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Molecular detection of *Arthrobacter* species isolated from sewage and soil samples

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Abstract
The most common housekeeping genetic marker to study bacterial phylogeny and taxonomy is 16S rRNA gene sequence. Hence, 16S rRNA gene sequencing has the potential to identify and diagnose bacteria. Forty-five samples of sewage and soil were collected from in and around Thane district. Organisms were isolated on *Arthrobacter* medium with pyridine. Eighty-five isolates were obtained from above mentioned samples. Preliminary identification of these isolates was done by gram staining and biochemical characteristics. Further confirmation was done by phase contrast microscopy and their morphogenesis also was studied subsequently. Morphology of thirty isolates matched with that of *Arthrobacter*. Sixteen isolates showed strong positive results of biochemical and morphogenetic characteristics. Qualitative biodegradative screening of various organic as well as inorganic pollutants, pesticide, dyes and lignocellulosic materials, by these isolates, was checked. Twelve potent isolates were also studied for quantitative analysis. The activities of these isolates were constantly compared with those of Standard strains of *Arthrobacter*. Out of 12 isolates isolate no. A11 the most potent probable *Arthrobacter* isolate, obtained from quantitative degradative analysis, was identified by performing the 16S rRNA gene analysis. Identification was confirmed with 16s rRNA technique. This isolate appeared to degrade wide variety of compounds and its overall degradation and decolourisation capability also was found to be better than other probable *Arthrobacter* isolates. Using NCBI blast similarity search tool and Sequence analysis of 16 rRNA indicated an isolate closely related to the *Arthrobacter viscosus strain* with the highest sequence similarity (100%).

Keywords: Sequence, morphogenesis, degradation, blast, similarity

Introduction
Arthrobacter belong to coryneform group of bacteria that exhibit pleomorphism. They have a life cycle marked by two stages, rods in exponential growth phase and coccoid morphology during their stationary growth phase [1]. They are versatile in nutrition, using a wide range of substrates in their oxidative metabolism such as various inorganic and organic pollutants, pesticides and herbicides. Several species of *Arthrobacter* are obligate aerobes, chemoorganotrophs, exhibiting total respiratory metabolism and not a fermentative one [2]. Ubiquitous in soil, several species have also been found in caves, sediments, sewage, saline and water deficient environments and can grow at 25-30ºC. Some psychrotropic strains can sustain life at low temperatures [3]. *Arthrobacter* are extremely competitive organisms with remarkable resistance to desiccation, starvation and can survive periods of oxygen limitation [3] *Arthrobacter* can degrade wide variety of substrates like food-processing wastes like fat, grease, detergents, dyes, industrial solvents, cosmetic products, petroleum hydrocarbons, fuel combustion products, insecticides and pesticides [4, 5]. Metals such as chromium, mercury, calcium, cobalt, iron, manganese, copper, potassium, magnesium, zinc, sodium and nickel are some of the inorganic pollutants can be degraded by *Arthrobacter* [6]. They can also degrade hydrocarbons, lignocellulosic wastes and decaying organic matter [7]. The major threat to our planet is pollution of environment and valuable water supplies. Pollution of both water and soil poses a significant hazard to human health and can lead to ecotoxicity [8]. A number of methods are used by industries and civic bodies to treat these polluted waters such as adsorption, biosorption, flocculation, membrane filtration and advanced oxidation, for removal of pollutants from the system. Biological methods include primary-secondary sludge treatments, anaerobic digestions, aerobic treatment, activated sludge processes of sludge [9] However physicochemical methods are very costly, less efficient and need specialized reactors.

Disposal of secondary sludge generated during treatment is an added problem.

Bioremediation is a process used to treat contaminated water, soil and subsurface material, by altering environmental conditions to stimulate growth of microorganisms and degrade the target pollutants. It is less expensive and more sustainable than other remediation alternatives [10, 11]. *Arthrobacter* is one such organism which can aid in bioremediation as it can utilize wide variety of pollutants as substrates. Bioremediation mercury, lead and cadmium by *Arthrobacter* [6]. Very few organisms can grow in the presence of hexavalent chromium. *Arthrobacter* can not only grow in the presence of hexavalent chromium but also reduce it to trivalent chromium its less toxic form [6]. As *Arthrobacter* is extremely competent microbe. It was explored to remediate subsurface pollution and environmental cleanup by bioremediation, a cost and treatment-effective, non-destructive technology [12]. Many conventional microorganisms are unable to operate as efficiently as these under t adverse conditions. Thus, *Arthrobacter* might promise to be a better organism for carrying out bioremediation of sewage and soil.

16S rRNA gene sequencing is an amplicon-based sequencing method that is used to identify and classify bacteria present in bulk and complex biological samples. This method uses the highly conserved nature of the 16S ribosomal RNA (rRNA) gene present in all prokaryotes that also contains variable regions that differentiate between species [13]. The 16s rRNA identification studies were carried out at Yaazh Genomics Research centre pvt Ltd, Chennai. The procedure followed by research centre at their laboratory for bacterial identification is described below and is outlined in Figure1.

Materials

Insta Gene TM Matrix Genomic DNA isolation kit
Universal primers
Research Peltier Thermal Cycler.
Montage PCR Clean up kit (Millipore).
Big Dye TM Terminator Cycle
Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme).
Bioinformatics tools
Purified colony of identified *Arthrobacter* isolate A11

Methods

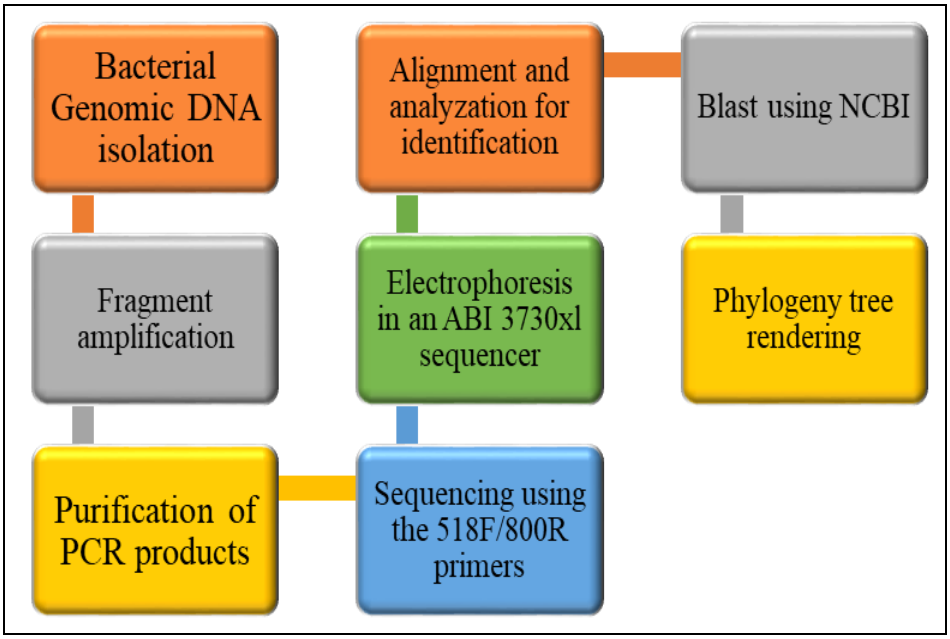


Fig 1: 16s rRNA identification methodology

- Genomic DNA of bacteria was isolated with the help of the Insta GeneTM Matrix Genomic DNA isolation kit.
- Using 16S rRNA Universal primers, gene fragment was amplified using MJ.

Research Peltier Thermal Cycler

- Purification of PCR products: The unincorporated PCR primers and dNTPs from PCR products were removed by using Montage PCR Clean up kit (Millipore).
- 518F/800R primers and ABI PRISM® Big DyeTM Terminator Cycle was used for carrying out the sequencing reactions and AmpliTaq® DNA polymerase (a FS enzyme) in Kits for Sequencing (Applied Bio systems). DNA sequencing reaction of PCR amplicon was carried out with 27F and1492R primers using BDT v3.1 Cycle sequencing kit on ABI 3500xl Genetic Analyzer.
- Sequencing protocol. Single-pass sequencing was performed on each template using 16s rRNA universal primers. The fluorescent-labelled fragments were purified from the unincorporated terminators with ethanol precipitation. The treated samples were suspended again in distilled water and electrophoresis was carried out in an ABI 3730xl sequencer (Applied Bio systems).
- Sequencing Primer Details. The data of sequence was aligned and then analysed to identify the sample.
- Bioinformatics protocol. The obtained 16s rRNA sequence was then subjected to BLAST, using similarity search tool called NCBI BLAST. The phylogeny analysis of sample sequence with the closely related sequence of blast results, was performed, followed by multiple sequence alignment. The program called MUSCLE 3.7 was employed/used for multiple

alignments of required sequences (Edgar 2004). Program Gblocks 0.91b was employed/used for curing of the resulted aligned sequences. For Phylogeny tree rendering the Tree Dyn 198.3 program was employed/used [14-20].

The primers 27F and 1492R and BDT v3.1 Cycle sequencing kit on ABI 3500xl Genetic Analyzer, were used for DNA sequencing reaction of PCR amplicon. Sequence with both the primers are given below. Details of primers i.e., names and details of universal and sequencing primer details with number of bases are described below in Table 1 and 2. NCBI BLAST similarity search tool was used to blast the 16s rRNA sequence. The phylogeny analysis of sample sequence with the closely related sequence of blast results was performed. It was followed by multiple sequence alignment. For multiple alignments of sequences, the program MUSCLE 3.7 was used (Edgar 2004). Using the program Gblocks 0.91b the resulting aligned sequences were cured. They are shown in Figure 2 and 4. Phylogeny tree rendering was done by the program Tree Dyn 198.3. (Dereeper *et al*.). It is depicted in Figure 3.

Isolate No A11

Universal Primer Details

Table 1: Details of the Universal primer used for the sequencing of isolate A11

Primer Name	Sequence Details	Number of Base
27F	AGAGTTTGATCMTGGCTCAG	20
1492R	TACGGYTACCTTGTTACGACTT	22

Sequencing Primer Details

Table 2: Details of the primer used for the sequencing of isolate A11

Primer Name	Sequence Details	Number of Base
785F	GGATTAGATACCCTGGTA	18
907R	CCGTCAATTCMTTTRAGTTT	20

>Contig 11

CGCAGCCTTACACTTGCAGTTCGAACGACTCCTTCGGAGTTCGTGGCGGACGGGTGAGTAATGGCTGGGAGCGT
GCCATTTGGTTCGAAATAGCCCCGGGAAACGGGGGCTAATACCGAAAGTGCCCCTCGGGGGAAAGATTCATCA
CCGTTAAAGCGGCCGGCGTCTGATAGCCTGTTGGTGATGTAACGGCTCTCAAGGCGACGATTGTAGGTGGTCTG
CTGGATGAGCAACCACACTGGGACTGATAGCGGCCCAGACACCTACGGGAGGCATGATGGGGAATCTTGCGCC
TGGGCGAGAGCCTGACGCAGCCATGCCTCGTGAATGATGACCGTCTTAGGATTGTAAAATTCTTTCACCGGAGA
CGATCTGACGGTACCCAGATAAGAAGCCCCGGCTAACTTCGTGCCAGCAACCGCAGTGATACTAAGGTGCTACG
TTGCTCGGACTTACTGGGCGTAGAGGGCGGTGCGCGGATCGTTAATCGGGGTGATTCCCAGGGCTCGGCTCGGA
ATTGCCCTTGAGCTGGCTATTTGAGTATGAGAGACTCGTGGACTCCTAGTGTAGACGTTAATTCGTAGACTTCTG
AGACCCCATGGCGAGGCACCTACTGTCTCAGTACTGACACTGAAGGTGCTAAGCATGTGAGGCACAGGTTTGCT
CCCTCGTAGTCTACGTACCGATAATGTTTCTTGCTGCTGTCATCATACATGATAACTTCCCATAAAAAGCAGTCA
GGGATACTTGAATATAACTCAAAAATTGAAGTTCCAGTAGAGGCGGATTGGCTGTATAAACAATCGCTAAATTC
CCTCACCTCCGACTACGTGGTCGCTTGCTGCCATTGCTGGTTAGAGCACCGCCGTCCGGTAGAGCCAATTCCGGT
GGTGTGACGGGCGGTGTGTAAAGGCCCGGGAACGTATTCACCGAGGCATGCTGATCCGCGATTACTAGCGATTC
CGACTTAATGGCCTAGAGTTGCACAAGACGATCCAAACTGAGAGGGCTTTTGAGGATTAACCCTCTGTACTCGC
CGTTGTACCACGTGTGTAACCCACCCTGTAAGGGCCATGATGACTTGACGTCGGCCACGACTTCCTCCCGCTTAC
CACCGGCAGTTCCGGTAGAGTTCCCAACTAAATGATGGCAACTAATGGCGAGGGTTGCACTCTTTGCGGGACTT
AACCCAACATCTCACGACACGAACTGACCACAGCCATGCAGCACCTGTCTCCTAGTCCCCCAAGGGAAAGCCCC
ATCTCCGTGGCCGTCCAGGCATGTCAAAGGGTGGTAAGGGTCTGCGCGTTGCTTCCAATTATACCACATGCTCCA
CCGCTTGTGCGGGCCCCCGGCATTTGCCTTGAGTTTTCTCGTGCGAGAGAACTCCCCGGGCGACCGCTTAAGCGG
TTAACTGCATCGCTAACTGAAGGCGCCCGAGGGGAGGAAAATCGTGGAGGAGGTGTGATACTGAGGTGCTAAA
CATGTTTAGGCCACAGGATTTGGCGCGCCAGCTGTTGTAATCAGTCAAGATGACGGCTACGTCGCTGGGGGTCT
CAAATAGTGACGAATACTACTCATAAATCAGGAGGTCAGGGTACTCTGCGACTGATGATCCATAGAATTGAAGG
TCGCCAGTAGAGGCGCGGATCAAGCGCTGGACTAAAGCGCTAAATGCTTCAACCCTAAATGCACGG

Fig 2: Multiple sequence alignment of isolate A11

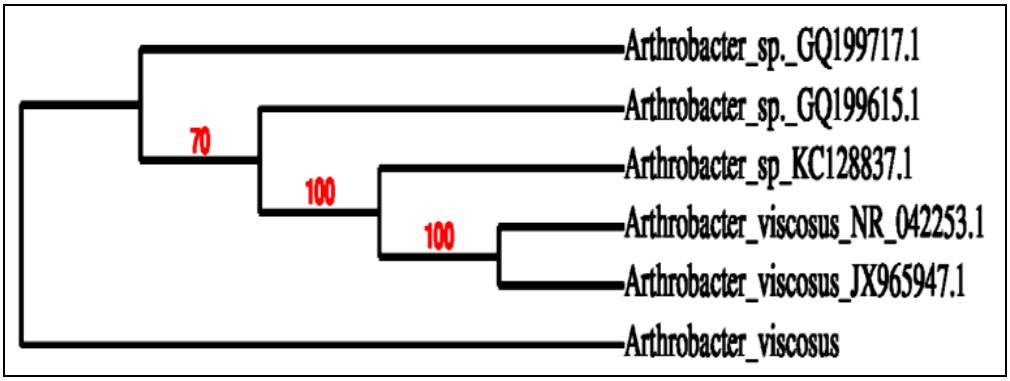
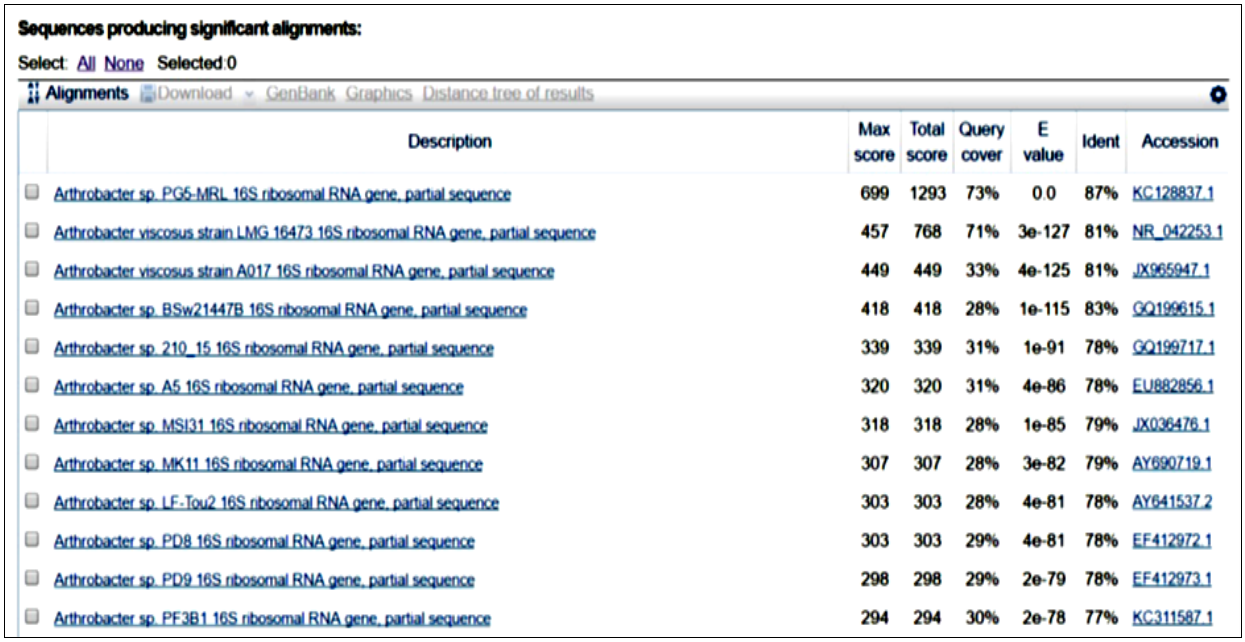


Fig 3: Phylogenetic tree of isolate A11



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Arthrobacter sp. PG5-MRL 16S ribosomal RNA gene, partial sequence	699	1293	73%	0.0	87%	KC128837.1
Arthrobacter viscosus strain LMG 16473 16S ribosomal RNA gene, partial sequence	457	768	71%	3e-127	81%	NR_042253.1
Arthrobacter viscosus strain A017 16S ribosomal RNA gene, partial sequence	449	449	33%	4e-125	81%	JX965947.1
Arthrobacter sp. BSw21447B 16S ribosomal RNA gene, partial sequence	418	418	28%	1e-115	83%	GQ199615.1
Arthrobacter sp. 210_15 16S ribosomal RNA gene, partial sequence	339	339	31%	1e-91	78%	GQ199717.1
Arthrobacter sp. A5 16S ribosomal RNA gene, partial sequence	320	320	31%	4e-86	78%	EU882856.1
Arthrobacter sp. MSI31 16S ribosomal RNA gene, partial sequence	318	318	28%	1e-85	79%	JX036476.1
Arthrobacter sp. MK11 16S ribosomal RNA gene, partial sequence	307	307	28%	3e-82	79%	AY690719.1
Arthrobacter sp. LF-Tou2 16S ribosomal RNA gene, partial sequence	303	303	28%	4e-81	78%	AY641537.2
Arthrobacter sp. PD8 16S ribosomal RNA gene, partial sequence	303	303	29%	4e-81	78%	EF412972.1
Arthrobacter sp. PD9 16S ribosomal RNA gene, partial sequence	298	298	29%	2e-79	78%	EF412973.1
Arthrobacter sp. PF3B1 16S ribosomal RNA gene, partial sequence	294	294	30%	2e-78	77%	KC311587.1

Fig 4: Sequences producing significant alignments

Conclusion

Based on the results of the sequence alignment, nucleotide homology analysis and phylogentic analysis, the isolate A11 was found to be *Arthrobacter viscosus.*

The isolate appeared to degrade wide variety of compounds and its overall degradation and decolourisation capability also were found to be better than other probable *Arthrobacter* isolates. Using NCBI blast similarity search tool they were identified as *Arthrobacter viscosus*. The morphology of the identified isolate *Arthrobacter viscosus* was reconfirmed by Scanning Electron Microscope (SEM).

Conflict of Interest

Not available

Financial Support

Not available

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