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Microbial Identification and Sequencing of Soil Bacteria from Indogangatic Plain

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Abstract
Motive of the present experimentation is to carryout Microbial Identification And Sequencing of Soil Bacteria from Indogangatic Plain. Systematic and scientific investigation was conducted for isolation, identification and characterization of selected soil bacteria. Identification was made on the basis of 16S rRNA sequencing. 16S rRNA sequencing showed that out of all 9 isolates;5 Bacillus sp., 1 achromobacter sp., 1 Brevundimonas sp., 1 Pseudomonas sp. and 1 Ochrobactrum sp. were identified. The further investigation shows that the isolates from Patna region of Indogangatic plain is having inhibitory effect on both Gram positive and Gram negative Bacteria. On the basis of sequencing, phylogenetic tree of each isolates was prepared and a comparative study was made.

Keywords: *Microbial identification, Indogangatic plain, 16S rRNA sequencing, Phylogenetic tree, soil bacteria.*

INTRODUCTION

One of the most distinguished features of bacteria is their biochemical versatility. Bacteriacan metabolize a wide range of chemicals, which can be utilized in many ways for the welfare of human beings/animals/environment. Some bacteria can even grow in the absence of oxygen, respiring anaerobically. One of the most notable characteristics of the bacteria is their ability to produce antibiotics. Streptomycin, Neomycin, Erythromycin and Tetracycline are only a few examples of these antibiotics which is of high therapeutic value.

Many soil bacteria have pharmaceutical and medical relevance, known as a source of bioactive secondary products influencing antibacterial, antiviral and cytotoxic activities. Recent work from some researchers shows active involvement of bacteria in the secondary metabolites production and this area needs to be investigated in depth. Soil of Indogangatic plain is rich in microorganisms capable of antibiotic synthesis in which the frequency of synthesis occurs at ecologically significant levels. About more than 5500 antibiotics have been discovered since now due to various researches made in this field (Adegboye et.al. 2013, Cook et.al. 2003 and Kokare et.al. 2004). Most antibiotics used today are isolated and extracted from microbial source. The emergence of antibiotic resistance and need

for better, broad-spectrum antibiotics which can be developed and formulated from antibacterial compounds found in soil bacteria.

Most soils are reservoir of biochemically active bacterial cells. Bacteria are simple and normally called as a bag of enzymes and/or soluble bags of fertilizer. Bacteria live under starvation conditions or soil water stress, they reproduce quickly when optimal water, food, and environmental conditions occur. Bacteria population may easily double in 15-30 minutes. Flourishing microbial populations increase soil productivity and crop yields over time. Bacteria are microscopic organisms with relatively simple and primitive forms of prokaryotic type.

MATERIALS AND METHODS

Sample collection and isolation of bacteria: A General Survey of the district of Patna was undertaken to understand the available soil characteristics in different areas. Whole Patna district was divided into four study sites selected on random selection basis with an objectives of getting a representative soil sample for this research work. These four areas selected were 1) Athmalgola, Barh; Patna 2) Rawaich, Bakhtiyarpur, Patna; 3) Arpana Bank Colony, Baily Road, Patna and 4) Mahendru, Ashok Rajpath, Patna.

Starch casein agar (SCA) medium was prepared and sterilized at 121°C in 15lbs pressure for 15 min. Then it was supplemented with streptomycin 30 µg/l and nystatin 50 µg/l to prevent the fungal growth (Gousterova *et.al*., 2005).The medium was poured into the sterile Petri plates. The collected soil samples were diluted upto 10-6 and 0.1 ml of the diluted samples was spread over the agar medium. The inoculated plates were incubated at 28±2°C for 7 to 10 days. Three replicates were maintained for each dilution. After incubation, the Bacillus colonies were purified by streak plate technique, sub-cultured and stored at 4°C on SCA slants for further investigation.

Biochemical characterization and 16S rRNA phylogenetic sequence analysis:

Colony morphology of the purified isolates on SCA medium were recorded with respect to colour of aerial spore mass, size and nature of the colonies, colour on the reverse side and diffusible pigmentation. Purified bacterial culture plates were prepared and 3 to 4 sterile cover slips were inserted at an angle of 45°. The plates were incubated at 28±2°C for4-8 days. The cover slips were removed at 2-3 days of interval and observed under the high power magnification. The structure and arrangement of conidiospores and arthospores on aerial and substrate mycelia were observed and compared with Bergey's Manual of Determinative Bacteriology.

Sequencing file generated: For each sample processed, the following files are provided:

Sample.ab1: The raw chromatogram trace file, as generated by the sequencing instrument

Sample.seq: A text file of sequence, as generated by the sequencing instruments

Base calling for 16S rRNA sequencing: The sequence files are basecalled from the chromatogram by the KB base calling soft ware which produces Phred-like basecalls. This program assesses the quality of each basecall and assigns a quality value using the QV20 score to each basecall. The quality values help to determine the reliability of the basecalls.

Sequence Trimming: The sequence file is the text file output from the sequencing instruments and contains the basecalls generated from the raw trace (ab1) file. There is no assessment of the quality basecall from the underlying sequence and good and bad quality bases are not discriminated. Therefore the sequence may contain inaccuracies, particularly towards both ends of the sequence. The sequence file has been generated by looking at the average QV20 scores within a 50 bp sliding window, starting at the beginning of the chromatogram. Once the average QV20 score for the base within this window falls below 20 a clip

is introduced. As long as the average QV20 score is above 20, the area being assessed within the sliding window continues to move along the length of the chromatogram. Thus the less reliable bases at the ends of the sequence was removed.

RESULTS AND DISCUSSION

A total of 12 Bacterial colonies were isolated from soil samples. Among the 12 Bacterial colonies, only 9 isolates were morphologically different. Out of 9 isolates; 5 Bacillus sp., 1 Achromobacter sp., 1 Brevundimonas sp., 1 Pseudomonas sp. and 1 Ochrobactrum sp. were identified. Based on the morphological, biochemical, physiological and quite significantly molecular features, antimicrobial compound producing *Bacillus* and others were identified. The findings and observations of the has been summarized in table no. 1. The diagrammatic representation of the Phylogenetic tree drawn on the basis of the research presented in table x has been presented in figure no. 1 to 9

Table 1: Microbial Identification And Sequencing File Generation Report

S.No.	Particulars	Results
1.	Organism reported	NAPT^{-1}(0) -*Alcaligenes/Achromobacter* NAPT^{-1}(0) New- *Lysinibacillus sp*. NABL10^{-2}(1)- *Lysinibacillussp* NABL10^{-3}(2)- *Ochrobactrumintermedium* AIABr10^{-3} – *Pseudomonas otitidis* AIA BT M- *Bacillus sp.* NA BL10^{-2} – *Brevundimonas terrae* NA-BL10^{-3}(1) – *Bacillus sp.* NA BK 10^{-4}- *Lysinibacillus sp.*
2.	Description of Sequencing Samples	No. of samples - 09 No. of primers - 00
3.	Quality control(presequencing QC by agarose gel electrophoresis)	No. of samples passed -09 No. of samples failed -09
4.	16s rRNA Sequencing	No. of samples - 09 No. of samples purified – 09 No. of reads – 18 No. of reads Passed – 18 No. of reads fail - 00
5.	Sequence remark	Pass>50% High quality value(QV) bars in electropherogram with non overlapping picks. Fail>50% low quality value(QV) bars in electropherogram with long overlapping picks

Figure no. 1: Phylogenetic tree of NA BL 10^{-2} (1)

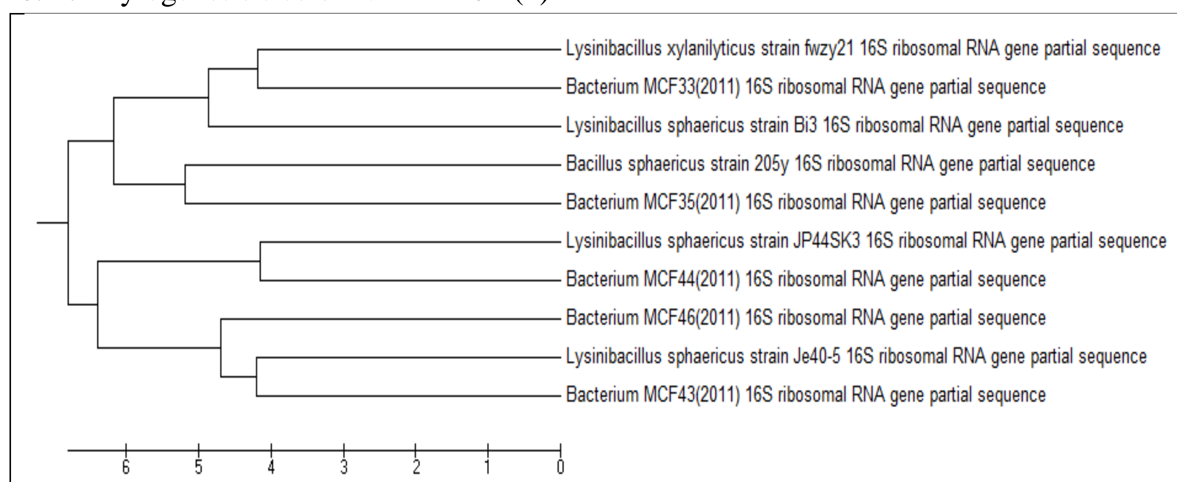


Figure no. 2: Phylogenetic tree of AIABr 10^{-3}

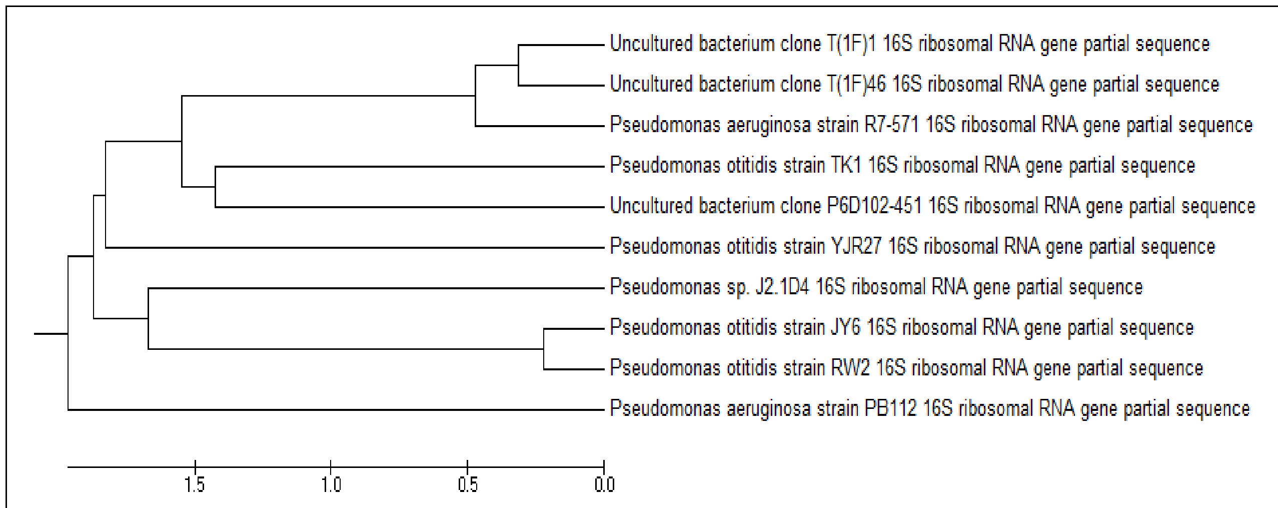


Figure no. 3: Phylogenetic tree of NA BL 10^{-3} (2)

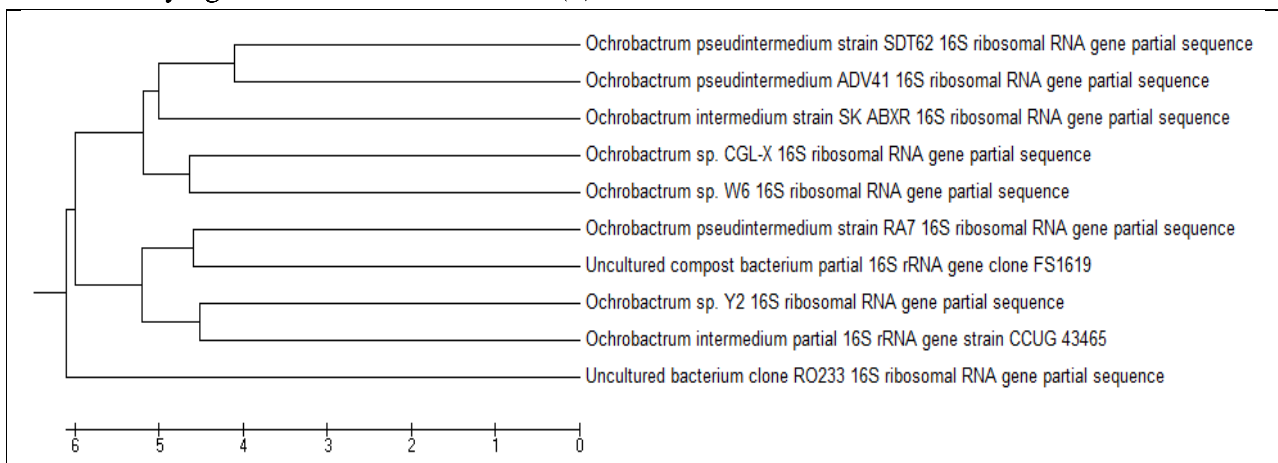


Figure no. 4: Phylogenetic tree of NA BL 10^{-2} (1)

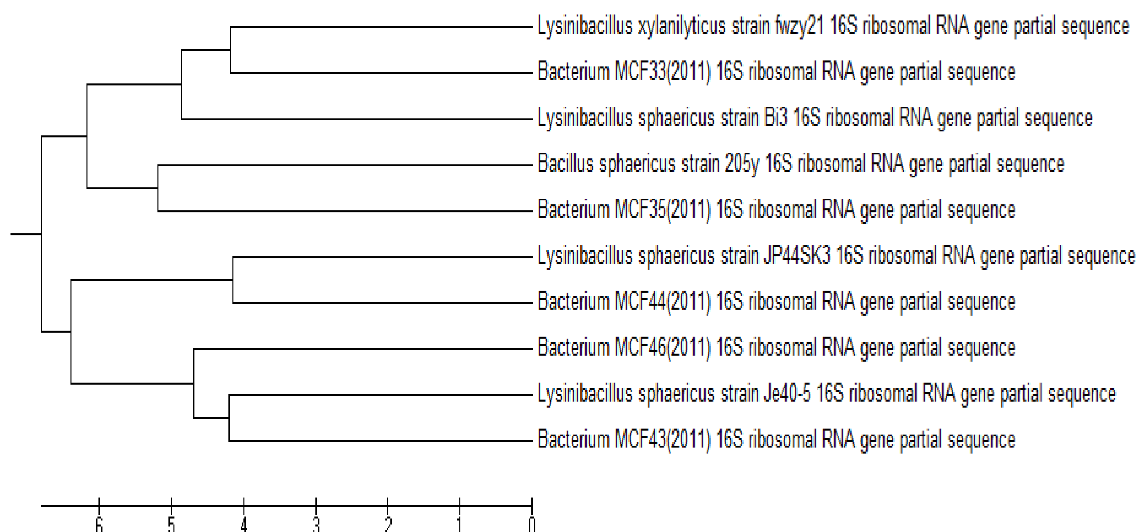


Figure no. 5: Phylogenetic tree of NA PT^{-1}(0)

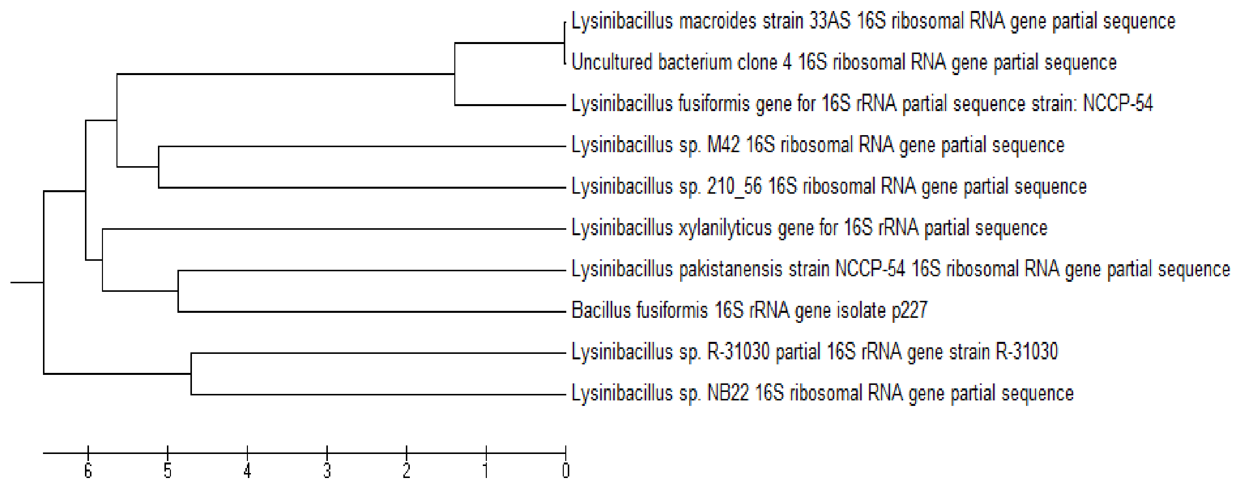


Figure no. 6: Phylogenetic tree of NA PT^{-1}(N)

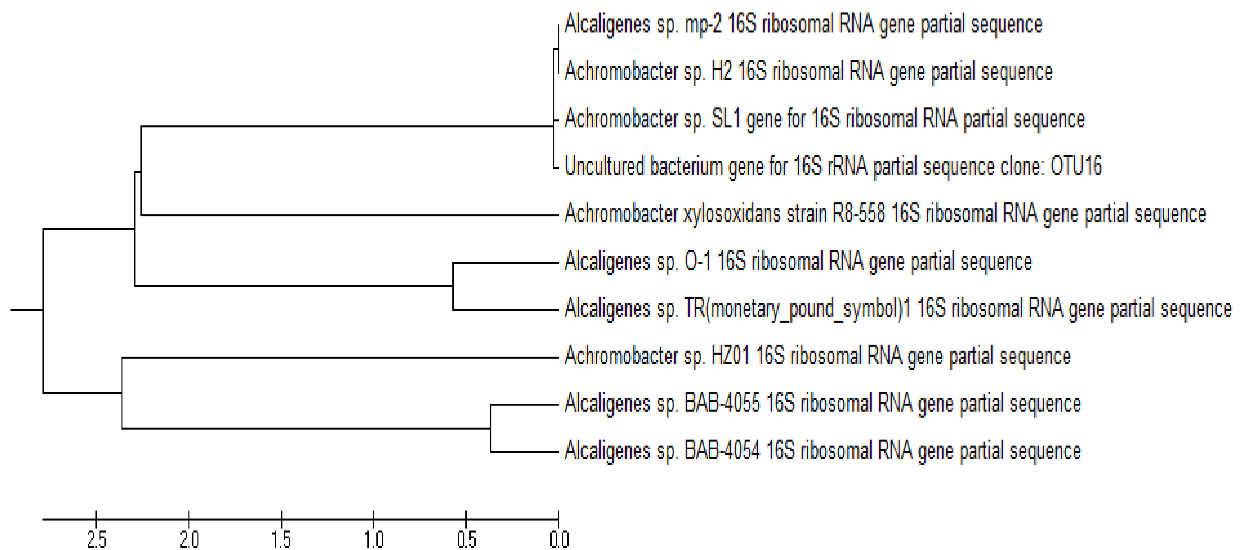


Figure no. 7: Phylogenetic tree of NABK 10^{-4}

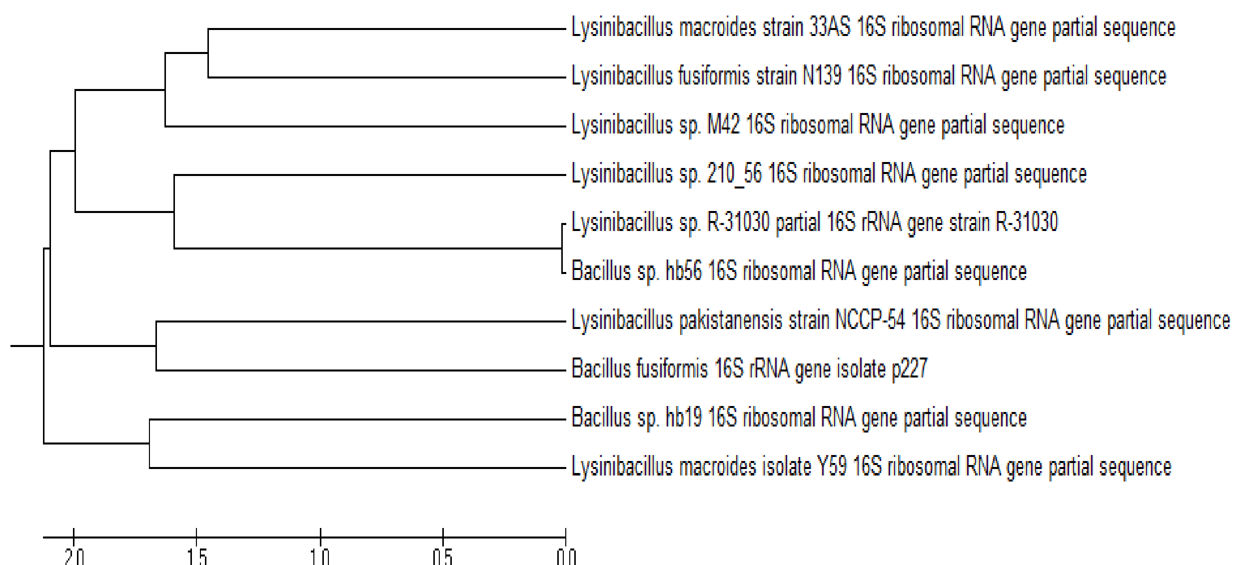


Figure no. 8: Phylogenetic tree of AIA BT M

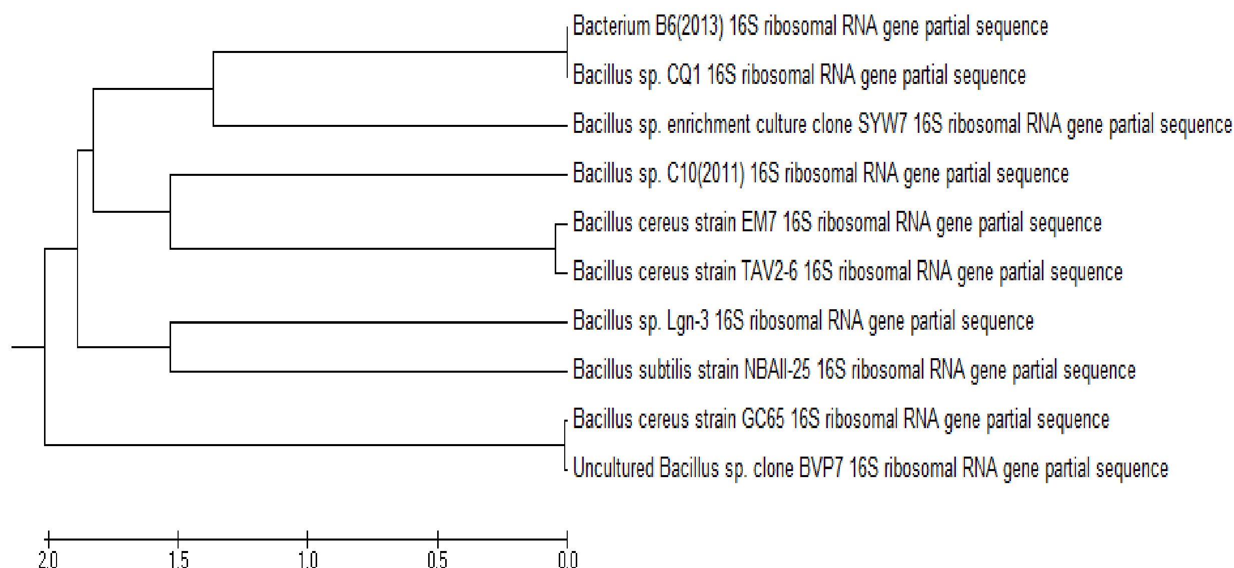
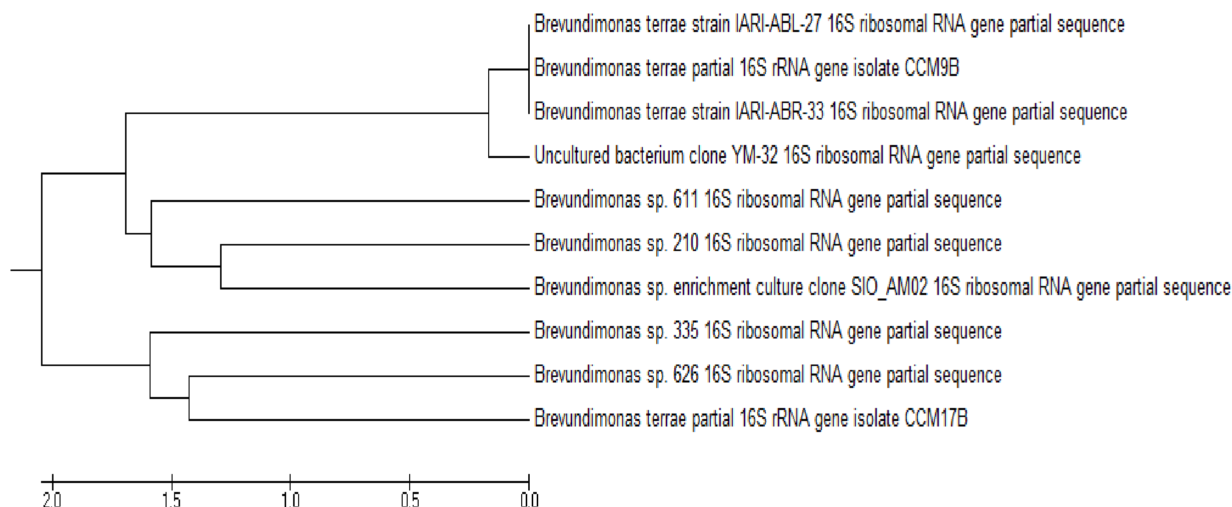


Figure no. 9: Phylogenetic tree of NA-BL10^{-2}(2) New



CONCLUSION

Physiological characteristics are important for the identification of Bacterial isolates at genus level but not helpful for species level identification. Though, they can be used atleast as markers by which an individual strain can be recognized. In the present study, the sensitivity pattern of isolates to the inhibitory compounds was studied. Although, preliminary identification of isolates can be made on the basis of morphology, yet reliable classification may not be possible using traditional approaches alone. Therefore under this study, identification based on 16S r RNA sequencing has been carried out using computerized instruments which was the most authentic approach to solve the taxonomic problems.

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