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Recognition of a Bacterial Isolate from "Rhizosphere" of a Tea Plant from a Tea Garden of Barak Valley, Assam (India).

[1*]Mrinal Kanti Bhattacharjee, P.B.Mazumder[2] and [3]G.D.Sharma.
[1, 2]Department of Biotechnology, Assam University, Silchar, Assam.*
[3]Bilaspur University.

Abstract: *A bacterium was detached from the "rhizosphere" part of tea plant from a tea garden of Barak Valley, Assam (India). The bacterial isolate was separated on an "N-free medium" and it was identified on the basis of its "external attributes". The "Gram staining" and "molecular features" were also conceded in. Further the molecular work through"16SrDNA scrutiny" of the isolate was decided out and on the foundation of that; "species level" identification of that bacterium was made. The "evolutionary tree" was constructed by taking the concerned bacterial isolate that was marked as "Azo-4"with taking ten other close strains that were available in the "NCBI" GenBank nucleotide database.*
Key words: *external attributes, detached, 16SrDNA scrutiny, species level.*

I. Introduction:

The "nitrogen" present in the atmosphere in massive amount as a gas and it gets reduced to some reducible forms of organic compounds through "biological reactions" with the help of microbes of prokaryotic nature [6]. As free living bacteria and cyanobacteria desire to live in a broad range of habitats with a varying degree of nutrients, oxygen, pH, etc [6]. Possessing of highest respiratory rates the "Azotobacters" are "aerobic bacteria" [6], as the members from this group happens to be "mesophilic bacteria" that have a preference to grow at temperature of around 30^0C [6]. It is been quit known that "microbes" hardly does any harm to the "environment", hence for better and safer agricultural outputs, microbes are thought to be a secured and a safer path.

This paper encompasses with a free living nitrogen fixing bacteria marked as "AZO-4" isolated from tea "rhizosphere" of a tea plant from a tea garden of Barak Valley, Assam (India).Some of the characteristics features of the isolate have been presented in the current paper. The scrutiny by "16SrDNA" for the bacterial isolate was also been made and then results were represented nicely.

II. Material and Methods:

Soil from the "rhizosphere" zone of tea plant was used for the separation of the bacterial isolate; the media chosen was "Ashby sucrose" medium [9] for isolating the bacterial isolate. The "Petri Plate" was brewed for almost a week at room temperature. Afterwards on recurring streaking on the identical medium "pure colony" was attained.

Morphology for the bacterial isolate:

The pure isolate marked as "AZO-4" was examined for colony form and shape on the same medium as per the procedures described by [2] also the further again morphology is ascertained by procedures described, as per [4]."Gram staining" was performed by following the method described in the "Practical laboratory Manual of Microbiology" by Dubey et al. (2002) [5].

"16SrDNA" Assessment:

By a standard scheme the genomic DNA was extracted from the isolate (AZO-4).After that from the isolated DNA, by using PCR a fragment of "16S rDNA" was allowed for amplification. After removing all the contaminants the PCR amplicon was thus purified. With the help of "8F" and "1492R" "primers" [7]; [3] the forward and the reverse DNA sequencing reaction of that purified PCR amplicon was made nicely. After that from the forward and reverse sequence data a "consensus sequence" of 1248bp of "16S rDNA" gene was created with the aid of "aligner software". In the next step, with that 16SrDNA gene sequence the BLAST [1] was carried out by making use of nrdatabase of NCBI (www.ncbi.nlm.nih.gov) GenBank database. Then, on the basis of the maximum identity score, first ten sequences were made to get selected and thereafter the sequences were made to get aligned with the help of the "multiple alignments" software program Clustal W [14].Further on generating distance matrix and then by using the MEGA4 [13] was utilized for generating the phylogenetic relationship, by following the Neighbor-joining method as being described by [12] and the Kimura's two-parameter nucleotide distances [11], as from 500 replicates the "bootstrap consensus" tree was concluded [8].

The "16SrDNA" sequence that has been obtained for the bacterial isolate (Azo-4) along with close ten strains that are available in the NCBI *GenBank* nucleotide database using those, the "phylogenetic tree" was build. The whole process was carried at Xcelris Labs.Ltd (Ahmedabad).

The sequencing Primers used were:
8F (AGAGTTTGATCCTGGCTCAG) - forward primer.
1492R (GGTTACCTTGTTACGACTT) - reverse primer

III. Results:

Morphology and Gram Staining result for the Isolate:

The morphology reveals that the isolate marked as "AZO-4" is round, circular in shape seen in "Petri plate". From the "Gram negative", rod shaped bacteria when seen under microscope.

"16SrDNA" Assessment Result:

The result of"16SrDNA" analysis, from "nucleotide" homology" and "phylogenetic" investigation of that strain, it has been come to know that the bacterial isolate marked as"Azo-4" is being identified as *Azotobacter beijerinckii strain ICMP8673***.** The sequence of that above mentioned bacterial strain (Azo-4) on doing BLAST showed homology with current bacterial isolate "*Azotobacter beijerinckii* strain ICMP 8673" which had an accession number EF100152.1.On submitting the current strain of the bacterial strain "Azo-4"to NCBI, an accession number KC 172855 was assigned. A "Phylogenetic tree" was built eventually based on comparing the 16SrDNA sequence of that bacterial strain (Azo-4) along with the other close ten strains that were available in the *GenBank* nucleotide database of NCBI.

Sequence producing significant Alignment (given below in the table) (carried out at Xcelris labs)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
GU372346.1	Beijerinckia indica strain SDSA-I30/2	2305	2305	100%	0.0	100%
EF100152.1	Azotobacter beijerinckii strain ICMP 8673	2305	2305	100%	0.0	100%
EF100151.1	Azotobacter beijerinckii strain ICMP 4032	2305	2305	100%	0.0	100%
NR_042071.1	Azotobacter beijerinckii	2305	2305	100%	0.0	100%
AB429526.1	Azotobacter beijerinckii , strain: C4	2276	2276	100%	0.0	99%
GQ397081.1	Uncultured bacterium clone AK4DE2_06E	2272	2272	100%	0.0	99%
JN641801.1	Azotobacter chroococcum strain A5	2198	2198	100%	0.0	98%
EU930421.1	Azotobacter chroococcum strain 10006	2198	2198	100%	0.0	98%
EF620440.1	Azotobacter chroococcum strain ISSDS-397	2198	2198	100%	0.0	98%
EF100153.1	Azotobacter chroococcum strain ICMP 4031	2198	2198	100%	0.0	98%

Distance Matrix (is given below) (carried out at Xcelris labs)**:**

AZO-4	1		0.000	0.000	0.000	0.000	0.001	0.002	0.003	0.003	0.003	0.003
GU372346.1	2	0.000		0.000	0.000	0.000	0.001	0.002	0.003	0.003	0.003	0.003
EF100152.1	3	0.000	0.000		0.000	0.000	0.001	0.002	0.003	0.003	0.003	0.003
EF100151.1	4	0.000	0.000	0.000		0.000	0.001	0.002	0.003	0.003	0.003	0.003
NR_042071.1	5	0.000	0.000	0.000	0.000		0.001	0.002	0.003	0.003	0.003	0.003
AB429526.1	6	0.002	0.002	0.002	0.002	0.002		0.002	0.003	0.003	0.003	0.003
GQ397081.1	7	0.005	0.005	0.005	0.005	0.005	0.006		0.003	0.003	0.003	0.003
JN641801.1	8	0.010	0.010	0.010	0.010	0.010	0.008	0.015		0.000	0.000	0.000
EU930421.1	9	0.010	0.010	0.010	0.010	0.010	0.008	0.015	0.000		0.000	0.000
EF620440.1	10	0.010	0.010	0.010	0.010	0.010	0.008	0.015	0.000	0.000		0.000
EF100153.1	11	0.010	0.010	0.010	0.010	0.010	0.008	0.015	0.000	0.000	0.000	

The Matrix is given in the above table the ten isolates with accession numbers are given along with the Azo-4 isolate.

Sequence of Azo-4 got by forward primer (8F) (836 bp):

TACGTCCTACGGGAGAAAGTGGGGGCTCTTCGGACCTCACGCTATCGGATGAGCCTAGGTCGGATT
AGCTAGTTGGTGGGGTAAAGGCCCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAG
TCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAAT
GGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTA
AGTTGGGAGGAAGGGCTGTAAGTTAATACCTTGCAGTTTTGACGTTACCGACAGAATAAGCACCG

GCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCG
TAAAGCGCGCGTAGGTGGTTTGGTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCA
TCCAAAACTGCCTGACTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCG
TAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCG
AAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGC
CGTTGGGCTCCTTGAGAGCTTAGTGGCGCAGCTAACGCATTAAGTCGACCGCCTGGGGAGTACGG
CCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAAT
TCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATCCTGCGAACTGGGTAGAG

Sequence of Azo-4 got by reverse primer (1492R) (881 bp):

CAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGACATT
CTGATTCGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACG
ATCGGTTTTCTGGGATTAGCTCCGCCTCGCGACTTGGCAACCCTCTGTACCGACCATTGTAGCACGT
GTGTAGCCCTGGCCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACC
GGCAGTCTCCTTAGAGTGCCCACCATGACGTGCTGGTAACTAAGGACAAGGGTTGCGCTCGTTACG
GGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTCTCTGCGCTCCC
GAAGGCACCCAGGTATCTCTACCCAGTTCGCAGGATGTCAAGGCCAGGTAAGGTTCTTC
GCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTT
AACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAATGCGTTAGCTGCGCCACTAAGCTCTCAAGG
AGCCCAACGGCTAGTCGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCC
CACGCTTTCGCACCTCAGTGTCAGTATCAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTA
TATCTACGCATTTCACCGCTACACAGGAAATTCCACCACCCTCTACCGTACTCTAGTCAGGCAGTTT
TGGATGCAGTTCCCAGGTTGAGCCCGGGGCTTTCACATCCAACTTACCAAACCACCTACGCGCGCT
TTACGCCCAGTAATTCCGATTAACGCT

Consensus Sequence of Azo-4 (1248 bp):

TACGTCCTACGGGAGAAAGTGGGGGCTCTTCGGACCTCACGCTATCGGATGAGCCTAGGTCGGATT
AGCTAGTTGGTGGGGTAAAGGCCCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAG
TCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAAT
GGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTA
AGTTGGGAGGAAGGGCTGTAAGTTAATACCTTGCAGTTTTGACGTTACCGACAGAATAAGCACCG
GCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCG
TAAAGCGCGCGTAGGTGGTTTGGTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCA
TCCAAAACTGCCTGACTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCG
TAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCG
AAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGC
CGTTGGGCTCCTTGAGAGCTTAGTGGCGCAGCTAACGCATTAAGTCGACCGCCTGGGGAGTACGG
CCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAAT
TCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATCCTGCGAACTGGGTAGAGATACCTGGGT
GCCTTCGGGAGCGCAGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT
AAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTCATGGTGGGCACTCTAAGGAG
ACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGG
GCTACACACGTGCTACAATGGTCGGTACAGAGGGTTGCCAAGTCGCGAGGCGGAGCTAATCCCAG
AAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAAT
CGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGG
AGTGGGTTG

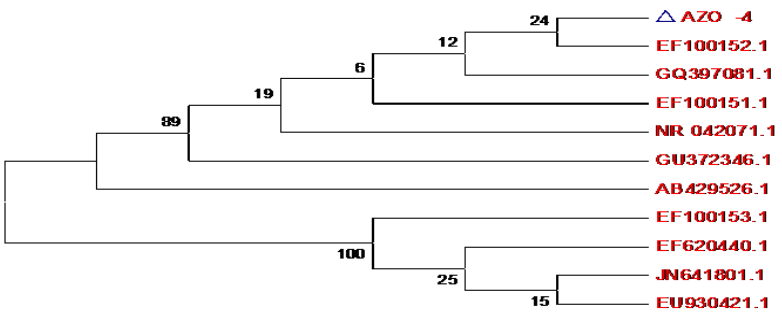

Figure: 1.Azo-4 evolutionary relationship with 10 other bacterial isolates**, 10** isolate sequences are taken from NCBI, GenBank database. (The whole method done at Xcelris labs)

IV. Discussion:

It has been come under consideration, the isolate was seen as rod shape under microscope .Accordingly from the results of "Gram staining", it has been seen that the isolate is of "Gram" negative type.

The above check of the isolate Azo-4 was compared with the results of Joseph et al. (2007) [10] for their similar isolates of *Azotobacter* and it was found that, the isolates showed similar result as the similar isolates of Azotobacter isolated by Joseph et al. (2007) were found to be seen as Gram negative by nature and rod shape in appearance when seen under microscope as of [10].Finally the isolate (Azo-4) was made to get identified and a GenBank Accession Number KC172855 has been provided by GenBank of NCBI for that isolate. Lastly, "phylogenetic tree" was set up.

V. Conclusion:

Clearly the study has brought a fascinating move in this part of Barak Valley, Assam (India).As on this trial an isolate was isolated and identified on basis of 'external attribute' and 'molecular' implementation by "16SrDNA".

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