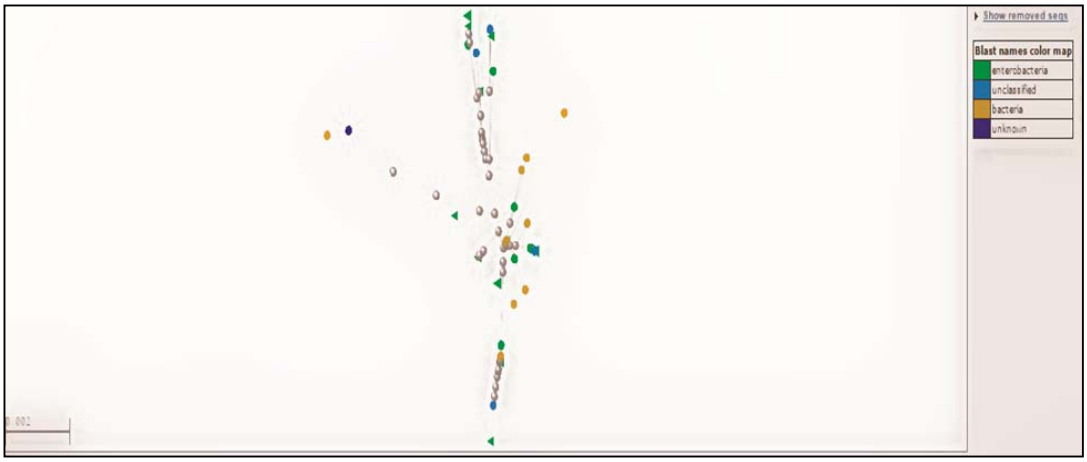


**Fig 6:** Phylogenetic tree of *Escherichia coli* strain ZH193.

From phylogenetic tree the distance value for the sample D from Ganga river result sequence CG20160320D strain ZH193 was related to very big tree and it is characterization are, a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium.

**Nucleotide sequence accession numbers**

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

| Sample No. | Sample I. D. | Sample Description | Code sequence | Accession No. |
|---|---|---|---|---|
| 1 | A | Ganga river | CG20160320A | LC144545 |
| 2 | B | Ganga river | CG20160320B | LC144546 |
| 3 | C | Ganga river | CG20160320C | LC144547 |
| 4 | D | Ganga river | CG20160320D | LC144548 |

**Discussions**

Metagenomics field is a set of technique and methods for the analysis of genomes, ubiquitous bacterial gathered without the need for culturing the elements in a way sporadic and considered this field a marriage between science microbiology and genomics, have allowed biologists to study genomes of organisms that cannot survive on their own, such as symbionts and pathogens symbionts (Tringe, 2005) [9].

Cutaneous anthrax accounts for more than 95% of human cases worldwide. Anthracis is an important pathogen that has been extensively studied not only for maintenance of public health worldwide but also for the defense against an effective biological weapon (Hirohito *et al* 2015) [4]. In the area concerned by the present study no treatment is applied to the abattoir effluent before it is released in the environment and spills into the small river Koreye. The water from the stream in turn is used to irrigate crops grown at a nearby farm. (Kabiru*et al.*, 2015) [5].

**References**

1. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI. Bacterial community variation in human body habitats across space and time. Science. 2009; 326:1694-1697.
2. Hale R, Scoggins M, Nathan J, Mucker S, Suchy A. Effects of climate on the expression of the urban stream syndrome. Freshwater Science. 2015; 35:421-428.
3. Hirohito O, Daisuke F, Miyuki O, Naomi O, Bernard M, Hang TE *et al*. A Novel Multiplex PCR Discriminates Bacillus anthracis and Its Genetically Related Strains from Other Bacillus cereus Group Species. PLoS ONE. doi:10.1371/journal.pone.0122004, 2015; 10(3):e0122004.
4. Kabiru LM, Bello M, Kabir J, Grande L, Morabito S. Detection of pathogenic Escherichia coli in samples collected at an abattoir in Zaria, Nigeria and at different points in the surrounding environment. International journal of environmental research and public health. 2015; 13-12(1):679-91.
5. Mark I, Jincai MB, Shelton EM. Bacterial community composition and structure in an Urban River impacted by different pollutant sources. Science of the Total Environment, 2016, 1176-1185.
6. May A, Allaf A. Isolation of Bacillus spp. from some sources and study of its proteolytic activity. Environmental & Pollution Control Research Center, Mosul university Mosul, Iraq, Tikrit Journal of Pure Science : ISSN: Published: March 16, 2015http://dx.doi.org/10.1371/journal.pone.0122004. 2010; 16(4).
7. Vorosmar TY, Mclntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P *et al*. Globle threats to human water security and river biodiversity. Invitrogen. 2010; 467:555-561.
8. Zinger L, Gobet A, Pommier T. Two decades of describing the unseen majority of aquatic microbial diversity. Molecular. Ecology. 2012; 21:1878-1896.