Comparative Analysis of Bacterial Diversity in the Water Samples from Domestic wastewater through Metagenomics Technique

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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. . Metagenomic is considered as a powerful method to study the bacterial diversity in environmental samples without using any cultural techniques. It provides a broader knowledge of bacterial diversity and avoid any risks. The present research was done on bacterial diversity in water samples from Domestic wastewater. Indian 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 results of this study identified 7 new strains that included 05 families: *Vibrio*, *Streptococcus* , *pseudomonas*, *Escherichia coli*, Mostly identified bacteria were pathogenic to humans. This study will help in reducing the water borne diseases which are to using several emerging bacterial pathogens.

This diversity study proves that most the species identified shows toxic characters.

Keywords: Metagenomic technique, Bacterial diversity, Domestic wastewater phylogenetic.

تحليل ومقارنة التنوع البكتيري في عينات المياه من مياه الصرف الصحي من خلال تقنية الميتا جيونومك

الخلاصة

في السنوات الأخيرة تطور علم الجينوم وأدخل تقنيات جديدة وإمكانيات أكبر في دراسة التنوع الميكروبي في البيئة.) Metagenomic(تعتبر طريقة الميتا جيونومك طريقة فعالة لدراسة التنوع البكتيري في العينات البيئية دون. وتوفر معرفة أوسع بالتنوع البكتيري مع جودة عالية في تحديد الانواع وتكلفة اقل واسرع في الوقت مع تجنب أي مخاطر . تم إجراء البحث الحالي على التنوع البكتيري في عينات المياه من مياه الصرف الصحي المنزلية. الهندي ، باستخدام التكنولوجيا الجديدة في مجال الهندسة الوراثية. تعتمد تسلسلات الحمض النووي الريبوزي و تحليل شجرة النشوء والتطور على جين واحد ، جين الحمض النووي الريبي الريبوسومي الصغير للوحدة الفرعية 16 الرنا الريباسي). تم تحليل تسلسل النوكليوتيدات DNA باستخدام 16 r RNA بواسطة BLAST وشجرة النشوء والتطور للحصول على العلاقة بين أنواع التسلسل البكتيري في الدراسة الحالية. حددت نتائج هذه الدراسة 7 سلالات جديدة تضمنت 04 عائلة: الضمة ، العقدية ، الزائفة ، الإشريكية القولونية ، البكتيريا التي تم تحديدها في الغالب كانت مسببة للأمراض للإنسان. ستساعد هذه الدراسة في الحد من الأمراض التي تنقلها المياه والتي تظهر سمات سامة على النظام البيولوجي و العديد من مسببات الأمراض البكتيرية الناشئة..

INTRODUCTION

Water represents big part on our planet that is essential to live on the earth. It is impossible to live without water. The different shapes of water body and chemical content from salt water are at oceans, sea and some ponds. Sweet water is present in rivers, lakes and ground water. We need to protect the blue surface i.e. water from pollution and the important pollutants are bacteria. Water is one of the most important bacterial habitats on Earth. Human activities led to the creation of the so-called urban water cycle, comprising different sectors. That diversity of bacteria eventually reached humans. In addition, bacteria can also transfer mobile genetic elements between different water types.Safe drinking water is a substantial human right and if polluted with pathogenic bacteria, it may have health implication for the consumers **(Fawell and Ahuijsen, 2003; WHO, 2004)** because water is the basic need of human and hence it should be clean, pure and free from pollutants (organic or inorganic) to protect Human health **(Volker *et al*., 2010).**

In rural communities, the surface water from streams, dams, and rivers is immediately used for domestic purpose and drinking without treatment **(Biyela *et al.*, 2004)**. These unprotected water sources can be polluted with agricultural sharing, mixing with sewage effluents and feces from wildlife which gets transferred with rain **(Obi *et al.*, 2002)**. These impacts are considered to pose risk to people's health in different forms of viral pathogens, polluted seafood and reduced well-being (**Fleming *et al.*, 2006; Kite *et al.*, 2008, Laws *et al*., 2008).**

1.1 Metagenomics technique

In recent years the genomics has developed and introduced new techniques, with greater possibilities in the knowledge of microbial diversity in the environment system, called

metagenomics **(Nold and Zwart, 1998; Cotner and Biddanda, 2002; Gessner *et al*., 2010).**

).Metagenomics is a new and exciting field of molecular biology which will be used as standard tool for gene fingerprinting method to describe microbial community profile and understanding the biological diversities **(Thomas *et al*., 2012).**

Unfortunately, scientists are able to grow less than 1% of all microorganisms observable in nature under standard laboratory conditions. This leaves scientists with more than 99% of the biological diversity to study in the environment. Metagenomics is a new field combining molecular biology and genetics in an attempt to identify, and characterize the genetic material from environmental samples. The genetic diversity is assessed by isolation of DNA followed by direct cloning of functional genes from the environmental sample.

In the past decade noteworthy development of microbial ecosystem represents in the field of Metagenomics. At first started with the cloning of extracted DNA, followed by sequence analysis **(Handelsman *et al*., 1998)** The 16s rRNA gene from clone libraries is a strong tool for alignment used for molecular approaches because it has a more comprehensive analysis of microbial assortment in water ecosystems **(Tringe, 2005)** and many research dependent on for the microbial classification analysis of environmental samples by the use of 16S rRNA and is considered as the golden criterion for taxonomic classification of bacteria.**(Liu *et al*., 2007; Wang *et al*., 2007).**

1.2 Bacterial diversity in wastewater

Bio-treatment of domestic wastewater has been combined since the early part of the 20th century and has contributed safely to preserve the quality of our circumference ecosystem and reduce of waterborne infection. A broad variety of combination of organic and inorganic waste can be energetically removed from industrial processed water by bio-treatment of wastewater, which is a preferable technology especially in comparison to alternatives such as chemical treatment and burning treatment **(Grady *et al*., 1980; Bitton, 1999**).

. In past decades the development of technique provides confidence for the safe reuse of treated water indifferently using helpful treatment station constitutes a decisive tool in the biodegradation of organic materials, diversion of nutrient removal of poisonous complexes and measures and to relieve pressure on freshwater **(Bramucci *et al*., 2006).**

Biological treatment processes are generally used for treating municipal and industrial wastewater in wastewater treatment plants (WWTPs) unpaid to their high capacity for removal of nutrient matters/various organic matters and low working cost, which represent to control microbial diversity **(Wagner *et al*., 2002).**

1.3 Research question

Water quality is an important public health issue given that the presence of pathogenic organisms in such waters can adversely affect human and animal health. Despite numerous studies conducted to assess the quality of environmental waters limited efforts have been put on investigating the microbial diversity because of the difficulty of the cultivation process or lack of knowledge of the conditions necessary for the cultivation of those microbial in the laboratory.

The present study focused on the determination of physio-chemical properties of different water samples and investigation of bacterial diversity in the aquatic environment. This

study will help in reducing the water borne diseases which are basically endorsed by the emerging bacterial pathogens.

1.4 Objectives:

On the basis of present study, the following objectives have been taken in consideration

1. To identify and validate bacterial DNA of microbial population in selected water samples by using metagenomic approach.
2. To predict genomic diversity and identify microbial strains by using in bioinformatics tools.
3. Reducing waterborne diseases that are mainly adopted by emerging microbial pathogens

REVIEW OF LITERATURE

The reports available in literature pertaining to various aspects of the present studies have been reviewed under the following headings:

2.1 Bacterial diversity in sweet water

2.2 Metagenomics technique

Cottrell *et al.*, (2005) observed that Bacterial diversity of metagenomic and PCR libraries from the Delaware River .Refers to determine whether metagenomic libraries sample adequately the dominant bacteria in aquatic environments, by examining the phylogenetic make-up of a large insert metagenomic library constructed with bacterial DNA from the Delaware River, The result of Delaware River libraries contained bacteria belonging to several widespread freshwater clusters, including clusters of *Polynucleobacternecessarius*, *Rhodoferax* species Bal47 and LD28 *beta-proteobacteria*, the ACK-m1 and STA2-30 clusters of *Actinobacteria*.

Christopher *et al.*, (2010) found the effect of human actions on microbial communities and their response to changing the environment. The metagenomic studies on the community of microbes residing in stressed groundwater revealed that excessive exposure to heavy metals, HNO_3, and organic solvents causes reduced number of bacterial species, allelic and metabolic diversity.

Jiang *et al.*, (2008) studied the Gaobeidian WWTP of Beijing for analyzing the bacterial communities present in it. A high bacterial diversity was observed in which clones found could be categorized into five different groups. The results conclude that the *proteobacteria* were most abundant constituting about 76.7% of all the communities, others being 39.8% (β-*proteobacteria*), 22.33% (the uncultured bacteria), 20.15% (γ-*proteobacteria*), 6.79% (α-*proteobacteria*) and 4.85% (the *σ-proteobacteria*).

Sanchez *et al.*, (2013) assessed the diverse microbial makeup of Almeria (Spain) WWTP activated sludge sample. The identification of sea water was based on 454-pyrosequencing. The study focused on the literature used to interpret the diversity of microbes in such seawater samples depending on the highly abundant species.

Wang *et al.*, (2014) characterized the structures and functions of nitrification and denitrification bacterial communities in aerobic and anaerobic sludge of two full-scale tannery WWTPs. Pyrosequencing of 16S rRNA genes showed that Proteobacteria and Synergistetes dominated in the aerobic and anaerobic sludge, respectively. The results showed that the denitrifiers mainly included the genera of Thauera, Paracoccus,

Hyphomicrobium, Comamonas and Azoarcus, which may greatly contribute to the nitrogen removal in the two WWTPs.

2.3 Metagenomics technique

Sebat *et al*., (2003) developed a novel technique where metagenomics library was combined for direct hybridization of DNA samples from various sources like groundwater and standard reference species. Functionally in this study the microarray was generated using microcosm of microbes from water, consisting of 672 cosmids and set of 16S rRNA. Thus metagenomics is considered a very useful method for defining clones and identifying the species.

Simon and Daniel, (2011) evaluated the revolutionary effect of metagenomic techniques. The applications of metagenomics lead to known the widest range of unculturablemicroorganisms have resided in extreme condition. This approach comprises of constructing and finding libraries which help to find new enzymes and compounds having a role in industries, for human health and many more.

Huson and Mitra, (2012) evaluated the comparison between datasets. This comparison was based on different work published on marine sample data which constitute of metagenomic, 16S rRNA data, meta-proteomic, meta-transcriptomic etc. Metagenomics comprises of studies on DNA samples directly extracted from the environment whereas meta-transcriptomics is a study of such RNA samples and meta-proteomics is the study of such protein samples. This paper is an amalgamation of these three types of data.

Teeling and Glockner, (2012) reviewed and compiled the advantages of NGS and the challenges faced by storages databases to process its data. Such studies require very efficient bioinformatic tools to tackle this complex data. The aim of this study was to provide readers compiled information from bioinformaticians point of view, which contains all the problems and opportunities in this area. This perspective will provide a better understanding of the data processing methods followed.

Neelakanta and Sultana, (2013) studied the role of metagenomics in determining the microbial diversity of any region in the environment. Microbes are the most abundant living organism on this earth, but there restrictions. This restriction is overcome by metagenomics method. The role of this technique extends from studying the diversity of microbes in oceans to the human microbiome. Metagenomics of microbes constitutes of taxonomy study, databases of huge and small sequences and lastly alignment tools.

Poretsky *et al*., (2014) assessed the bacterial community and its dynamics in the freshwater samples. 16S rRNA gene amplification was done from 04 different samples. The amplicons obtained showed around 1.5 times variation in phylum while 10 times more variation in general. His work focused on the amplification of 16S rRNA and the results which interpret the dynamics of microbes present in any kind of environment.

Bag *et al*., (2016) developed metagenomics method the nucleic acid extracted from this method was found to have high recovery and very low impurities and thus useful for further downstream processing. A combination of the physical, chemical and mechanical method of lysis is used for efficient breakdown of cells of microbes.

MATERIALS AND METHODS

3.1 Place of study

The present study entitled "Comparative Analysis of Bacterial Diversity in the Water Samples from Domestic wastewater Allahabad and Lucknow, India through Metagenomic Technique" and the research was carried out in CytoGene Research & Development, Lucknow, U.P. India.

3.2 Sample collection

7 samples of surface water were collected from 02 different sources in India (Domestic wastewater from Naini, Allahabad and Lucknow). including 04 samples from Naini, Allahabad and 03 samples from Lucknow during December 2015 at range of temperature (20-22 ^{0}C)

3.3 Molecular analysis of water sample

3.3.1. Isolation of DNA by Meta-G-Nome™ DNA Isolation Kit from Water Samples

Reagent

Listed in Appendix 1

3.3.2 Quantification of DNA isolated from water samples

DNA was quantified by using UV-Vis double beam spectrophotometer.

3. 3.3 Amplification of DNA by Polymerase Chain Reaction

All equipment required is listed in appendix 10.

PCR reaction mixture composition

The PCR conditions and reaction mixture are listed in Appendix 10

Temperature Cycle of PCR

The temperature cycle of PCR is given listed in appendix 10

Table 3.1: Primers 16S rRNA using for amplification of DNA isolated from water samples

Primer name	Primer sequence (5'-3')
27F	5' AGAGTTTGATCMTGGCTCAG 3'
1492R	5' TACGGYTACCTTGTTACGACTT 3'

3.4 Sequence analysis by using Bioinformatics tools

3.4.1 Sequencing:

Amplified 16S rRNA PCR products were send for the High throughput sequencing by using the Sanger method. After sequencing sequence analysis was done by using bioinformatics tool like BLAST server.

RESULTS AND DISCUSSION

4.1. DNA isolation and Molecular analysis of water samples:

This research focuses on methodological advances that have allowed the sequencing of natural microbial populations in water environmental samples by Meta-G-Nome™ DNA Isolation Kit was separately as per the tow sources for selected water samples of Domestic waste water from Naini, Allahabad and Lucknow.

4.1.1 DNA isolation from Domestic waste water:

Seven Domestic waste water samples from Naini, Allahabad and Lucknow have been taken and DNA was extracted and analyzed on agarose gel electrophoresis **(Figure 4.1)** The genomic DNA was observed more than 10kb in size.

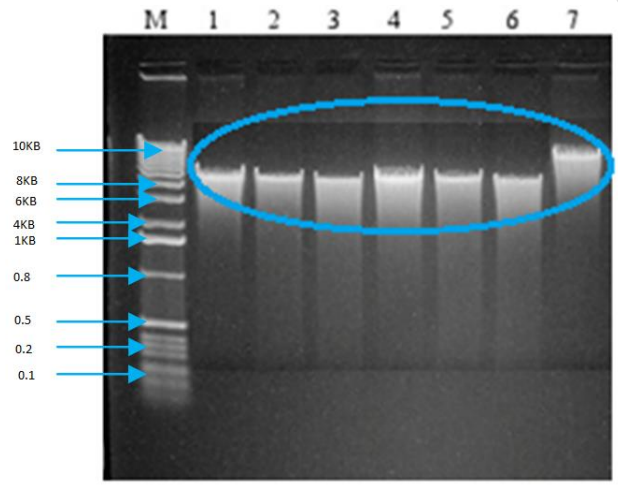

Figure 4.1: Agarose gel electrophoresis for the genomic DNA extracted from the Domestic waste water samples from Naini Allahabad and Lucknow as. Lane1: Sample 1; Lane 2: Sample 2; Lane 3: Sample 3; Lane 4: Samp 4, Lane 5: Sampl 5; Lane 6: Sample 6; Lane 7: Sample 7; Lane M: 10kb DNA Ladder4.2 Quantification of DNA samples **isolated from different water samples:**

After isolation of DNA, it was used to determine the quantification by using UV – Vis Double Beam Spectrophotometer, a Nano drop-1000 spectrophotometer (one of the most commonly used methods to determine the quantity of nucleic acids). Quantification step was performed by using UV-Vis double beam spectrophotometer of wave length between 260-280 nm and the significant DNA concentration of different samples are shown in **(Table 4.1).**

Table 4.1: DNA concentration (µg/ml) of Naini, Allahabad and Lucknow.

S. No	Sample	Ratio 260/280nm	DNA concentration *(µg/ml)*	Protein concentration *(µg/ml)*
1	Naini, Allahabad Domestic waste water	0.91	0.95	2.63
2	Naini, Allahabad Domestic waste water	0.67	0.01	0.06
3	Naini, Allahabad Domestic waste water	0.89	0.46	1.32
4	Naini, Allahabad Domestic waste water	0.86	0.68	2.08
5	Lucknow, Domestic waste water	1.08	0.62	1.27
6	Lucknow, Domestic waste water	1.31	0.07	0.10
7	Lucknow, Domestic waste water	0.93	1.23	3.26

The observed protein concentration was directly proportional with DNA concentration whenever increased the DNA concentration the protein concentration increased by double.

4.3 16S-rRNA amplification of Genomic DNA extracted from different selected water samples:

After DNA isolation, all samples were used to amplify the 16S rRNA region (which is the signature region for every bacterial species) (Filloux *et al*., 1997). The 16S rRNA gene is1500 bp large and enough for the bacterial diversity information purposes (Patel, 2001).

4.3.1 16S-rRNA amplification of DNA extracted from Domestic wastewater samples of Allahabad and Lucknow

Seven Domestic wastewater samples including four samples from Naini, Allahabad and three samples from Lucknow were used for extraction of DNA by using Meta-G-Nome™ DNA Isolation Kit and amplified 16S rRNA gene by using PCR and analyzed in agarose gel electrophoresis **(Figure 4.2).**

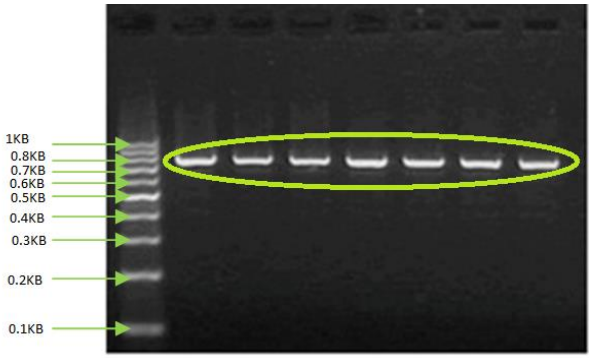

Figure 4.2: Agarose gel electrophoresis of amplified 16S-rRNA products for the Domestic waste water of Naini, Allahabad and Lucknow samples. Lane 1 = Sample 1; Lane 2 = Sample 2; Lane 3 = Sample 3; Lane 4= Sample 4; Lane 5= Sample 5; Lane 6= Sample 6; Lane 7= Sample 7; Lane L = DNA ladder (1kb).4.4 Sequencing of 16S-rRNA for the different water samples

Bacterial 16S rRNA gene was amplified by PCR with 16s rRNA universal primers. The amplified PCR product was sent for the sequencing at **Chromus Biotech, Bengaluru**. The sequencing result of all water samples are shown in **(Table 4.2)** .

Table 4.8: Sequence details of different water samples of India.

Sample No.	Sample description	Code sequence
1	Naini, Allahabad Domestic wastewater	CG20160320I
2	Naini, Allahabad Domestic wastewater	CG20160320J
3	Naini, Allahabad Domestic wastewater	CG20160320K
4	Naini, Allahabad Domestic wastewater	CG20160320L
5	Lucknow Domestic wastewater	CG20160320M
6	Lucknow Domestic wastewater	CG20160320N
7	Lucknow Domestic wastewater	CG20160320O

4.5: Sequencing analysis by Bioinformatics Tools

The full length nucleotide sequences were analyzed and compared with the reported sequences by BLAST server. The closest relatives that showed in subhead in the following tables identified with the sequence reported in the database were assigned.

4.5.1 BLAST results for Naini, Allahabad domestic wastewater isolate (CG20160320I):

The BLAST results were showing percentage of similarity for the Eighth sequence from Naini, Allahabad sewage water. The largest percentage of similarity was 97% with *Vibrio alginolyticus* strain NIOTVA 05.16S rRNA gene. The design of phylogenetic tree of *Vibrio alginolyticus strain* NIOTVA 051 6S rRNA gene and relation with other bacterial species is shown in **(Figure 4.3).**

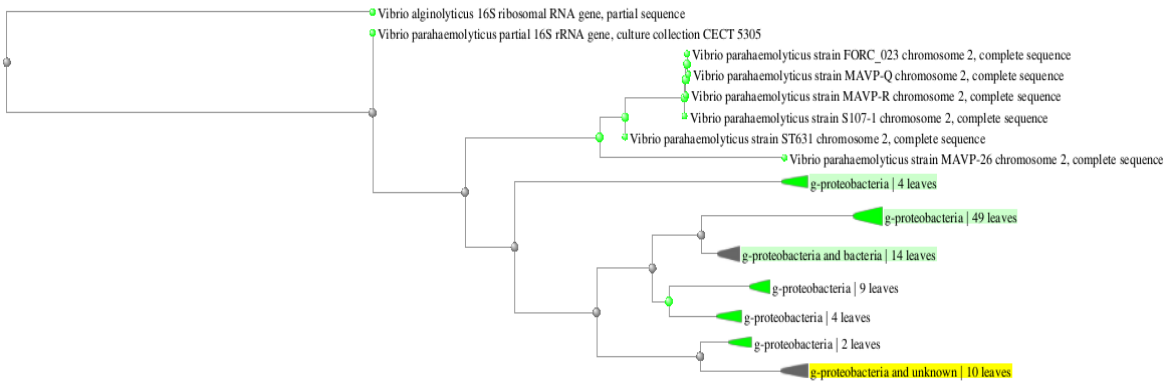

Figure 4.3: Phylogenetic tree of Vibrio alginolyticus strain NIOTVA 05 16S rRNA gene.

It is a gram-negative marine bacterium. *V. alginolyticus* was first identified as a pathogen of humans and is the leading cause of seafood-associated bacterial gastroenteritis worldwide. It occasionally causes eye, ear and wound infections **(Depaola *et al.*, 2000).**

4.5.2 BLAST results for Naini, Allahabad domestic wastewater isolate (CG20160320J):

The BLAST results were showing percentage of similarity for the ninth sequence. The largest percentage of similarity was 98% with *Vibrio tritoniusstrin* JCM 1645616S rRNA gene**.** The phylogenetic tree of *Vibriotritoniusstrin* JCM 16456 16S rRNA gene and relation with another bacterial species is shown in **(Figure 4.4).**

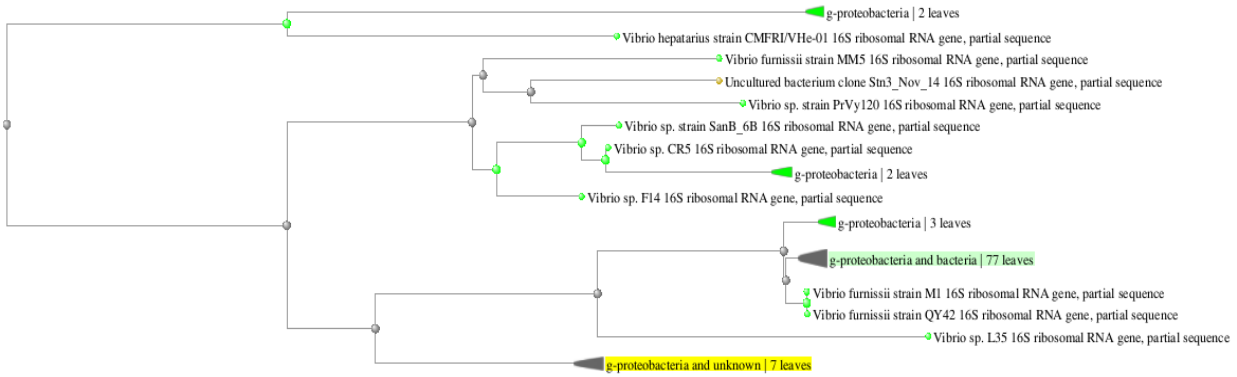

Figure 4.4: Phylogenetic tree of Vibrio tritonius strinJCM 1645616s r RNA gene.

The phylogenetic tree was found similarity with *Vibrio tritonius strain* JCM 16456.16S rRNA gene. It is a genus of Gram-negative bacteria, possessing a curved-rod shape (comma shape). Several species of which can cause foodborne infection, usually associated with eating undercooked seafood. Typically found in salt water. Recent phylogenies have been constructed based on a suite of genes (multi-locus sequence analysis) **(Thompson *et al*., 2005).**

4.5.3 BLAST results for Naini, Allahabad domestic wastewater isolate (CG20160320K):

The BLAST results were showing percentage of similarity for the tenth sequence. The largest percentage of similarity was 98% with *Vibrio vulnificus* strain ATCC27562 16S ribosomal RNA gene complete genome. The design of phylogenetic tree of *Vibrio vulnificus* strain ATCC27562 16S r RNA gene and its relation with another bacterial species is shown in **(Figure 4.5).**

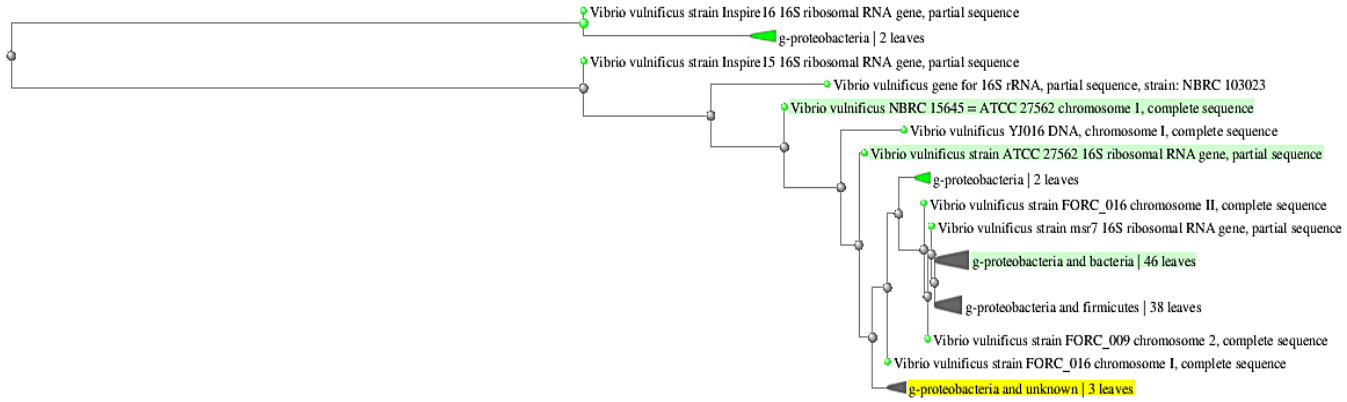

Figure 4.5: Phylogenetic tree of Vibrio vulnificus strain ATCC27562 16s r RNA gene

These types are considered as toxic, Gram-negative, halophilic bacterium, natural inhabitant of estuarine and coastal waters. In healthy individuals, this pathogen may cause gastroenteritis or severe wound infections, leading to necrotizing cellulitis. *Vibrio vulnificus* caused infections often leads to septicemia especially in immune compromised individuals, case-fatality rates are greater than 50% for primary septicemia and about 15% for wound infections. *V. vulnificus* has been recovered from fish, shellfish, **(Irene *et al.*, 2010).**

4.5.4 BLAST results for Naini, Allahabad domestic wastewater isolate (CG20160320L):

The BLAST results were showing percentage of similarity for the eleventh sequence. The largest percentage of similarity was 98% with *Pseudomonas putidas* strain KF715 16S rRNA gene partial sequence. The design of phylogenetic tree of *pseudomonas putida* strain KF715 16S rRNA gene partial sequenc**e** and relation with other bacterial species is shown in **(Figure 4.6).**

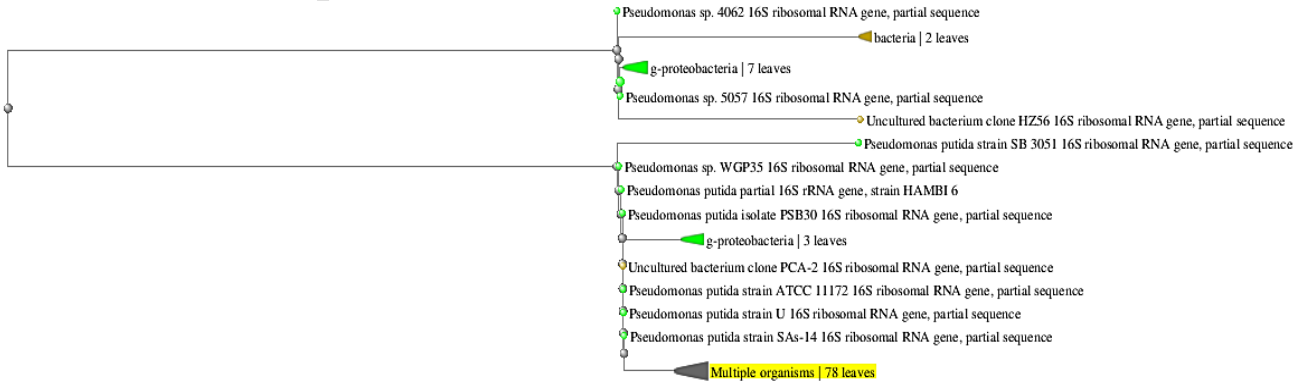

Figure 4.6: Phylogenetic tree of pseudomonas putida strain KF 715 16S rRNA gene partial sequence.

Certain strains of *Pseudomonas putida* are not pathogenic due to lack of certain genes including those for enzymes that digest cell membranes and walls of humans and plants cell. The bacterium is found in the ear, nose and throat **(Harwood *et al.*, 1989).**

4.5.7 BLAST results for Lucknow domestic wastewater isolate (CG20160320M):

The BLAST results were showing percentage of similarity for the twelfth sequence. The largest percentage of similarity was 98% with *E. coli* strain MS7163 chromosome complete genome**.** The design of phylogenetic tree of *E. coli* strain MS7163 chromosome complete genome and relation with other bacterial species is shown in **(Figure 4.7).**

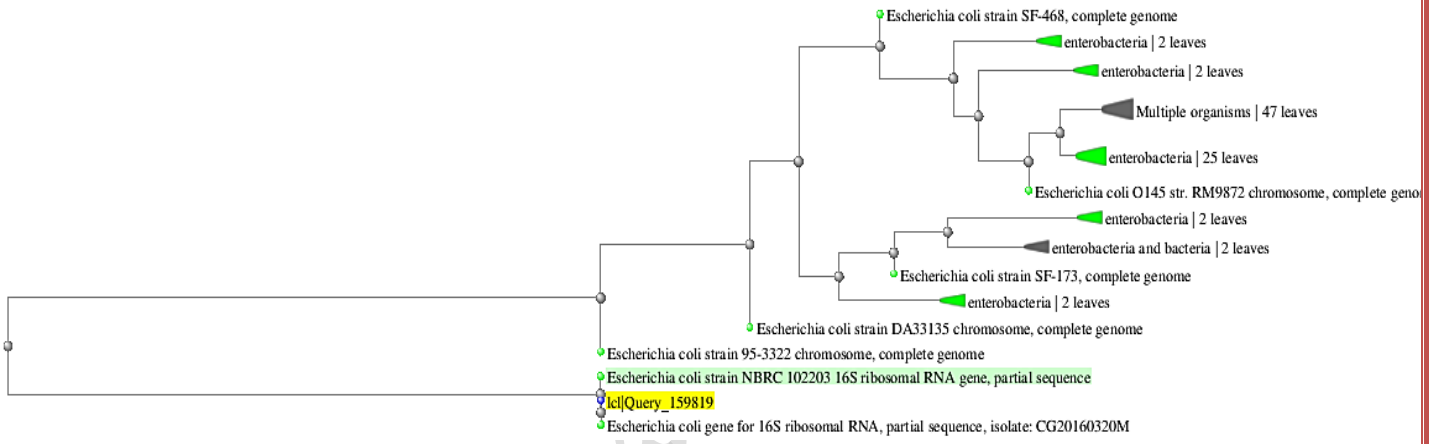

Figure 4.7: Phylogenetic tree of E. coli strain MS 7163 chromosome complete genome.

Pathogenic *E. coli* with an inter-human circulation represents a leading cause of diarrhea, often with high mortality rates, in developing countries. *E. coli* is part of the natural microflora of the digestive tract of human and contaminates the surrounding environment. 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. The water from the stream in turn is used to irrigate crops grown at a nearby farm **(Kabiru *et al.*, 2015).**

4.5.6 BLAST results for Lucknow domestic wastewater isolate (CG20160320N):

The BLAST results were showing percentage of similarity for the thirteenth sequence. The largest percentage of similarity was 98% with *Streptococcus mutans* strain ATCC 25175 16S rRNA partial sequence**.** The design of phylogenetic tree of *Streptococcus mutans* strain ATCC 25175 16S rRNA partial sequence and its relation with other bacterial species is shown in **(Figure 4.8):**

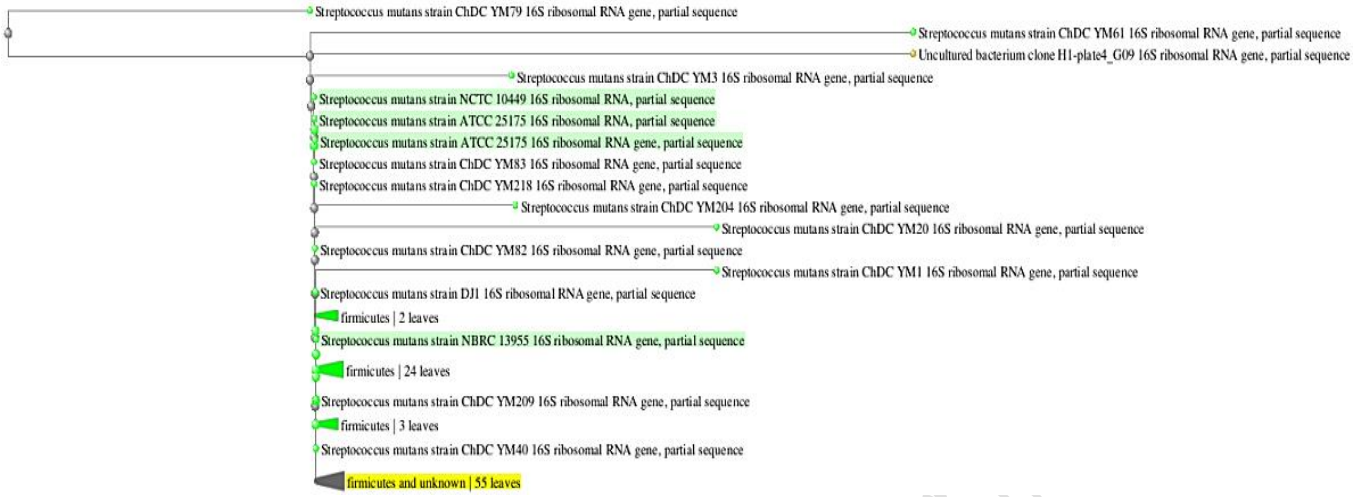

Figure 4.8: Phylogenetic tree of Streptococcus mutans strain ATCC 25175 16S rRNA partial sequence

The phylognetic tree results showed similarity with *Streptococcus mutans* strain chDc YM116S rRNA gene partial sequence. *Streptococcus mutans* are facultative anaerobes gram positive and coccus (round bacterium) commonly found in the human oral cavity and is a significant contributor to tooth decay. The microbe was first described by **(Kilian, 1924).**

4.5.7 BLAST results for Lucknow domestic waste water isolate (CG20160320O):

of similarity was 97% with *streptococcus anginosus* strain J4211 complete genome .The design of phylogenetic tree of *streptococcus anginosus* strain J4211 complete genome and relation with other bacterial species is shown in **(Figure 4.9).**

The BLAST results were showing percentage of similarity for the fourteen the sequence. The largest percentage

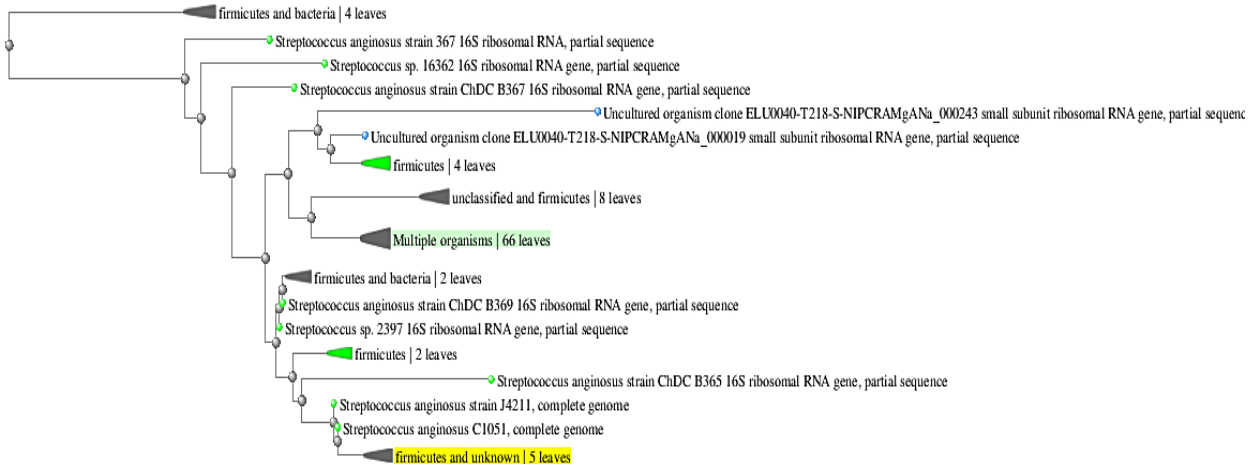

Figure 4.9: Phylogenetic tree of Streptococcus anginosus strain J4211 complete genome.

The phylogenetic tree showed similarity with *streptococcus anginosus* strain ChDC B365 16S rRNA partial sequence. This is gram-positive, intermedius and Streptococcus constellates constitute the *anginosu*s group. *Streptococcus anginosus* is a part of the human bacteria flora, but can cause diseases including brain and liver abscesses under certain circumstances **(Bert *et al.*, 1998).**

SUMMARY AND CONCLUSION

The microbial diversity in different environment has importance to bacteriological processes and development characters special in aquatic system and living organism. Diversity in the aquatic environment is very vast and different contingent depends on the type of water source like sweet and salty. The Understanding of diversity intimate an ecosystem is an important, primary step in studying the survivability and adaptation of halophiles at diverse stages of opposition. Microorganisms participate safely to the earth's biological diversity, so far comparatively few study of the microorganisms existing in nature have been cultured and described. The traditional technique is dependent on culture-based for the identification of microbes in ecological samples. A broad understanding of microbial diversity was not provided as a result of complying with the necessary conditions for culture and at other times difficult to be provided or may not be known at all. In recent years the development of genomics and introduction of new techniques provides greater possibilities in the study of microbial variety in the environment. These techniques are known as Metagenomics. Metagenomics is the name of new technology and represent a key constituent for understanding microbial diversity in ecosystem.

After the amplification of 16S rRNA gene segment, PCR products obtained by Cyto Gene lab Research and Development, Lucknow, U.P. India were sent for the Sanger sequencing to Chromus Biotech, Bengaluru. 16S rRNA sequences were analyzed with the help of NCBI-BLAST and CLUSTAL-*W* tools. To construct the phylogenetic tree CLUSTAL OMEGA server was used.

The main step of the study was bacterial analysis of the water samples and the extraction of DNA by using DNA isolation kit. Genomic DNA was analyzed with the help of agarose gel electrophoresis with different sizes of DNA bands. All DNA samples were used for the amplification of 16S rRNA by using PCR and standard primer sequences. After that PCR products were sent for the Sanger sequencing. Sequences were characterized by using two approaches of sequence analysis. First method was based on the BLASTN comparison of 16s rRNA sequences by NCBI database and second method was based on phylogenetic approach by using phylogenetic tree.

CONCLUSION

1 . In this study 7 new nucleotide sequences were sequenced and these sequences were not showing 100% similarity with the known sequences. It means these bacterial strains were uncharacterized sequences. But these 7 bacterial strains were showing evolutionary relationship with 04 types of bacterial . These bacterial strains were: *Escherichia coli, , Pseudomonas, Vibrio* and *Streptococcus.*

2 .The bacterial groups found in this study are mostly toxic because these species cause different types of diseases in aquatic organisms, land organisms and humans. It can resist antibiotics and should supply new novel approaches to the diagnosis and treatment of

infectious diseases. The bacterial diversity in water samples was huge and all of them were unknown because they were difficult to culture. The results obtained from this study confirmed that new strains were related to five types of bacteria in fourteen different strains. All sequences which were obtained from this study are submitted to the international DDBJ database.

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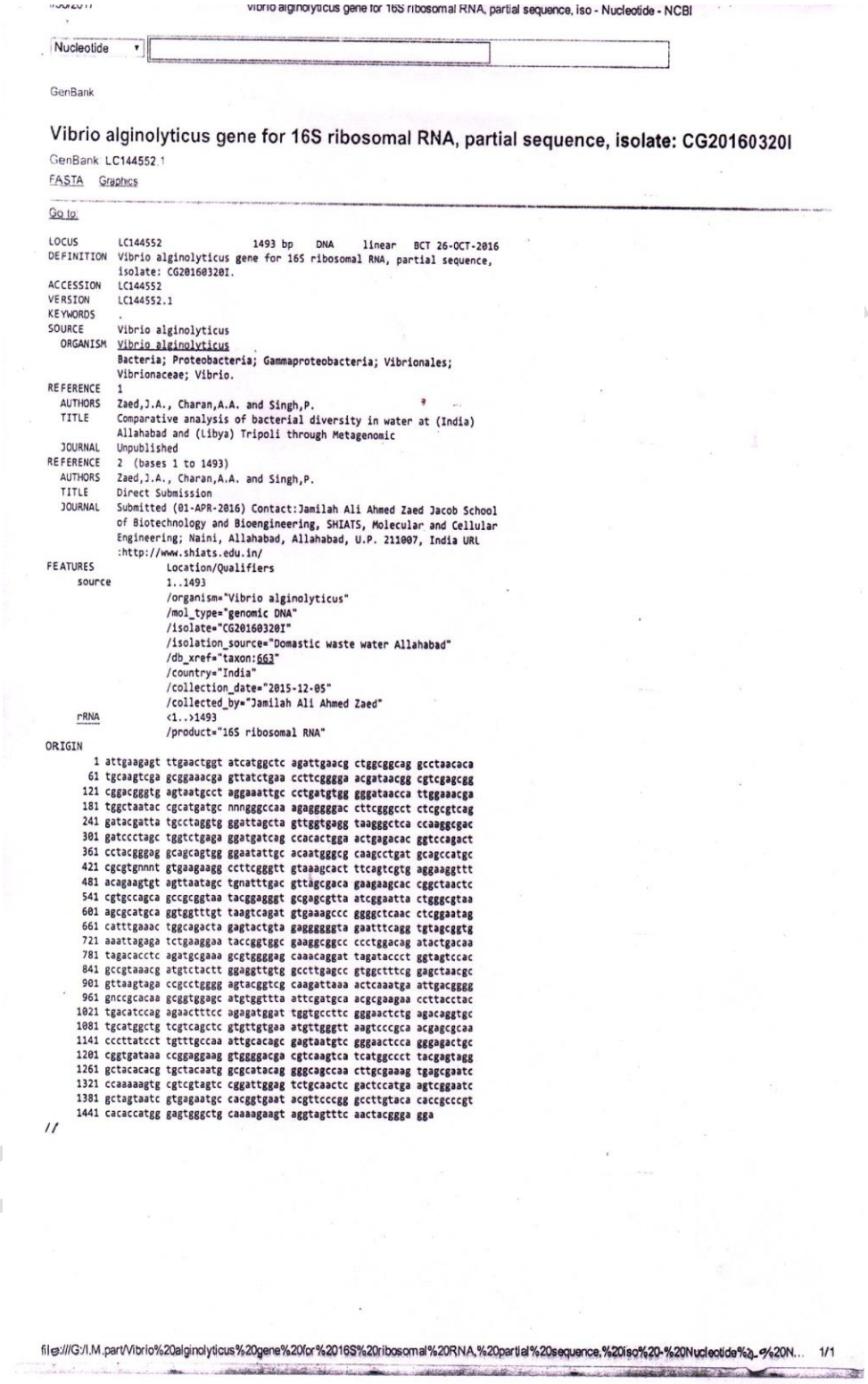
ABBREVIATIONS

ml	Milliliter
mm	Millimeter
mg	Milligram
M	Molarity
µg	Microgram
µM	Micro molar
µl	Micro liter
µm	Micro meter
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
N	Normality
ng	NanoGram
nm	Nanometer
O_2	Oxygen
OTUs	Operational taxonomic unit
%	Percent
PCI	Domain proteins interact
PCOA	Principal Coordinates Analysis
PCR	Polymerase chain reaction
PH	Potential of hydrogen ion
pM	Picometre
Ppm	Parts per million
R	Refers word
RNA	Ribonucleic acid
Rpm	Revolution per minute
rRNA	ribosomal Ribose Nucleic Acid
SSU	Small Sub Unit
TAE buffer	Tris Base, Acetic acid and EDTA
TE Buffer	Tris EDTA Buffer
Tris	Tris (Hydroxyl methyl) amino ethane
U.P.	Uttar Pradesh
USA	United States of America
UV - Vis	Ultraviolet-visible spectroscopy
U	Unit
V	Volume
Wt	Weight
WWTPs	Wastewater Treatment plants

LIST OF TABLES

LIST OF FIGURES



Vibrio alginolyticus gene for 16S ribosomal RNA, partial sequence, iso - Nucleotide - NCBI

Nucleotide

GenBank

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GenBank LC144552.1

FASTA Graphics

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GenBank: LC144553.1

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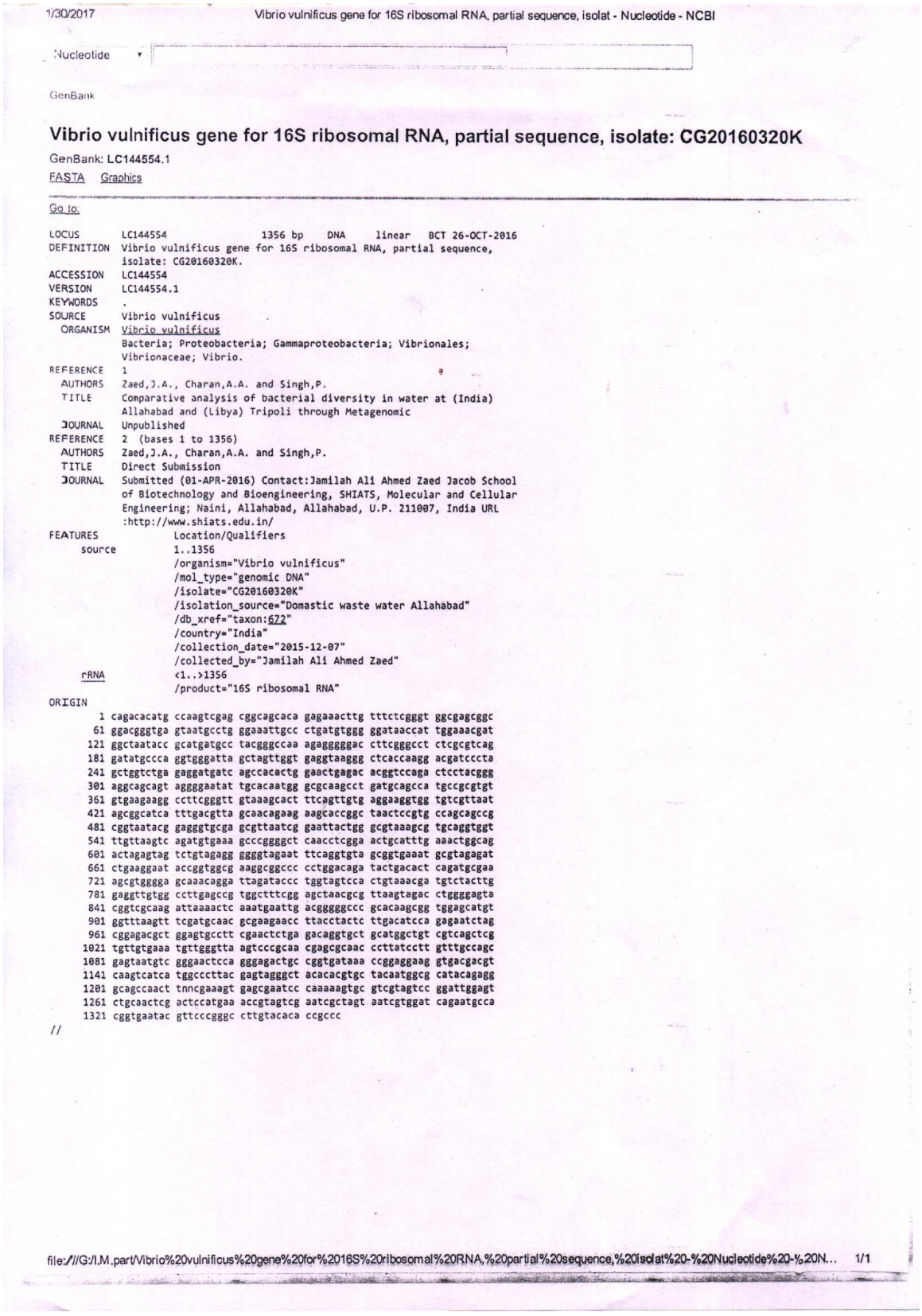

1/30/2017 Vibrio vulnificus gene for 16S ribosomal RNA, partial sequence, isolat - Nucleotide - NCBI

Nucleotide

GenBank

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GenBank: LC144554.1

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Nucleotide

GenBank

Escherichia coli gene for 16S ribosomal RNA, partial sequence, isolate: CG20160320M

GenBank: LC144556.1

FASTA Graphics

Go to:

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REFERENCE   1
  AUTHORS   Zaed,J.A., Charan,A.A. and Singh,P.
  TITLE     Comparative analysis of bacterial diversity in water at (India)
            Allahabad and (Libya) Tripoli through Metagenomic
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1464)
  AUTHORS   Zaed,J.A., Charan,A.A. and Singh,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-APR-2016) Contact:Jamilah Ali Ahmed Zaed Jacob School
            of Biotechnology and Bioengineering, SHIATS, Molecular and Cellular
            Engineering; Naini, Allahabad, Allahabad, U.P. 211007, India URL
            :http://www.shiats.edu.in/
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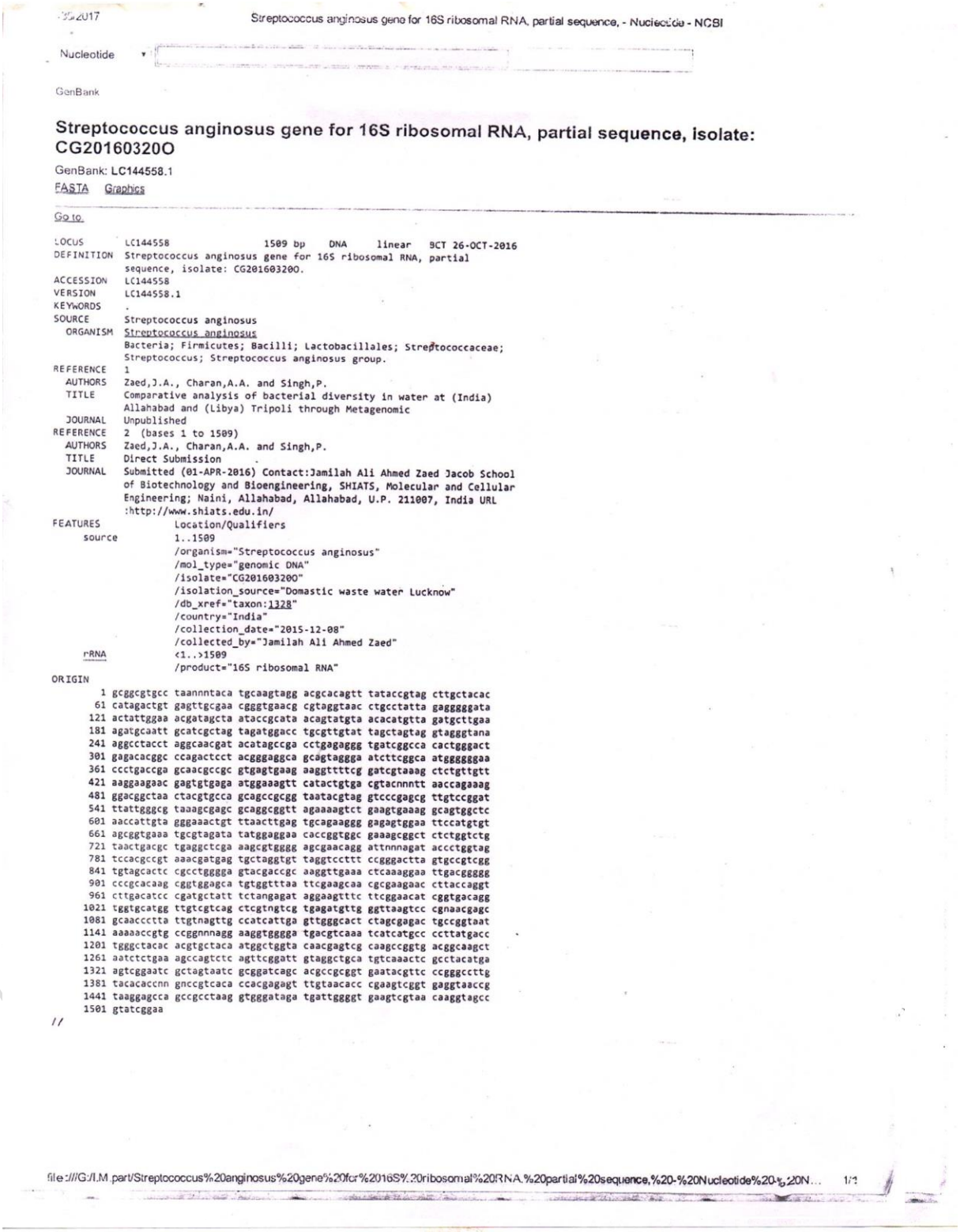

2017 Streptococcus anginosus gene for 16S ribosomal RNA, partial sequence, - Nucleotide - NCBI

Nucleotide

GenBank

Streptococcus anginosus gene for 16S ribosomal RNA, partial sequence, isolate: CG20160320O

GenBank: LC144558.1

FASTA Graphics

Go to:

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REFERENCE   1
  AUTHORS   Zaed,J.A., Charan,A.A. and Singh,P.
  TITLE     Comparative analysis of bacterial diversity in water at (India)
            Allahabad and (Libya) Tripoli through Metagenomic
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1509)
  AUTHORS   Zaed,J.A., Charan,A.A. and Singh,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-APR-2016) Contact:Jamilah Ali Ahmed Zaed Jacob School
            of Biotechnology and Bioengineering, SHIATS, Molecular and Cellular
            Engineering; Naini, Allahabad, Allahabad, U.P. 211007, India URL
            :http://www.shiats.edu.in/
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