# Computational Analysis of Distance based Phylogenetic Tree for Azotobacter Species

## Akansha Sharma, R.S Thakur, Shailesh Jaloree

Abstract: Phylogenetic tree is a pictorial representation of evolutionary relationships between organisms. It is important method to analyze the biological data. Phylogenetic trees are based on two methods : Distance based and Character based. Phylogenetic tree are used comparative analysis of any organism like human Beings, Animals, Bacteria, Viruses and Fungi's etc. In this paper we compare 12 different nucleotide sequences of Azotobacter species having linear DNA of 999 BP as maximum size using substitution model and phylogenetic model. In this study two different models name P-Distance and Jukes cantor model are used and helped in finding UPGMA or Neighbour joining method efficiency in evaluating the similarity and dissimilarity of bacterial species. This paper gives influence in reconciliation of Azotobacter species to produce phylogram with informative branch lengths. This further leads to analyze and understand various expressive characters of Azotobacter in agriculture field.

Index Terms: Phylogenetic tree, UPGMA, Neighbour joining, P-Distance, Jukes cantor

#### I. INTRODUCTION

A phylogenetic tree is a branching tree that gives similarity and dissimilarity of organisms based on physical and genetic characteristics[1]. Phylogenetic tree can be represented in two ways rooted tree and unrooted tree. Rooted tree gives direction tree with the unique node called root node and the unrooted tree give the relation of leaf nodes without ancestral root. It is very useful in the field of bioinformatics [2]. When clustering algorithms of data mining is included in the phylogenetic tree, it gives more detailed or accurate relatedness. UPGMA and Neighbour joining clustering algorithms are mostly used to make distance based phylogenetic tree[3][4]. There are so many software like phylip and MEGA for constructing and analyzing the phylogenetic tree [5]. In MEGA software, there are so many models which can be used to analyze the tree [6]. Some models are P-distance, Jukes Cantor, Poisson etc. The MEGA software has the ability to make distance matrix based as Character based matrix on different models [7]. We are interested in comparing distance based matrices and worked over it. Data mining is the method of extracting information for the patterns and models from large broad datasets [8]. Before implementing data mining algorithms, Some preprocessing steps have to be done like data cleaning process, data selection and transformation process[9]. Association, Classification, Clustering are the different methods which are applied in analysis of pattern in data

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**Dr.R.S. Thakur**, MCADept. MANIT, Bhopal, India. **Dr. Shailesh Jaloree**, Department of mathematics, SATI, Vidisha, India. mining. To draw the conclusion of biological data or analyze the biological data, data mining method are commonly used and they play important role. There are various clustering or algorithms classification are implemented in that bioinformatics [10]. V.Sohpal et.al using different substitution models to compare the capsid protein of HHV to find relationship between proteins. They also analyze the effect of poisson correction with shape parameter. They give the computational analysis of phylogenetic tree for both distance and character based tree. Finally they conclude that taxon separation of proteins give maximum similarities and can use as a drug for human therapy. A.Smith et.al gives the methods for aligning, synthesizing and analyzing rooted phylogenetic trees within a graph, called a tree alignment graph (TAG). This model can be used for large scale analysis and resolve the problem for find common node and edges. Mahapatro et.al (2012) constructed the phylogenetic tree for DNA sequence using different clustering methods. In this work they use three different clustering algorithms named K-mean medoid and DBSCAN. They conclude that the DBSCAN is performing better in many respects in future.

## **II.** METHODOLOGY

Phylogenetic tree construction and analysis have four phases: 1. Select the sequences and download in fasta format. 2. Align the sequences from any selected method that are used in multiple sequence alignment. 3. Construct the distance matrix for different parameters. 4. Tree building and evaluation In Phylogenetic tree analysis, the important source of data is NCBI (National Center for biotechnology Information).NCBI has different databases like gene bank for DNA sequences and many more . It is having thousands of nucleotides sequences for almost all organisms in two different classifications linear and non linear with different sizes, which are useful in the construction of phylogenetic tree[11]. In this paper we are using 12 different nucleotide sequences of Azotobacter chrococcum strains with whole genome shotgun sequences with different nodes in them. They all are the family of Azotobacter chrococcum species. But some where they are different from each other at molecular genetic level. Following nucleotide sequence are downloaded in Fasta format. Among many soft wares available to make a phylogenetic tree, we have chosen MEGA Software that constitutes of all features to make align, make distance matrix, tree evaluation and so on[12]. Here in this study P-Distance and Jukes cantor model were used as a different parameters and compare it with result.

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Fig 1. Showing the steps of creating distance matrix and phylogenetic tree

## III. RESULT

## A. Substitution models

Distance measures are the important tool by which evolution is studied at molecular level, phylogenetic reconstruction. In this study azotobacter chrococcum has undergone estimation by using P-distance and jukes cantor model for analyzing the evolutionary divergence of base pair sequences of whole genome. Table.1 shows the data of distance against 12 whole genome sequence of azotobacter chrococcum for P-distance model. In continuation of the study table.2 gives the data for distance against 12 whole genome sequence of azotobacter chrococcum for jukes cantor model. The study gives a critical evaluation of distance between Azotobacter chroococcum strain ATCC 9043 Node 115 and Azotobacter chroococcum strain ATCC 9043 Node 113 has minimum distance in both the substitution models. The distance divergence among these two strains is 0.255 and 0.312 for the P-distance and jukes cantor model respectively(Table.3). On the other side Azotobacter chroococcum strain ATCC 9043 Node 106, Node 104 and Node 111 shows maximum distance divergence of 0.617 and 1.297 with Azotobacter chroococcum strain ATCC 9043 Node 110 in both the models respectively.All tables.1,2and 3 shows that results are qualitatively consistent irrespective of models used. Lower divergence and the higher divergence between the strains signifies the similar and dissimilar relationship among them.

Table	1. P-Distance matrix	K

Species No./Name	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]
[1]Azotobacter_chroococcum_str ain_ATCC9043NODE117											
[2]Azotobacter_chroococcum_str ainATCC9043NODE114	0.539										
[3]Azotobacter_chroococcum_str ainATCC9043NODE110	0.596	0.553									
[4]Azotobacter_chroococcum_str ainATCC9043NODE109	0.504	0.511	0.553								
[5]Azotobacter_chroococcum_str ainATCC9043NODE106	0.574	0.511	0.617	0.546							
[6]Azotobacter_chroococcum_str ainATCC9043NODE104	0.582	0.468	0.617	0.546	0.553						
[7]Azotobacter_chroococcum_str ainATCC9043NODE101	0.518	0.447	0.539	0.475	0.489	0.447					
[8]Azotobacter_chroococcum_str ainATCC9043NODE111	0.539	0.567	0.617	0.560	0.567	0.582	0.553				
[9]Azotobacter_chroococcum_str ainATCC9043NODE113	0.511	0.539	0.546	0.525	0.546	0.496	0.489	0.539			
[10]Azotobacter_chroococcum_st rainATCC9043NODE115	0.553	0.546	0.553	0.553	0.539	0.553	0.553	0.560	0.255		
[11]Azotobacter_chroococcum_st rainATCC_9043_NODE_112	0.567	0.525	0.596	0.596	0.589	0.553	0.546	0.532	0.525	0.567	
[12]Azotobacter_chroococcum_st rain ATCC 9043 NODE 103	0.589	0.511	0.617	0.617	0.603	0.454	0.496	0.553	0.532	0.553	0.574



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Species No/Name	[1]	[2	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	
[1]Azotobacter_chroococcum_strain _ATCC9043NODE117												
[2]Azotobacter_chroococcum_strain ATCC9043NODE114	0.951											
[3]Azotobacter_chroococcum_strain ATCC9043NODE110	1.186	1.003										
[4]Azotobacter_chroococcum_strain ATCC9043NODE109	0.835	0.857	1.003									
[5]Azotobacter_chroococcum_strain ATCC9043NODE106	1.089	0.857	1.297	0.977								
[6]Azotobacter_chroococcum_strain ATCC9043NODE104	1.120	0.734	1.297	0.977	1.003							
[7]Azotobacter_chroococcum_strain ATCC9043NODE101	0.879	0.679	0.951	0.753	0.793	0.679						
[8]Azotobacter_chroococcum_strain ATCC9043NODE111	0.951	1.059	1.297	1.031	1.059	1.120	1.00	)3				
[9]Azotobacter_chroococcum_strain ATCC9043NODE113	0.857	0.951	0.977	0.902	0.977	0.813	0.79	93 (	).951			
[10]Azotobacter_chroococcum_strai nATCC9043NODE115	1.003	0.977	1.003	1.003	0.951	1.003	1.00	)3 1	1.031	0.312		
[11]Azotobacter_chroococcum_strai nATCC_9043_NODE_112	1.059	0.902	1.186	1.120	1.152	1.003	0.97	(	).926	0.902	1.059	
[12]Azotobacter_chroococcum_strai n_ATCC_9043_NODE_103	1.152	0.857	1.297	1.059	1.221	0.697	0.81	3 1	1.003	0.926	1.003	1.089

Table 2. Jukes cantor model

Table:-3 Comparision of Models

Model	Lower divergence	Higher divergence	Species of Lower divergence	Species of higher divergence		
P-Distan ce	0.255	0.617	Node 115-Node1 13	Node 103-Node 109,Node11 1,Node104, Node 106		
Jukes Cantor	0.312	1.297	Node 115-Node 113	Node 103-node 111,104,106		

## B. Distance Based Phylogenetic tree

This phylogenetic method measures the distance between pair of sequences observing dissimilarities as well as similarities computed on the basis of sequence alignment. The primary thought of distance based method is homology among the sequences and are summed up by help of tree



branches. The neighbor joining and UPGMA Method based on clustering algorithm are used in this paper for 12 whole genome sequence of azotobacter chrococcum species. The tree shown in fig 2 and fig 3 is output of neighbour joining methodology and for which the p-distance and jukes cantor distance are shown in table.1 and table.2 respectively. On comparing the fig2 and fig.3 with table1 and table2, distance matrix the two closest operational taxonomic units(OTUs) i.e Azotobacter chroococcum strain ATCC 9043 Node 115 and Azotobacter chroococcum strain ATCC 9043 Node 113 has the lowest taxon separation and 100%, 99% similarity in neighbour joining tree. The tree shown in fig 4 and fig 5 is an observation of UPGMA method for which the p-distance and jukes cantor distance are shown in table.1 and table.2 respectively. On comparative analysis of the fig4 and fig.5 with table1 and table2, distance matrix the two closest OTUs i.e Azotobacter chrococcum strain ATCC 9043 Node 115 and Azotobacter chrococcum strain ATCC 9043 Node 113 has the lowest taxon separation and 100% similarity in UPGMA tree.

SJAA01000015.1 Azotobacter chroococcum strain ATCC 9043 NODE 113 whole genome shotgun sequence AD1000017.1 Azotobacter chroococcum strain ATCC 9043 NODE 115 whole genome shotgun sequence 01000012.1 Azotobacter chroococcum strain ATCC 9043 NODE 110 whole genome shotgun sequence AAD1000019.1 Azotobacter chroococcum strain ATCC 9043 NODE 117 whole genome shotgun sequence NZ SJAA01000010.1 Azotobacter chroococcum strain ATCC 9043 NODE 109 whole genome shotgun sequence NZ SJAAD1000007.1 Azotobacter chroococcum strain ATCC 9043 NODE 106 whole genome shotgun sequence NZ SJAA01000002.1 Azotobacter chroococcum strain ATCC 9043 NODE 101 whole genome shotgun sequence NZ SJAA01000016.1 Azotobacter chroococcum strain ATCC 9043 NODE 114 whole genome shotgun sequence NZ SJAAD1000005.1 Azotobacter chroococcum strain ATCC 9043 NODE 104 whole genome shotgun seguence SJAA01000004.1 Azotobacter chroococcum strain ATCC 9043 NODE 103 whole genome shotgun sequence SJAA01000013.1 Azotobacter chroococcum strain ATCC 9043 NODE 111 whole genome shotgun sequence SJAA01000014.1 Azotobacter chroococcum strain ATCC 9043 NODE 112 whole genome shotgun sequence

Fig2. Neighbour joining method for P distance model (bootstrap consenses)



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## Fig3. Neighbour joining method for Jukes cantor modele (bootstrap consenses)



## Fig4. UPGMA method for P distance model (bootstrap consenses)



Fig5. UPGMA method for Jukes cantor model (bootstrap consenses



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## **IV. DISCUSSION**

Substitution model proofs to be a powerful tool for reconstructing distance based phylogenetic trees . The present study support this argument with proficiency and authenticity. The study shows that UPGMA method is efficient to obtain correct tree on the basis of distance data for Azotobacter chrococcum. Several lines of evidence suggest that both the UPGMA and NJ estimates are robust in calculating evolutionary distances.

# v. CONCLUSION

The research of specific biological dataset of Azotobacter chrococcum reveals the importance of different methods used to analyze evolutionary a likeness or differences . Both the methods used In this present study gives us a presumption that they are able to recreate distance based phylogenetic trees for the given bacterial species with utmost efficiency leading to proficient analysis of the relationships among the bacterial species

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