

16S rDNA Sequence based Phylogenetic Analysis of Some Bacterial Phytoplasma



Microbiology

KEYWORDS : Phytoplasma, Plant diseases, Phylogenetic analyses, 16SrRNA.

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ABSTRACT

Phytoplasma are the bacteria without cell wall and are associated with plant diseases. 26 strains of phytoplasma sequences were analysed by 16s rRNA based Phylogenetic which are widely infected economically important plants. Among the five different Iranian phytoplasma strains, the two strains like Iranian phytoplasma strains HAY2 and HAY4 shows the maximum bootstrap value of (98%) which means both the species have been originated from a single ancestor. The sequence similarity among this monophyletic group is higher in comparison with other three species of Iranian strains Iranian potato purple top, Plum phytoplasma PJ15, Peach phytoplasma PP49. The study was conducted to understand the major evolutionary relationship among the different phytoplasma strains which are pathogens to a wide variety of symptoms that range from mild yellowing to death of infected important plants.

Introduction:

The microorganisms like Phytoplasma which are specialised bacteria that are obligate parasites of plant phloem tissue and transmitting insects (vectors). They were first discovered by scientists in 1967 and were named Mycoplasma-like organisms or MLOs (Doi et al.1967). Phytoplasmas are bacteria without cell walls and are responsible for plant diseases that have large economic impacts (Glick,2003;Nynne et al, 2005). Phytoplasmas have adapted intimately to their host cells and thus they are regarded as an important factor for sieve element manipulation. They cannot be cultured in vitro in cell-free media. They are characterised by their lack of a cell wall, a pleiomorphic or filamentous shape, normally with a diameter less than 1 micrometer, and their very small genomes. They are minute bacteria (200–800 µm) that have no cell wall and inhabit phloem sieve elements in infected plants. These noncultivable plant pathogens belong to the class of Mollicutes (Bai et al, 2004; Garnier et al., 2001). Comparison of 16S rDNA sequences showed the MLOs to be phylogenetically close to the acholeplasma/anaeroplasm group of the Mollicutes, and the trivial name phytoplasma was adopted in 1994 to replace MLO (Garnier et al., 2001).

Phytoplasmas are pathogens of important plants, including coconut, sugarcane, sandal wood, causing a wide variety of symptoms that range from mild yellowing to death of infected plants. They are most prevalent in tropical and sub-tropical regions of the world. Phytoplasmas require a vector to be transmitted from plant to plant, and this normally takes the form of sap sucking insects such as leaf hoppers in which they are also able to replicate (Bove et al.2003).

16S rDNA sequences were determined and used in the late 1990s to classify the phytoplasmas into 20 phylogenetic clusters (Seemuller et al.,2002). They have been associated with diseases in more than 300 plant species belonging to 98 families. Plants infected by phytoplasmas exhibit an array of symptoms suggesting profound disturbances in the normal balance of plant metabolism, including yellowing, chlorosis, or bronzing of foliage, stunting (reduction of internodes and leaf size), virescence (the development of green flowers and the loss of normal pigments), phyllody (the development of floral parts into leafy structures), sterility of flowers, proliferation of secondary auxiliary buds often resulting in a witch-broom effect, proliferation of secondary roots, abnormal fruits and seeds, and abnormal elongation of internodes leading to slender shoots (Garcia et al.2004). The lifecycle of phytoplasmas includes an intracellular phase in both the vector insect and the sugar-conducting phloem of the host plant. Phloem contains highly specialized plant cells that are enucleate and totally dependent upon their neighbour cells. Phytoplasmas have adapted intimately to their host

cells, which makes them 'experts' in sieve element manipulation (Junqueira et al.2004). Thus, a study was carried for a brief analysis of the sequences using bioinformatics tools and 16s rRNA based Phylogenetic analysis of Phytoplasmas which are widely infected economically important plants.

Material and Methods:

Data Collection:

In the present study, 26 strains of Phytoplasma from National Center for Biotechnology Information (NCBI) nucleotide database were downloaded and were examined and edited with the Bioedit Sequence Alignment Editor. The Basic Local Alignment Tool (Blast) at the National Center for Biotechnology Information was used to search for similar known sequences. The sequences were aligned with Clustal X2. Phylogenetic and molecular evolutionary analysis with MEGA version 4.1. Neighbour joining consensus trees were obtained with Kimura two-parameter substitution model and bootstrap test.

Results and Discussion:

The names of the Downloaded species with their Accession Numbers have been mentioned in the **Table 1**. The lengths of DNA of all downloaded sequences with their ssDNA and dsDNA have been estimated. The average of (G+C) content and (A+T) contents were found to have **46.67%** and **53.30%** respectively using the standard BioEdit software tools. The average values of all the nucleotide bases are also estimated for G, C, A, T and the values are found as 27.65%, 19.02%, 29%, 45% and 23.87% respectively (**Tables 2 & 3**).

Table 1: Name of the phytoplasma strains with their Accession Number

| SL No: | Name of the species | Accession number |
|--------|---|-------------------------------|
| 1. | Candidatus_Phytoplasma_solani | (gi 312922521 gb HQ402915.1) |
| 2. | Stolbur-Rus_phytoplasma | (gi 308525069 gb GU004375.1) |
| 3. | Phytoplasma sp. BN-Ab175 | gi 237683092 gb F 409894.1 |
| 4. | Russia potato purple top phytoplasma Rus-PPT109 | gi 169248246 gb EU344888.1 |
| 5. | Candidatus_Phytoplasma_solani isolate 06PS085 | gi 156186196 gb EU086529.1 |

| | | |
|-----|---|-------------------------------------|
| 6. | Iranian potato purple top phytoplasma | gi 194400539 gb EU661607.1 |
| 7. | Allium cepa' phytoplasma strain OnM-2 | gi 266706187 gb GU129974.1 |
| 8. | Papaya phytoplasma TW | gi 197096951 emb AJ919994.2 |
| 9. | Mollicutes (from V.Vinifera) | gi 433908 emb X76428.1 |
| 10. | Mollicutes (from C.anuum to C.roseus) | gi 433907 emb X76427.1 |
| 11. | Grapevine stolbur phytoplasma strain GrIRAN08 | gi 257123757 gb GQ403235.1 |
| 12. | Stolbur phytoplasma | gi 13649444 gb AF248959.1 |
| 13. | Sorghum phytoplasma | gi 226935072 gb AY725232.2 |
| 14. | Conyza phytoplasma | gi 226935071 gb AY725231.2 |
| 15. | Corn-reddening phytoplasma | gi 78709995 gb DQ222972.1 |
| 16. | Iranian hemp phytoplasma strain HAY4 | gi 329047274 gb JF441273.1 |
| 17. | Iranian peach phytoplasma PP49 | gi 209168754 gb FJ204394.1 |
| 18. | Iranian hemp phytoplasma strain HAY2 | gi 329047275 gb JF441274.1 |
| 19. | Turkish potato stolbur phytoplasma | gi 298715940 gb HM485579.1 |
| 20. | Sugarcane phytoplasma | gi 37496534 emb AJ539182.1 |
| 21. | Iranian plum phytoplasma PJ15 | gi 212292623 gb FJ409624.1 |
| 22. | Nasturtium officinale phytoplasma strain WatM-2 | gi 266706189 gb GU129976.1 |
| 23. | Stolbur phytoplasma clone b | gi 52789115 gb AY739653.1 |
| 24. | Bindweed yellows phytoplasma | gi 3288105 emb Y16391.1 |
| 25. | Strawberry green petal phytoplasma | gi 5139643 emb AJ243044.1 |
| 26. | Phormium yellow leaf phytoplasma | gi 1171226 gb U43570.1 P SU43570 |

Table 2: The Phytoplasma strains with their Length of DNA and Molecular Weights in Daltons.

| SL No: | Name of the Phytoplasma Strains | Length of DNA (in bps) | Molecular weight in Daltons | |
|--------|---------------------------------|------------------------|-----------------------------|-----------|
| | | | ssDNA | dsDNA |
| 1. | Candidatus_Phytoplasma_solani | 1243 | 376264.00 | 755053.00 |
| 2. | Stolbur-Rus_phytoplasma | 1467 | 444016.00 | 891102.00 |
| 3. | Phytoplasma sp. BN-Ab175 | 1244 | 376443.00 | 755601.00 |

| | | | | |
|-----|---|------|-----------|------------|
| 4. | Russia potato purple top phytoplasma Rus-PPT109 | 1754 | 531067.00 | 1065239.00 |
| 5. | Candidatus Phytoplasma solani isolate 06PS085 | 1782 | 539487.00 | 1082174.00 |
| 6. | Iranian potato purple top phytoplasma | 1243 | 376155.00 | 754985.00 |
| 7. | Allium cepa' phytoplasma strain OnM-2 | 1524 | 461015.00 | 925575.00 |
| 8. | Papaya phytoplasma TW | 1581 | 478425.00 | 960165.00 |
| 9. | Mollicutes (from V.Vinifera) | 1501 | 454448.00 | 911746.00 |
| 10. | Mollicutes (from C.anuum to C.roseus) | 1494 | 452296.00 | 907517.00 |
| 11. | Grapevine stolbur phytoplasma strain GrIRAN08 | 1633 | 494198.00 | 991726.00 |
| 12. | Stolbur phytoplasma | 1783 | 539750.00 | 1082775.00 |
| 13. | Sorghum phytoplasma | 1509 | 456833.00 | 916661.00 |
| 14. | Conyza phytoplasma | 1509 | 456833.00 | 916661.00 |
| 15. | Corn-reddening phytoplasma | 1648 | 498928.00 | 1000836.00 |
| 16. | Iranian hemp phytoplasma strain HAY4 | 1244 | 376523.00 | 755601.00 |
| 17. | Iranian peach phytoplasma PP49 | 1243 | 376163.00 | 754951.00 |
| 18. | Iranian hemp phytoplasma strain HAY2 | 1244 | 376482.00 | 755584.00 |
| 19. | Turkish potato stolbur phytoplasma | 1243 | 376043.00 | 755036.00 |
| 20. | Sugarcane phytoplasma | 1251 | 378584.00 | 759813.00 |
| 21. | Iranian plum phytoplasma PJ15 | 1481 | 448130.00 | 899460.00 |
| 22. | Nasturtium officinale phytoplasma strain WatM-2 | 1513 | 457945.00 | 918880.00 |
| 23. | Stolbur phytoplasma clone b | 1544 | 467313.00 | 937716.00 |
| 24. | Bindweed yellows phytoplasma | 1599 | 483516.00 | 971032.00 |
| 25. | Strawberry green petal phytoplasma | 1715 | 519117.00 | 1041369.00 |
| 26. | Phormium yellow leaf phytoplasma | 1483 | 448847.00 | 900779.00 |

Table 3: A Brief Analysis of (G+C) and (A+T) Content and the Molecular weights by using The Bioedit Tools of Phytoplasma Strains

| SL No: | G+C Content (%) | A+T Content (%) | Nucleotide | | | | Molecular weight (%) | | | |
|--------|-----------------|-----------------|------------|-----|-----|-----|----------------------|-------|-------|-------|
| | | | A | C | G | T | A | C | G | T |
| 1. | 47.14 | 52.86 | 366 | 238 | 348 | 291 | 29.44 | 19.15 | 28.00 | 23.41 |
| 2. | 47.10 | 52.90 | 433 | 279 | 412 | 343 | 29.52 | 19.02 | 28.08 | 23.38 |
| 3. | 46.86 | 53.14 | 365 | 236 | 347 | 296 | 29.34 | 18.97 | 27.89 | 23.79 |
| 4. | 46.47 | 53.53 | 525 | 334 | 481 | 414 | 29.93 | 19.04 | 27.42 | 23.60 |
| 5. | 46.24 | 53.76 | 535 | 337 | 487 | 423 | 30.02 | 18.91 | 27.33 | 23.74 |
| 6. | 46.82 | 53.18 | 365 | 236 | 346 | 296 | 29.36 | 18.99 | 27.84 | 23.81 |

| | | | | | | | | | | |
|-----|-------|-------|-----|-----|-----|-----|-------|-------|-------|-------|
| 7. | 46.52 | 53.48 | 443 | 288 | 421 | 372 | 29.07 | 18.90 | 27.62 | 24.41 |
| 8. | 46.43 | 53.57 | 446 | 299 | 435 | 381 | 29.48 | 18.91 | 27.51 | 24.10 |
| 9. | 47.04 | 52.90 | 444 | 288 | 418 | 350 | 29.58 | 19.19 | 27.85 | 23.32 |
| 10. | 46.99 | 52.68 | 439 | 286 | 416 | 348 | 29.38 | 19.14 | 27.84 | 23.29 |
| 11. | 46.36 | 53.64 | 481 | 310 | 447 | 395 | 29.45 | 18.98 | 27.37 | 24.19 |
| 12. | 46.21 | 53.79 | 534 | 337 | 487 | 425 | 29.95 | 18.90 | 27.31 | 27.31 |
| 13. | 47.25 | 52.68 | 444 | 290 | 423 | 351 | 29.42 | 19.22 | 28.03 | 23.26 |
| 14. | 47.25 | 52.68 | 444 | 290 | 423 | 351 | 29.42 | 19.22 | 28.03 | 23.26 |
| 15. | 46.36 | 53.64 | 488 | 316 | 448 | 396 | 29.61 | 19.17 | 27.18 | 24.03 |
| 16. | 46.86 | 53.14 | 365 | 238 | 345 | 296 | 29.34 | 19.13 | 27.73 | 23.79 |
| 17. | 46.66 | 53.34 | 367 | 235 | 345 | 296 | 29.53 | 18.91 | 27.76 | 23.81 |
| 18. | 46.78 | 53.22 | 365 | 237 | 345 | 297 | 29.34 | 19.05 | 27.73 | 23.87 |
| 19. | 47.06 | 52.94 | 362 | 235 | 350 | 296 | 29.12 | 18.91 | 28.16 | 23.81 |
| 20. | 46.68 | 53.32 | 367 | 238 | 346 | 300 | 29.34 | 19.02 | 27.66 | 23.98 |
| 21. | 46.52 | 53.48 | 432 | 282 | 407 | 360 | 29.17 | 19.04 | 27.48 | 24.31 |
| 22. | 46.46 | 53.54 | 442 | 291 | 412 | 368 | 29.21 | 19.23 | 27.23 | 24.32 |
| 23. | 46.50 | 53.50 | 452 | 296 | 422 | 374 | 29.27 | 19.17 | 27.33 | 24.22 |
| 24. | 46.15 | 53.78 | 462 | 299 | 439 | 398 | 28.89 | 18.70 | 27.45 | 24.89 |
| 25. | 45.38 | 54.17 | 517 | 321 | 465 | 412 | 30.15 | 18.72 | 27.11 | 24.02 |
| 26. | 46.93 | 53.07 | 439 | 281 | 415 | 348 | 29.60 | 18.95 | 27.98 | 24.32 |

From the above reconstructed neighbor joining tree, it was found that phromium yellow leaf phytoplasma is the most primitive and Stolber phytoplasma is more advanced. Among the five different Iranian phytoplasma strains, the two strains like Iranian phytoplasma strains HAY2 and HAY4 shows the maximum bootstrap value of (98%) which means both the species have been originated from a single ancestor. The sequence similarity among this monophyletic group is higher in comparison with other three species of Iranian strains Iranian potato purple top, Plum phytoplasma PJ15, Peach phytoplasma

According to the 16srRNA based phylogenetic tress, it can be interpreted that the strains like Mollicutes from *C.anum* showed a different phylogenetic trait with 93% bootstrap value, thus diverged into a different taxa than other 4 strains of phytoplasma like mollicutes from *Vvincifera*, *Conyza* phytoplasma, *Sorghum* phytoplasma and *Papaya* phytoplasma (Figure 1) (Liefing,2004; Christensen et al,2004).

Conclusion:

The present study of phylogenetic relationship between various microorganisms like Phytoplasma gives us a clear idea of evolutionary relationships between various strains which are responsible for several hundred plant diseases worldwide, including many diseases of economically important plants. The use of bioinformatics tools are very helpful for generating the correct evolutionary relationship between different taxa of Phytoplasma. It is therefore, aims at to get a very authentic output for a given data with molecular and phylogenetic relationship. Thus, it will be of great interest to investigate the phylogenetic analyses of the important strains of phytoplasma for molecular studies and their mechanisms with the various host plants.

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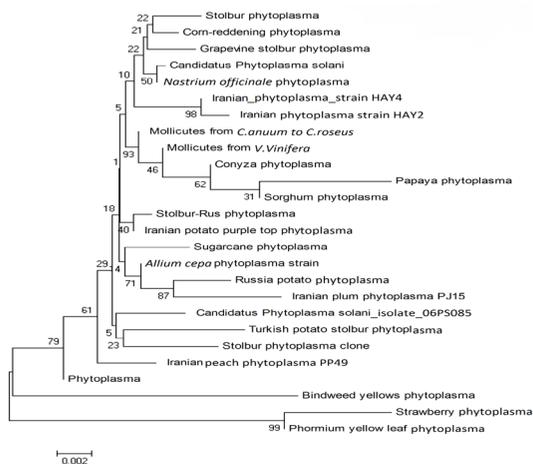


Figure 1: Neighbour-joining tree of of 16S rRNA gene sequences of 26 strains of phytoplasma obtained from gen-bank.

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