

# Molecular characterization and identification of unknown bacteria from waste water

Brindha V<sup>1\*</sup>, Ani Ann Mathew<sup>2</sup>

<sup>1,2</sup>Department of Biotechnology, Hindustan College of Arts & Science, Padur, Kelambakkam -603103, India

<sup>1\*</sup>brindhav2@gmail.com; <sup>2</sup>aninibin@gmail.com

## Abstract

Water plays a very important role in supporting all forms of life. However if it contaminated, it has a great potential for transmitting a variety of diseases and illnesses. Waste water can serve as a source of infectious bacteria, viruses and other pathogens. The transmission of these diseases through waste water is a constant public threat. The purpose of this study was to identify the unknown bacteria, which cause dreadful disease to humans. The sample was collected from household waste water and the organism was screened using selective media and the taxonomy status was identified based on biochemical test. The molecular characterization is done by isolating DNA from the selected organism. Universal primer was designed based on 16srRNA. The conserved region was amplified and the amplified product was sequenced based on 16s rRNA.

**Keywords:** Molecular Biology; Bacterial Characterization; Waste water; sRNA.

## Introduction

Large number of water borne microorganisms are pathogenic to humans, cause many diseases. The transmission of these diseases through waste water is a constant public threat (Gray, 1989). Bacteria are the most important group of microorganisms found in this biological contact process, since they are responsible for the structural and functional activity of the activated sludge (Seviour *et al.*, 1999).

There are many practical applications for identifying unknown bacteria. During research, it is of utmost importance to identify unknown bacteria if diversity is being studied. One of the main responsibilities is to determine the identity of pathogenic bacteria. Identification of bacteria by diagnostic laboratories is based on phenotypic characteristics. Primary identification involves a few simple tests such as morphology, growth on various types of culture media catalase and oxidase tests (Boddinghaus *et al.*, 1990).

Molecular techniques are major tools for the analysis of microorganisms from various sources. Molecular techniques are effective and fast technology for identification of microbial diversity

in different environments (Pace, 1997, Hatamoto *et al.*, 2008). Genetic diversity can identify individual organisms from some unique part of their DNA or RNA providing definitive information on its biodiversity (Elsayed and Elbestawy, 2008). Traditional microbiological methods are not suitable for the study of the full microbial diversity. The use of molecular techniques in the characterization and identification of bacteria has many advantages (Mills *et al.*, 2007).

16s ribosomal RNA is a component of the 30s subunit of prokaryotic ribosomes. One of the most attractive potential uses of 16s rRNA gene sequence informatics is to provide genus and species identification for isolates that do not fit any recognized biochemical profiles for strains generating only a low likelihood or acceptable identification according to commercial systems or for taxonomy that are rarely associated with human infectious diseases (Malik *et al.*, 2008).

## Materials and methods

Water samples are collected in a sterile container from the domestic waste water. It was then plated on the nutrient agar medium for the growth of microbes.

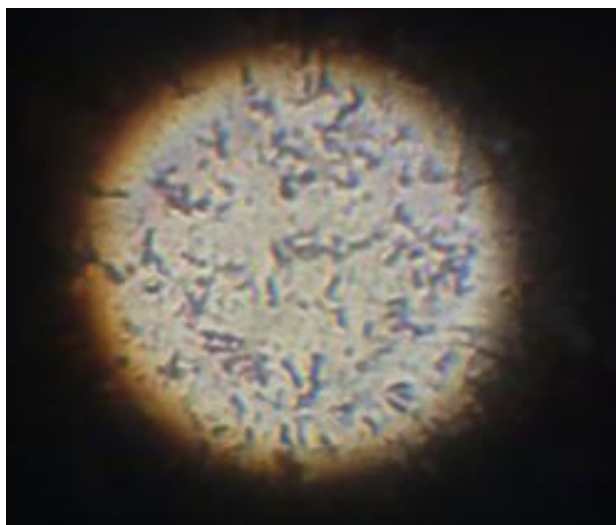
<i>Table 1.</i> Taxonomy status of the selected unknown microorganism	
Gram staining	Negative cocobacilli
Motility	Non motile
Indole	Negative
Methyl red	Positive
Voges proskauer	Negative
Citrate utilization	Positive
Catalase	Negative
Oxidase	Negative
Urea hydrolysis	Positive
Nitrate reductase	Negative
Gas production	Positive

### Screening of microbes

From the nutrient agar plates the organism was isolated and screened using selective media such as MacConkey, Eosin methylene blue and Mannitol salt agar.

### Taxonomy status

Various physiological confirm characterizations of the selected isolates and biochemical test according to Mackie and McCarthy practical



*Fig .1.* Microscopic identification of the microbe

medical microbiology.

### Genomic DNA isolation and quantification

Culture was centrifuge at 8000rpm for 5 min. Collect the pellet and add 30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase-K to give a final concentration of 100 µg/ml proteinase-K in 0.5% SDS. Mix thoroughly and incubate 1 hr at 37°C. Add 80 µl of CTAB/NaCl solution. Mix thoroughly and incubate 10 min at 65°C. Add an approximately equal volume (0.7 to 0.8 ml) of chloroform/isoamyl alcohol, mix thoroughly, and spin 4 to 5 min in a micro-centrifuge. Collect aqueous phase to a fresh micro-centrifuge tube, add an equal volume of phenol/chloroform/isoamyl alcohol, and spin for 5 min. Transfer the supernatant to a fresh tube and add 0.6 vol., isopropanol to precipitate the nucleic acids. Wash the DNA with 70% ethanol to remove residual CTAB and re spin 5 min at room temperature to re-pellet it. Re-dissolve the pellet in 100-µl TE buffer and quantify using NanoVue.

### Amplification of DNA based on 16s rRNA

The extracted DNA was purified and used for PCR amplification. Prepare DNA amplification mix, place the tubes in thermo cycler, and program as follows.

<b>Initial Denaturation:</b>	94°C for 3 min	} 30 cycles
<b>Denaturation:</b>	94°C for 1 min	
<b>Annealing:</b>	58°C for 1min	
<b>Extension:</b>	72°C for 1min	
<b>Final extension:</b>	72° C for 5 min	

### Sequence analysis

Amplified DNA was sequenced based on 16s rRNA.

## Result and Discussion

### Taxonomy Status

Various physiological confirm characterizations of the selected isolates and biochemical test. The taxonomy status of the unknown microorganism is



Fig. 2. Quantification of DNA using NanoVue

given in Table 1. Fig.1 shows the microscopic identification of the microorganism.

```

Primer3 Output

PRIMER PICKING RESULTS FOR /tmp/readseq.in.1338 [Unknown form], 783 bases, E16 checksum.

No mispriming library specified
Using 1-based sequence positions
OLIGO      start  len   tm    gct   any   3' seq
LEFT PRIMER 275    20   60.03 45.00 4.00 3.00 gtggggaattttggacaatg
RIGHT PRIMER 479    20   60.10 45.00 4.00 2.00 attccgattaacgcttgcac
SEQUENCE SIZE: 783
INCLUDED REGION SIZE: 783

PRODUCT SIZE: 205, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 1.00
    
```

Fig. 4. Primer-3 output

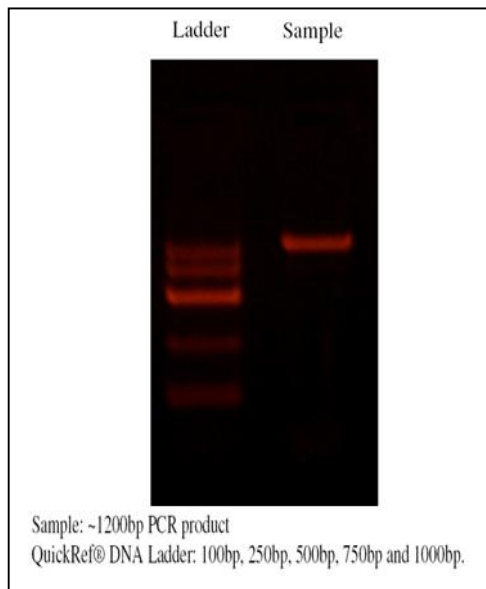


Fig. 5. Amplified DNA in agarose gel electrophoresis

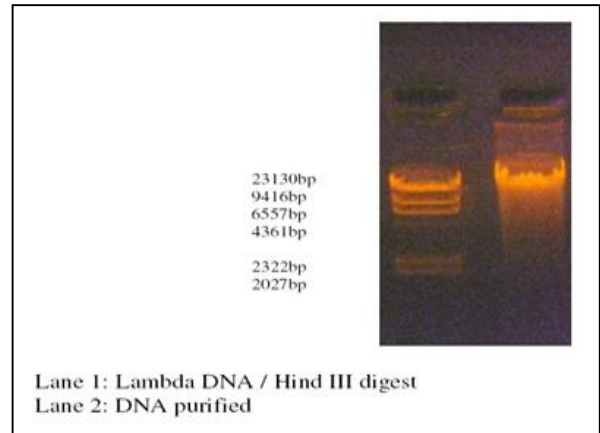


Fig. 3. Purified DNA in agarose gel electrophoresis

The isolated DNA was quantified using NanoVue. Fig.2 shows the purity of the DNA. The purity is observed as 1.750 and the concentration is 378 ng/μl. The purified DNA was run in agarose gel electrophoresis along with lambda DNA/Hind III digest marker. The band formation was shown in Fig.3.

The purified DNA sample was amplified using PCR. The amplified product was shown in Fig.5. The molecular weight of the sample is calculated based on Rf value. Rf value is the ratio of the distance migrated by the molecule to that migrated by a marker dye front.

### Design a primer for the amplification of DNA

To amplify the desired gene universal species-specific primer has used. The universal primer was designed using primer3 and the output was shown in Fig.4.

Sequence analysis of the 16s rRNA sequences is done with the help of several primers called universal primers. These primers target the conserved region of the 16s rRNA gene and amplify the target in parts. Finally the several amplified parts could be assembled together to have the entire sequence. The sequencing of the amplified gene is shown in Fig.6. Fig.7 shows the phylogenetic relationship between the organisms

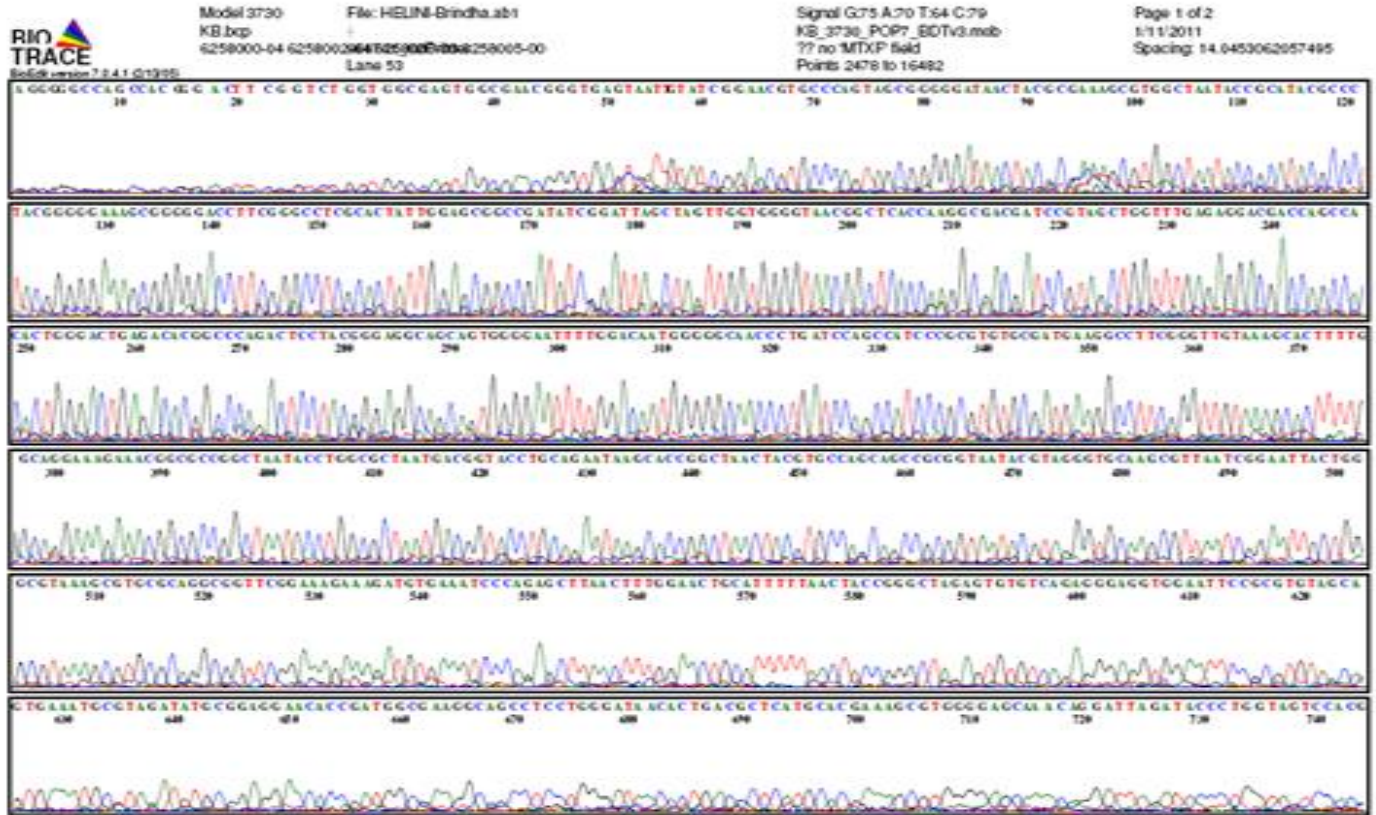


Fig. 6. Sequencing of amplified gene based on 16s rRNA

Guide Tree

[View Guide Tree File](#)

```
(
(
gi|257072963|gb|GQ416682.1|:0.29046,
(
gi|223955061|gb|FJ658206.1|:0.32275,
Brindha:0.35639)
:0.03007)
:0.00663,
(
gi|257073439|gb|GQ417158.1|:0.29703,
gi|257073290|gb|GQ417009.1|:0.29857)
:0.00109,
gi|257073291|gb|GQ417010.1|:0.29568);
```

Phylogram

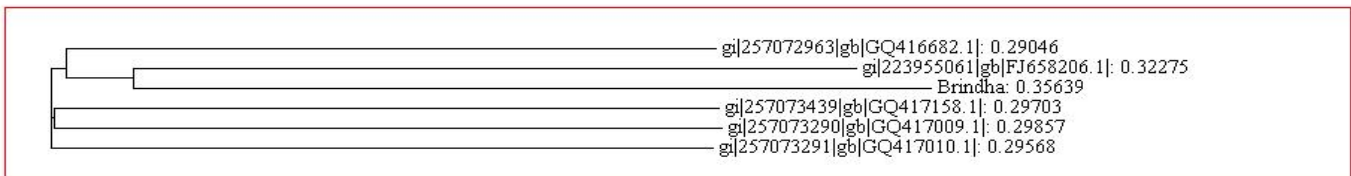


Fig. 7. Phylogenetic analysis of the sequence

and the distance between the selected species was identified by dendrogram graphical representation.

## Conclusion

16s rRNA gene sequencing provide genus and species identification for isolates that do not fit any recognized biochemical profiles for strains generating only a low likelihood or acceptable identification according to commercial systems or for taxa that are rarely associated with human infectious diseases. Based on the microbiological identification and molecular characterization it was found that the identified organism belongs to *Bordetella* sps.

*Bordetella* sps., colonize the mammalian respiratory tract and cause whooping cough in infants and young children. It also causes hypoglycemia, increased histamine and endotoxin sensitivity. The toxin inhibits many leukocyte functions, including chemotaxis, phagocytosis and respiratory burst and impairs NK cell killing. Treatment is by routine acellular vaccine immunization.

## References

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