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Molecular identification of Phytoplasma associated with yellows disease of *Eucalyptus tereticornis* in Central India

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Abstract

Eucalyptus tereticornis has economic importance for industrial and Ayurvedic purposes and has been recently affected by a disease named phytoplasma. During a survey conducted in April 2025 in Madhya Pradesh, India, the leaf yellowing with little leaf symptoms was observed in *Eucalyptus tereticornis*. In SEM micrograph structural alternations were observed in the leaf tissue of a symptomatic sample in comparison with healthy leaf tissue sample of *Eucalyptus tereticornis*. For identification of the associated disease, total DNA was extracted from seven symptomatic leaf samples, along with an asymptomatic leaf sample, and subjected to PCR assay utilizing phytoplasma 16S rRNA gene specific primers. The achieved ~1.2 kb amplicons from Nested PCR, were sequenced, aligned and submitted to NCBI GenBank under the accession numbers PX442052 (EL01), PX442053 (EL02), PX909886 (EL03), PX909887 (EL04), PX909888 (EL05), PZ610277 (EL06), PZ610278 (EL07). Sequence analysis of all the identified understudy strains revealed the highest 99% nucleotide sequence identities and close phylogenetic relationships with the strains of '*Candidatus* Phytoplasma cynodontis' (16SrXIV-A subgroup). For differentiation *in silico,* RFLP analysis was carried out and showed the highest similarity to the reference strain of the '*Ca.* P. cynodontis' of the 16SrXIV-A subgroup (AJ550984), with similarity coefficients ranging from 0.88 to 0.97. To the best of our knowledge, this is the first report of '*Ca.* Phytoplasma cynodontis' group (16SrXIV) associated with leaf yellows disease in economically important *E. tereticornis* trees from India and worldwide.

Keywords:

E. tereticornis · SEM analysis · '*Ca.* Phytoplasma cynodontis' · Leaf Yellows disease · 16SrXIV group

Introduction

Eucalyptus is the fastest-growing species and is popularly known as gum tree, red iron tree, safeda or nilgiri tree and belongs to the family Myrtaceae. It is planted for fuel wood, poles, timber, biomass and essential Ayurvedic oil purposes. Moreover, it also provides ecological, socioeconomic and industrial services. Eucalyptus growing countries are: China (170 million ha), India (2.5 million ha), and Brazil (3.7 million ha) [1,2,3]. In India, major eucalyptus (mainly *E. tereticornis*) growing states are Andhra Pradesh, Bihar, Goa, Gujarat, Punjab and Haryana, Madhya Pradesh, Maharashtra, Uttar Pradesh, Tamil Nadu, Kerala, West Bengal and Karnataka, with a global 20.2% total plantation area. Trade of Eucalyptus wood in India alone has been valued at INR 480 billion [4]. Eucalyptus trees are susceptible to a variety of fungal, bacterial, and environmental diseases. One of them is phytoplasma disease; infected plants show symptoms like proliferation of axillary buds leading to witches ' broom behaviour, reduction in leaf size, shortening of internodes and general yellowing of the foliage [5]. Phytoplasmas are intracellular obligate prokaryotes which lack cell wall, have small genome and are mainly transmitted in nature by insect vector [6].

Hence, the objective of the present study was to study the surface morphology of infected tissues and to identify the suspected phytoplasma disease associated with leaf yellows disease of *E. tereticornis* trees collected from the Bhopal and Raisen districts of Madhya Pradesh, India by utilizing the phytoplasma 16S rRNA gene specific primers. Furthermore, the study was aimed to determine the phylogenetic group and subgroup affiliation of the detected phytoplasma strains, thereby providing valuable information for understanding the epidemiology of the diseases and supporting the development of effective diseases management strategies.

Materials and methods

Survey and collection of plant samples

In April 2025, a survey was conducted in Bhopal- Narmadapuram highway corridor (NH46) of Madhya Pradesh and during survey, an unusual leaf yellowing with little leaf symptoms was observed in an *E. tereticornis* tree planted under the Government Green highways policy 2015. The infected leaf samples showing symptoms related to phytoplasma presence were collected from symptomatic *E. tereticornis* tree planted at Bagsewaniya, Misrod, 11 Mile of District Bhopal and Mandideep Industrial Road of District Raisen, along with healthy leaf samples from asymptomatic *E. tereticornis* tree for DNA isolation.

Disease incidence

A total of 97 *E. tereticornis* trees planted on the roadside of NH46 Highway, covering a distance of approx. 18 km were inspected, and out of which only 13 trees showing leaf yellowing with little leaf symptoms suspected of phytoplasma disease were observed. Disease incidence was evaluated based on visual inspection by counting the number of symptomatic plants (13) in relation to the total number of plants inspected (97) using the formula: (number of infected plants / total plants inspected) x 100.

SEM analysis

Scanning electron microscopy (SEM) was applied to observe the variations in surface morphology of healthy and phytoplasma-infected phloem tissues by following the method described by Lebsky [26]. Leaf midrib samples from symptomatic and asymptomatic trees were collected and samples were prepared according to the standard SEM protocol, and the prepared specimens were examined using a JCM-6000Plus scanning electron microscope at 10 kV in Banaras Hindu University, Varanasi. Micrographs were captured at x300 and x100 magnification power.

Sample preparation: Samples from leaf midribs, fixed in 2.5% solution of glutaraldehide with sodium cacodilate buffer 0.2 M (pH 7.2-7.4) at 4°C during 24-48 hours. The specimens were washed in the same buffer (20 min) and then dehydrated in solutions of alcohols at 30, 50, 70, 96 y 100%, followed by absolute acetone or hexamethyldisilazane, for 20 min in each solution. After the dehydration, the specimens were dried and attached to SEM stubs and coated with palladium in an ion sputter, for 40 seconds.

Genomic DNA isolation and PCR amplification:

A total of seven symptomatic samples DNA was extracted from 100 mg of leaf tissues following a described procedure [7]. The phytoplasma 16S ribosomal RNA gene was amplified from isolated total DNA using P1/P6 primers [8] by direct PCR, then the resulting PCR products were diluted (1:10) and used for nested PCR using R16F2n/R16R2 primers [9], respectively. Each PCR reaction consists of nuclease-free water, 10x PCR Buffer, dNTPs (10 mM), Taq polymerase of 5 U/ul (Takara Bio Inc., Japan), forward and reverse primers (25 pmol) and DNA template (100 ng), with PCR conditions including initial denaturation at 94 ^{0}C for 5 min. and 35 cycles of denaturation at 94 ^{0}C for 1 min, annealing at 60 ^{0}C (for direct PCR) and 55 ^{0}C (for nested PCR) for 1 min. and extension at 72 ^{0}C for 90 sec. The

resulting products of direct PCR and nested PCR were electrophoresed on 1.0% agarose gel with 1.0 kb DNA ladder (GeneDireX, Inc., Taiwan) as a marker for comparison and assessing the size of the amplicons and the image was documented using a gel documentation system (BIORAD, USA). The resulting amplicons obtained from nested PCR (~1.2 kb) were purified (Hi-Media Purification Kit) and sequenced by Hi-Media, Mumbai, India.

Sequence analysis, Phylogenetic tree construction, Percent nucleotide identity and Virtual RFLP analysis

The sequence data obtained were aligned and analysed using the Basic Local Alignment Search Tool (BLAST) for nucleotide identity within and with other reported phytoplasma strains, and submitted to the National Centre for Biotechnology Information (NCBI) GenBank database.

A phylogenetic tree was subsequently constructed using MEGA software (V.12) based on aligned phytoplasma conserved gene sequences. The tree was generated by the Neighbour-Joining (N-J) method, and the stability of the tree topology was assessed with 1000 bootstrap replicates [10].

The aligned sequences were loaded into SDT (Sequence Demarcation Tool) v1.3 [11], in fasta format to construct a matrix of pairwise nucleotide identity comparisons (alignment by MUSCLE) between understudy strains and selected representative 16S rRNA reference strains of XIV group/subgroup of phytoplasma retrieved from NCBI GenBank database (Table 1).

To confirm the 16Sr group and subgroup affiliations of the identified phytoplasma strains, their 16S F2nR2 sequences were analyzed with the online tool *i*PhyClassifier [12]. Furthermore, *in silico* restriction fragment length polymorphism (RFLP) analysis was performed with 17 restriction enzymes that are routinely used for phytoplasma 16S rRNA gene RFLP analysis, including *AluI, BamHI, BfaI, BstUI, DraI, EcoRI, HaeIII, HhaI, HinfI, HpaI, HpaII, KpnI, Sau3AI, MseI, RsaI, SspI and TaqI.*

Results and Discussion

Survey and Disease Incidence

During survey conducted in April 2025, symptomatic leaf samples of *E. tereticornis* trees exhibiting leaf yellowing with little leaf symptoms, were collected and compared with

healthy leaf sample of *E. tereticornis* tree. The observation revealed that the symptomatic leaf express visible morphological alternation including leaf chlorosis, pale foliage and relatively smaller leaf size. In construct, the healthy plant leaf sample exhibited uniformly green, well- developed and elongated leaf with normal morphological characteristics (Fig. 1). The total area of approx. 18 km was surveyed of Bhopal-Narmadapuram highway and a disease incidence of less than 15% was evaluated.

SEM analysis

SEM observations revealed marked changes in the surface morphology of infected tissues in comparison with healthy tissues. Healthy sample tissues exhibited a smooth, intact, and well-organized surface structure, whereas the infected sample tissues exhibited rough, irregular, and aggregated structures, and spherical to pleomorphic bodies in clusters (indicated by arrows as 'ph') which are consistent with putative phytoplasma-associated structures (Fig. 2). The observation suggests that the phytoplasma infection is associated with significant ultrastructural alterations in host tissues. Both samples (symptomatic and asymptomatic) were viewed at x100 and x300 magnification in SEM (JCM-6000Plus) at 10 kV. However, SEM observation alone is insufficient to confirm the identity of phytoplasma; hence, molecular detection using PCR and sequencing was used for confirmation.

Phytoplasma identification and sequence analysis

The isolated DNA of all seven symptomatic samples employed positive results with direct (P1/P6) and nested PCR (R16F2n/R16R2), except the asymptomatic sample (negative) used in the same PCR cycle. A band size of ~1500bp (P1/P6) and ~1200bp (R16F2n/R16R2) was achieved. The nested PCR amplicons of ~1200 bp were sequenced, aligned and submitted to NCBI GenBank under Acc. No. PX442052 (EL01), PX442053 (EL02), PX909886 (EL03), PX909887 (EL04), PX909888 (EL05), PZ610277 (EL06), PZ610278 (EL07). The BLASTn analysis results of all query strains (EL01, EL02, EL03, EL04, EL05, EL06, EL07) showed close identities with *'Ca.* Phytoplasma cynodontis' group (16SrXIV). The query strain EL05 shared the highest above 99%, and EL04 shared the lowest below 98% sequence similarity with *'Ca.* Phytoplasma cynodontis' (KF234570) identified from Bermuda grass in India. The query strains EL01, EL02, EL03, EL07 shared the highest above 99% and EL06 shared the lowest below 98% sequence similarity with *'Triticum turgidum'* phytoplasma (MK829201, MK829202, MK829203) identified from *"Triticum turgidum var. durum*" in India.

Phylogenetic relationship and Percent nucleotide identity (colour demarcation)

A phylogenetic tree of the 16S rRNA gene sequences of identified phytoplasma from Eucalyptus trees was constructed using the neighbour-joining method in MEGA 12 software with a 1000 bootstrap value to assess branch reliability and was compared with representative 16S rRNA reference strains of XIV group/subgroup along with distinct groups/subgroups of phytoplasma retrieved from NCBI GenBank database. The resulting phylogenetic tree clearly demonstrated that the understudy strains (EL01, EL02, EL03, EL04, EL05, EL06, EL07) identified from Eucalyptus tree clustered with the 16SrXIV-A subgroup branch, representing its close affiliation with members of the 16SrXIV-A subgroup. However, the constructed tree clearly showed that the isolated query strains are not 100% identical to each other but closely related to the '*Ca.* Phytoplasma cynodontis'; 16SrXIV-A subgroup. The query strains EL01 (PX442052), EL02 (PX442053), and EL04 (PX909887) representing close affiliation with reference strains of BGWL Acc. No PX442054, PX442055 of the 16SrXIV-A subgroup, whereas EL05 (PX909888), EL06 (PZ610277) form a single, well-supported clade with each other and the EL03 (PX909886), EL07 (PZ610278) clustered separate branches under the 16SrXIV-A subgroup. Furthermore, the undersudy strains (EL01, EL02, EL03, EL04, EL05, EL06, EL07) formed a distinct lineage from other '*Ca.* phytoplasma species' groups and subgroups included in the phylogenetic analysis (Fig. 3).

Percent nucleotide identity of selected 16SrRNA strains of XIV-A subgroups shared the highest above 99% sequence similarity with EL07 strain. The EL01, EL02, EL03 and EL05 strains shared sequence similarity above 98% with selected 16SrRNA strains of XIV-A subgroups. The lowest below 97% sequence similarity was represented by EL06 strain with strains of XIV-A subgroups. The colour demarcation also revealed that the understudy strains are not 100% identical to each other, as representing variation in their colour matrix with each other (Fig. 4).

Virtual RFLP by using the *i*PhyClassifier tool

In silico RFLP analysis was performed using *i*PhyClassifier with a set of 17 restriction endonuclease enzymes. The virtual RFLP patterns of Query strains EL01, EL02, EL03, EL04, EL05, EL06, and EL07 showed the highest similarity with the reference pattern of the 16SrXIV group of subgroup A (AJ550984), with a similarity coefficient factor ranging 0.97, 0.90, 0.88, 0.71, 0.91, 0.88 and 0.97. This representation of understudy strains EL02, EL03,

EL04, EL05, and EL06 with less than 0.97 coefficient factor indicating that these strains may represent sequence variations within the 16SrXIV-A subgroup, with all previously recognized strains in this group/subgroup with difference in their restriction profiles (Fig. 5 & Table 2).

Discussion

Phytoplasma associated with leaf yellows disease of *Eucalyptus tereticornis* was confirmed by PCR assay and SEM analysis also revealed the marked changes in the surface morphology of infected tissues in comparison with healthy tissues. So, mainly based on combined results of 16S rRNA gene sequence comparison, phylogenetic analysis, and *in silico* RFLP patterns, the identified phytoplasma strains are the member of '*Candidatus* Phytoplasma cynodontis' belonging to the 16SrXIV group, most closely aligned with the 16SrXIV-A subgroup.

In the literature, phytoplasmas infecting diverse species of Eucalyptus tree are reported worldwide with various symptoms such as the presence of small leaves, yellowing, and the growth of broom-like structures from axillary buds but no reports are identified from *Eucalyptus tereticornis* tree. In early times, Eucalyptus little-leaf disease was initially observed in India in 1971 [13,14], and then reported in China [15], Sudan [16], and Syria [17], by examining the phytoplasma presence in diseased eucalyptus tree through the use of electron and light microscope observations. As per updated literature total four phytoplasma group (16SrI, 16SrII, 16SrV & 16SrXII) were reported affecting eucalyptus trees worldwide; 16SrI-B and 16SrI-C subgroup phytoplasmas were identified in Italy; 16SrI-B phytoplasma infecting *E. camaldulensis* trees identified in Iran; 16SrII-D subgroup phytoplasma infecting *E. camaldulensis* with leaf roll symptoms identified in Iran [18], and 16SrII-C subgroup infecting *Eucalyptus urophylla* trees with witches' broom and little leaf symptoms identified in Brazil [19]; 16SrII- D subgroup with *E. Camaldulensis* little leaf and leaf yellowing symptoms identified in Haryana, India [20]; 16SrV group infecting Eucalyptus tree with little leaf disease identified in Italy [21]; 16SrXII-A subgroup phytoplasma infecting *Eucalyptus camaldulensis* with witches' broom, little leaf and general yellowing of the foliage identified in Iran [5,22]. Although four different phytoplasma groups (16SrI, 16SrII, 16SrV and 16SrXII) identified earlier in different species of eucalyptus trees representing identical symptoms as described in the present study with *Eucalyptus tereticornis*.

Phytoplasmas belonging to the 16SrXIV group have previously been reported infecting monocot and dicot hosts, including grasses such as Bermuda grass and cultivated crops like linseed in India. Recently, *'Ca.* Phytoplasma cynodontis' group (16SrXIV) identified from India in Chilli (*Capsicum annuum*), Bermuda grass and durum and bread wheat genotypes [23,24,25].

However, presently no such reports from India and worldwide were identified related to the association of *'Ca.* Phytoplasma cynodontis' group (16SrXIV) in *Eucalyptus tereticornis* tree exhibiting leaf yellowing with little leaf symptoms. Hence, this is the first report from India and worldwide of the association of *'Ca.* Phytoplasma cynodontis' group (16SrXIV); 16SrXIV-A subgroup in *Eucalyptus tereticornis*, a economic important tree playing a key role in industrial fuel woods, poles, timber, biomass, and essential oil purposes in Ayurveda treatment.

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Author Contributions

S.M. designed the study, drafted the experiments and prepared the manuscript; A.S. wrote, reviewed, and edited the manuscript; R.P.K. provided supervision; S.S. involved in investigation; A.K. provided resources; S.K. supervised, revised and approved the manuscript for submission; all authors reviewed and approved the submission.

Declarations

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Ethics declaration

Ethical approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the author(s). The leaf samples of *Eucalyptus tereticornis* were collected from previously established trees planted along the roadside of Bhopal- Narmadapuram highway (NH46), Madhya Pradesh, India. The sampling procedure was non-destructive, and no trees were cut, uprooted or damaged during the collection process. No whole plants or seeds were collected. The trees were part of an existing roadside plantation and were not collected from a protected forest or wildlife area. No whole plants or endangered species were involved and no specific permits or licences were required. The collection and use of plant material were undertaken in accordance with applicable local and national guidelines with no need for further affirmation. Not applicable.

Competing interest

The author(s) declare that they have no competing interests

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figure Legends:

Figure 1. (a) Healthy *E. tereticornis* tree; (c) close view of collected symptomatic sample of *E. tereticornis* showing leaf yellowing and little leaf symptoms and compared with healthy sample collected; (b, d, e & f) field view of symptomatic *E. tereticornis* tree showing leaf yellowing and little leaf symptoms.

Figure 2. SEM micrograph of phytoplasmas (ph) distribution in tissue of eucalyptus leaf midribs samples (*E. tereticornis*). (a & b) Healthy sample image; (c & d) Infected sample image. Arrows indicate phytoplasma associated bodies (ph).

Figure 3. Phylogenetic tree of query strains constructed by neighbour-joining method with bootstrap value of 1,000 replicates using 47 taxa of other selected phytoplasma strains on the basis of partial 16Sr RNA gene sequences with their accession numbers, showing the relationships with the query strains. Evolutionary analyses were conducted in MEGA12 software. The newly identified phytoplasma (query strains) is highlighted by blue colour with their Acc. No. in 16SrXIV-A subgroup branch.

Figure 4. Colour-coded pairwise identity matrix generated from 23 'Ca. Phytoplasma' isolates of 16SrXIV groups/subgroup sequences (see details in Table 1). Each coloured cell represents a percentage identity score between two sequences (one indicated horizontally to the left and the other vertically at the bottom). A coloured key indicates the correspondence between pair wise identities and the colours displayed in the matrix.

Figure 5. Virtual RFLP patterns of query strains from *in silico* digestion of 16S rDNA F2nR2 fragments by 17 restriction enzymes. The 16SrXIV-A subgroup reference strain

(AJ550984) (a) used in the *iPhy*Classifier system showing maximum similarity with under study query strains: EL01, EL02, EL03, EL04, EL05, EL06, EL07 (b, c, d, e, f, g & h) associated with the *E. tereticornis* tree.

Table 1 Distinct predefined 16S rRNA Phytoplasma XIV groups retrieved from NCBI gene Bank used for comparisons with under study strains of *E. tereticornis* in phylogenetic relationships and Percent nucleotide identity (Colour demarcation)

GenBank Accession number	***'Candidatus Phytoplasma'* strains**	**Country /Region**	**Host**	**16SrGroup /Sub group**	**Nucleotide similarity with under study strains in NCBI**
AB741630	'*Candidatus* Phytoplasma cynodontis'	Myanmar	"*Cynodon dactylon* (bermuda grass)"	XIV-A	99%
LT558777	'*Candidatus* Phytoplasma cynodontis'	India	"*Cynodon dactylon* (bermuda grass)"	XIV-A	97-99%
EU294011	Malaysia Bermuda grass white leaf phytoplasma	Malaysia	"*Cynodon dactylon* (bermuda grass)"	XIV-A	97-98%
MK829203	*'Triticum turgidum'* phytoplasma	India	"*Triticum turgidum* var. durum"	XIV-A	97-98%
KF234570	'*Candidatus* Phytoplasma cynodontis'	India	"*Cynodon dactylon* (bermuda grass)"	XIV-A	97%
AJ550984	Bermuda grass white leaf phytoplasma	Italy	"*Cynodon dactylon* (bermuda grass)"	XIV-A	97%
MK829206	*'Triticum turgidum'* phytoplasma	India	"*Triticum turgidum* var. durum"	XIV-A	98-99%
MK829201	*'Triticum turgidum'* phytoplasma	India	"*Triticum turgidum* var. durum"	XIV-A	98-99%
MT862162	*Brachiaria* grass white leaf phytoplasma	Sri Lanka	Signal grass	XIV-A	99%

PX442054	‘*Candidatus* Phytoplasma cynodontis’	India	"*Cynodon dactylon* (bermuda grass)"	XIV-A	98%
PX442055	‘*Candidatus* Phytoplasma cynodontis’	India	"*Cynodon dactylon* (bermuda grass)"	XIV-A	98%
EU999999	‘*Candidatus* Phytoplasma cynodontis’	China	"*Cynodon dactylon* (bermuda grass)"	XIV-A	97%
KX641016	*'Chrysanthemum morifolium'* little leaf and yellowing phytoplasma	India	"*Chrysanthemum morifolium"*	XIV-A	98%
MK829202	*'Triticum turgidum'* phytoplasma	India	"*Triticum turgidum* var. durum"	XIV-A	97-99%
MK041924	*‘Candidatus* Phytoplasma cynodontis’	India	"*Citrus maxima*"	XIV-A	97%
KJ873877	*'Urochloa distachya'* white leaf phytoplasma	India	"*Urochloa distachya*"	XIV-A	97%

Table 2 The representation of understudy strains with difference in their restriction profiles in comparison with the reference strain of Acc. No. AJ550984; 16SrXIV-A subgroup, analyzed in *i*phyclassifier with 17 restriction enzymes.

Under study strains	**Key enzymes representing difference in their restriction profiles with reference strain (AJ550984);16SrXIV-A subgroup**
EL01	*BstUI, MseI*
EL02	*AluI, BstUI, MseI*
EL03	*AluI, BstUI, HaeIII, Sau3AI*
EL04	*AluI, BfaI, BstUI, DraI, HpaII, Sau3AI, MseI, TaqI*
EL05	*AluI, HpaII, TaqI*
EL06	*AluI, HaeIII, HpaII, TaqI*
EL07	*AluI, Sau3AI, MseI*

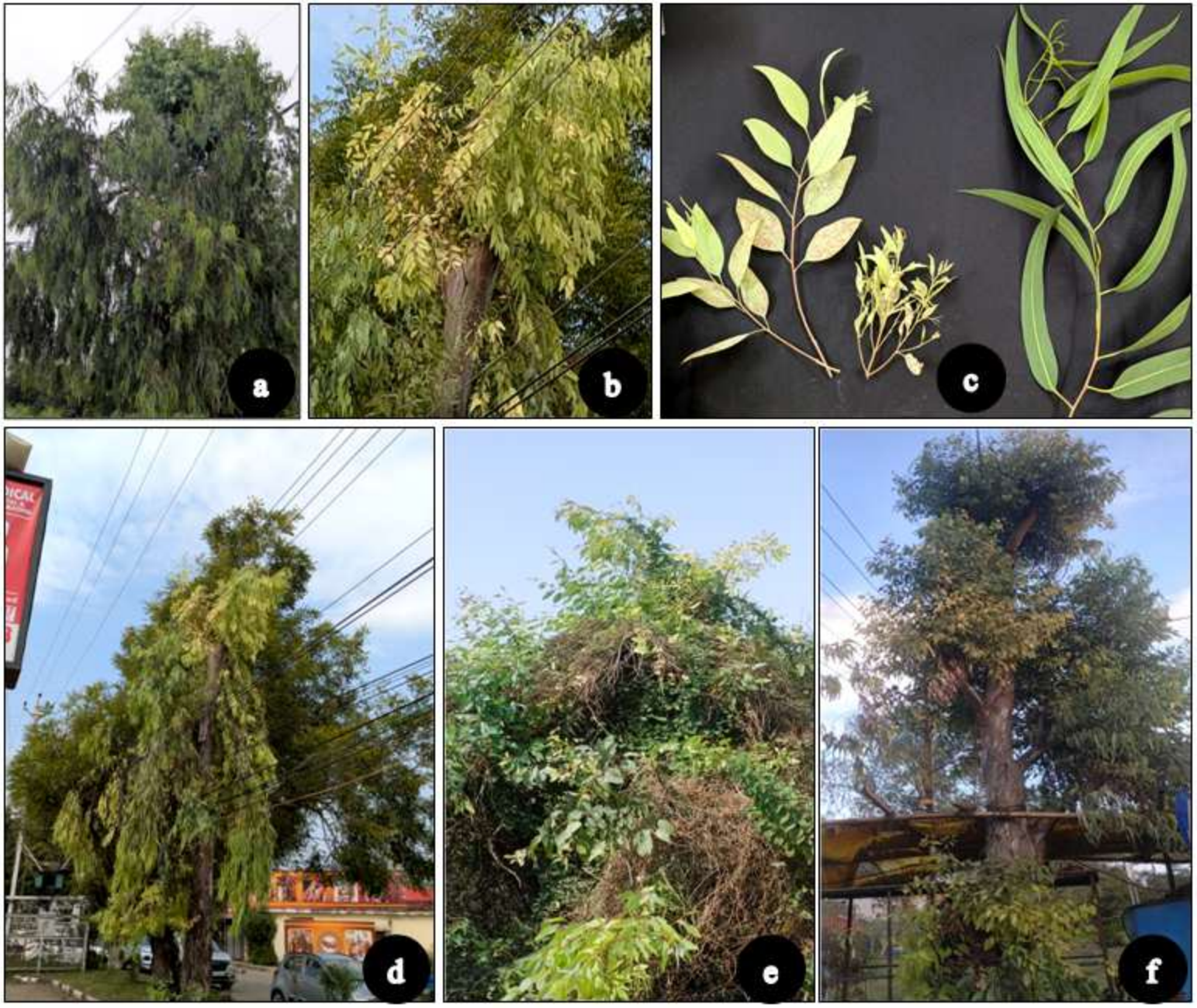


Fig. 1

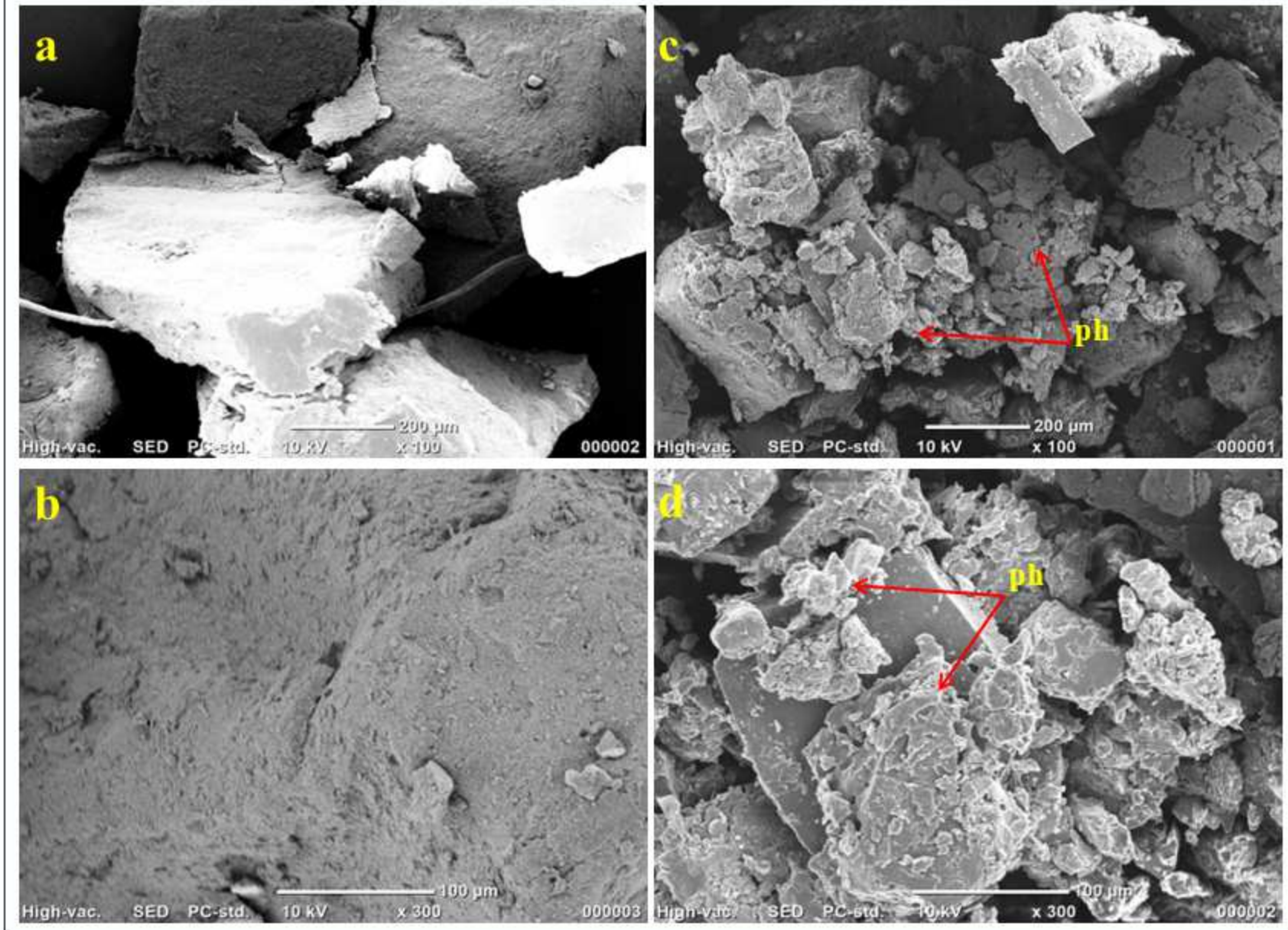

a
200 µm
High-vac. SED PC-std. 10 kV x 100 000002
c
ph
200 µm
High-vac. SED PC-std. 10 kV x 100 000001
b
100 µm
High-vac. SED PC-std. 10 kV x 300 000003
d
ph
100 µm
High-vac. SED PC-std. 10 kV x 300 000002

Fig. 2

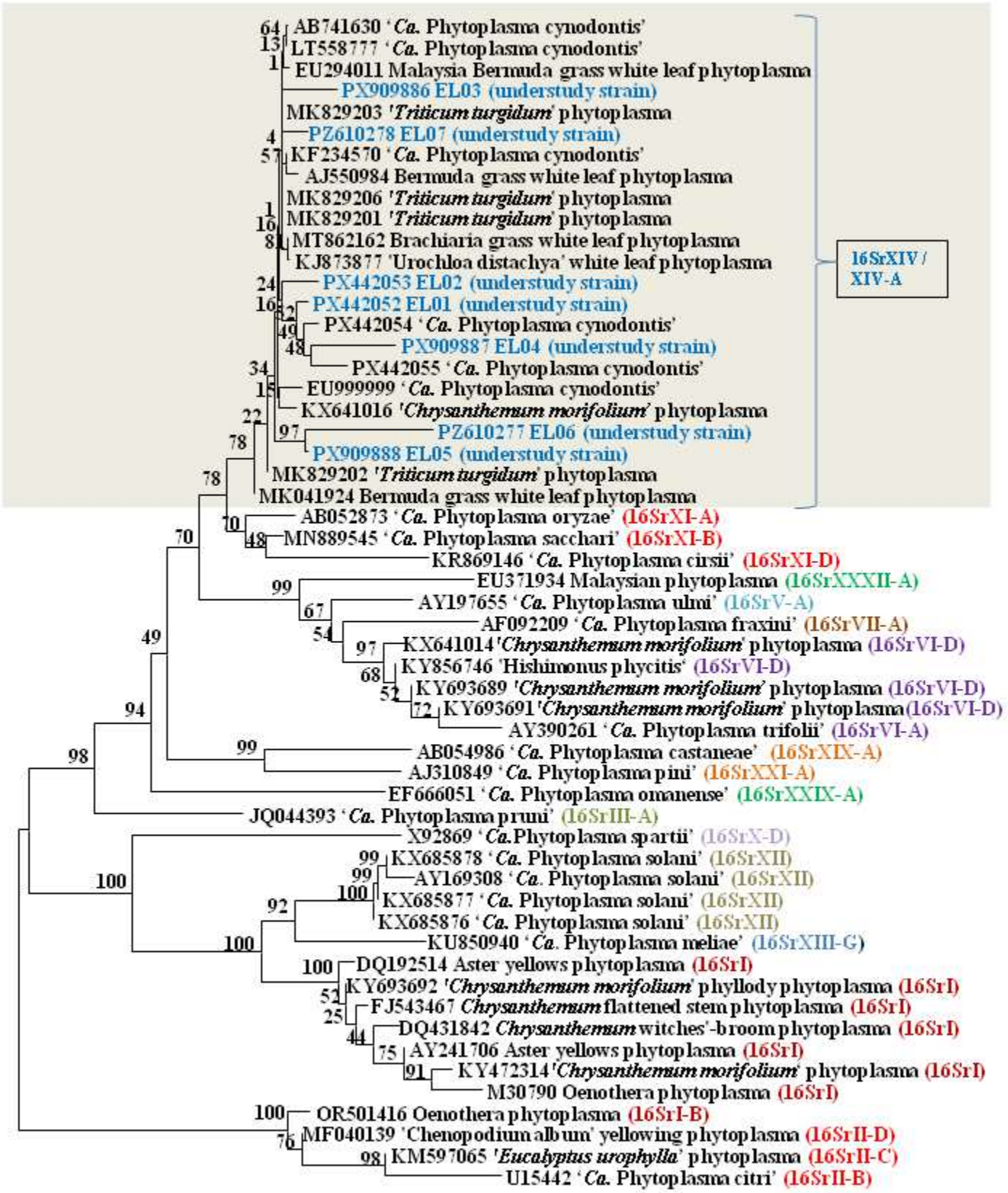

AB741630 'Ca. Phytoplasma cynodontis'
LT558777 'Ca. Phytoplasma cynodontis'
EU294011 Malaysia Bermuda grass white leaf phytoplasma
PX909886 EL03 (understudy strain)
MK829203 'Triticum turgidum' phytoplasma
PZ610278 EL07 (understudy strain)
KF234570 'Ca. Phytoplasma cynodontis'
AJ550984 Bermuda grass white leaf phytoplasma
MK829206 'Triticum turgidum' phytoplasma
MK829201 'Triticum turgidum' phytoplasma
MT862162 Brachiaria grass white leaf phytoplasma
KJ873877 'Urochloa distachya' white leaf phytoplasma
PX442053 EL02 (understudy strain)
PX442052 EL01 (understudy strain)
PX442054 'Ca. Phytoplasma cynodontis'
PX909887 EL04 (understudy strain)
PX442055 'Ca. Phytoplasma cynodontis'
EU999999 'Ca. Phytoplasma cynodontis'
KX641016 'Chrysanthemum morifolium' phytoplasma
PZ610277 EL06 (understudy strain)
PX909888 EL05 (understudy strain)
MK829202 'Triticum turgidum' phytoplasma
MK041924 Bermuda grass white leaf phytoplasma
16SrXIV / XIV-A
AB052873 'Ca. Phytoplasma oryzae' (16SrXI-A)
MN889545 'Ca. Phytoplasma sacchari' (16SrXI-B)
KR869146 'Ca. Phytoplasma cirsii' (16SrXI-D)
EU371934 Malaysian phytoplasma (16SrXXXII-A)
AY197655 'Ca. Phytoplasma ulmi' (16SrV-A)
AF092209 'Ca. Phytoplasma fraxini' (16SrVII-A)
KX641014 'Chrysanthemum morifolium' phytoplasma (16SrVI-D)
KY856746 'Hishimonus phycitis' (16SrVI-D)
KY693689 'Chrysanthemum morifolium' phytoplasma (16SrVI-D)
KY693691 'Chrysanthemum morifolium' phytoplasma (16SrVI-D)
AY390261 'Ca. Phytoplasma trifolii' (16SrVI-A)
AB054986 'Ca. Phytoplasma castaneae' (16SrXIX-A)
AJ310849 'Ca. Phytoplasma pini' (16SrXXI-A)
EF666051 'Ca. Phytoplasma omanense' (16SrXXIX-A)
JQ044393 'Ca. Phytoplasma pruni' (16SrIII-A)
X92869 'Ca. Phytoplasma spartii' (16SrX-D)
KX685878 'Ca. Phytoplasma solani' (16SrXII)
AY169308 'Ca. Phytoplasma solani' (16SrXII)
KX685877 'Ca. Phytoplasma solani' (16SrXII)
KX685876 'Ca. Phytoplasma solani' (16SrXII)
KU850940 'Ca. Phytoplasma meliae' (16SrXIII-G)
DQ192514 Aster yellows phytoplasma (16SrI)
KY693692 'Chrysanthemum morifolium' phyllody phytoplasma (16SrI)
FJ543467 Chrysanthemum flattened stem phytoplasma (16SrI)
DQ431842 Chrysanthemum witches'-broom phytoplasma (16SrI)
AY241706 Aster yellows phytoplasma (16SrI)
KY472314 'Chrysanthemum morifolium' phytoplasma (16SrI)
M30790 Oenothera phytoplasma (16SrI)
OR501416 Oenothera phytoplasma (16SrI-B)
MF040139 'Chenopodium album' yellowing phytoplasma (16SrII-D)
KM597065 'Eucalyptus urophylla' phytoplasma (16SrII-C)
U15442 'Ca. Phytoplasma citri' (16SrII-B)
0.02

Fig. 3

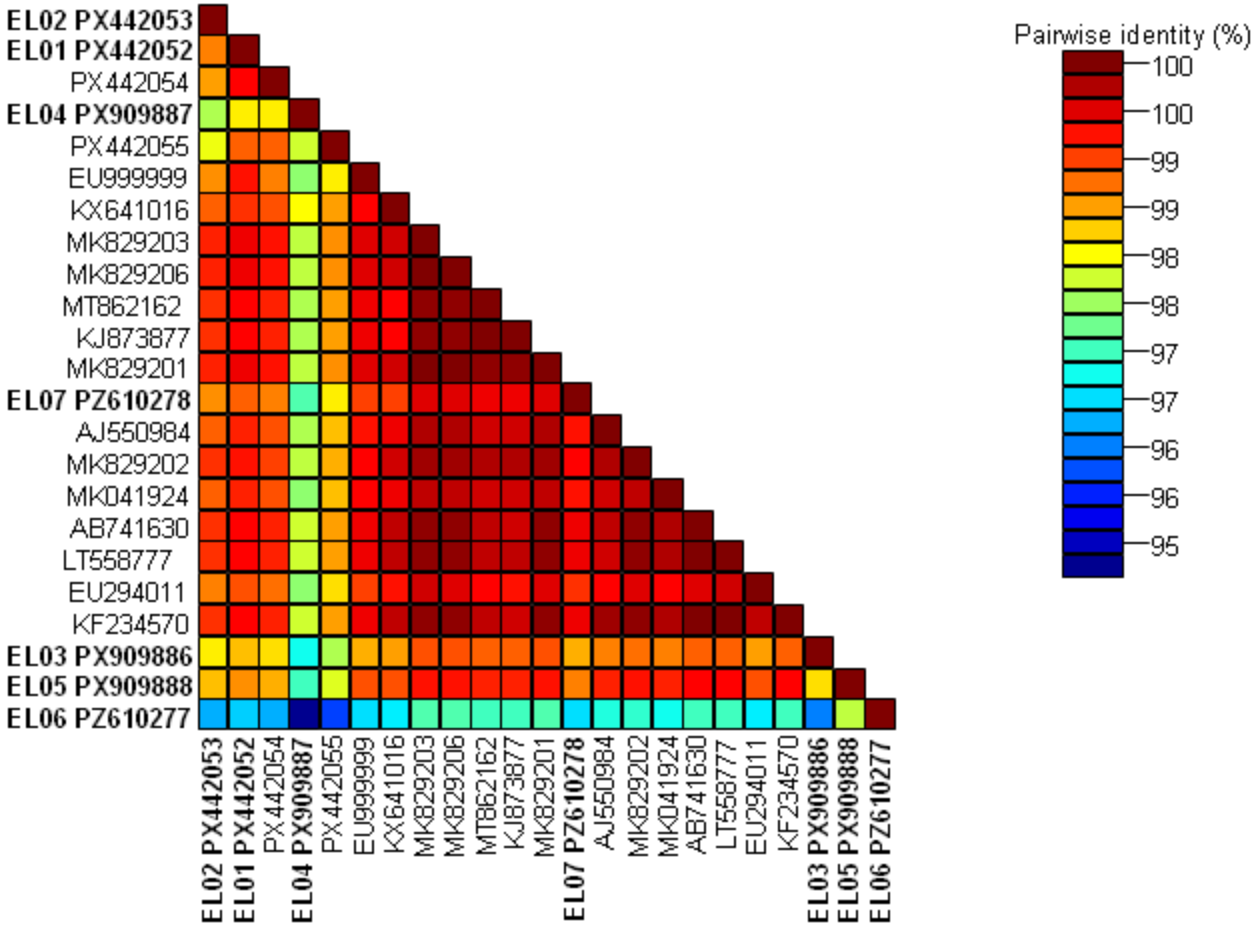
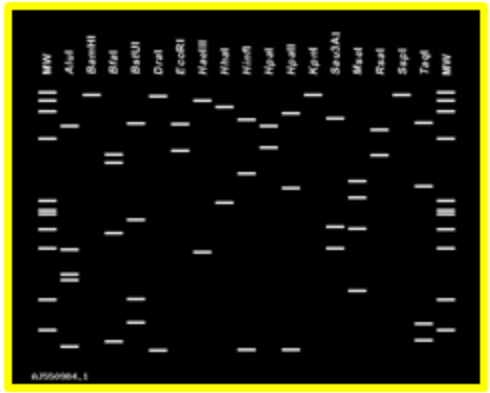
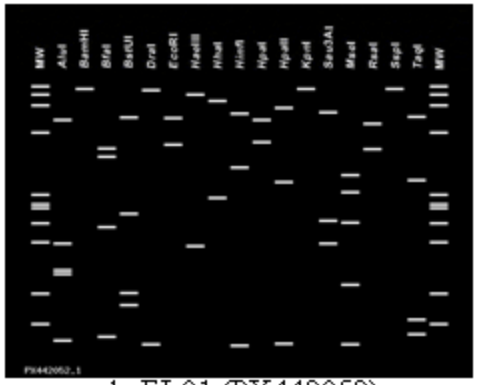

EL02 PX442053
EL01 PX442052
PX442054
EL04 PX909887
PX442055
EU999999
KX641016
MK829203
MK829206
MT862162
KJ873877
MK829201
EL07 PZ610278
AJ550984
MK829202
MK041924
AB741630
LT558777
EU294011
KF234570
EL03 PX909886
EL05 PX909888
EL06 PZ610277
Pairwise identity (%)
100
100
99
99
98
98
97
97
96
96
95

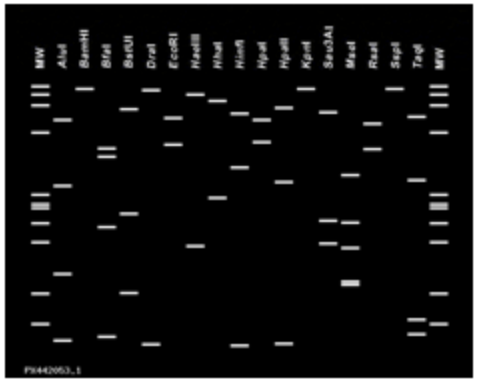
Fig. 4



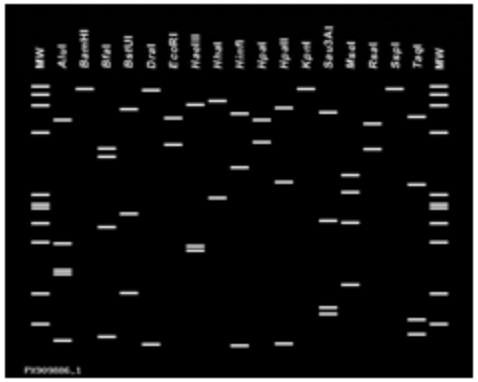
a. XIV-A subgroup (Reference strain)



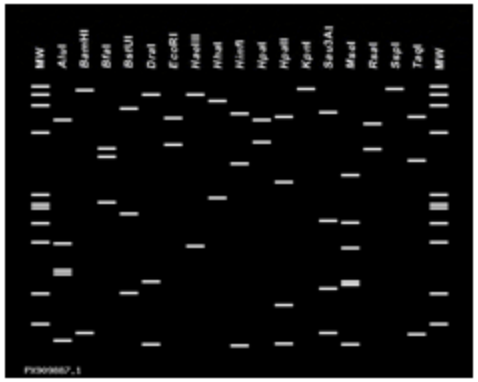
b. EL01 (PX442052)



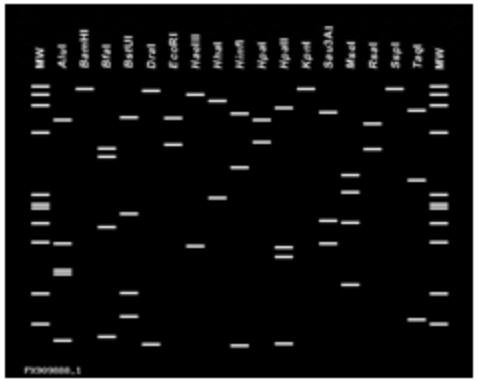
c. EL02 (PX442053)



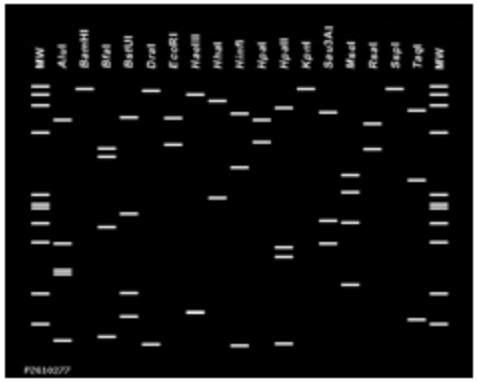
d. EL03 (PX909886)



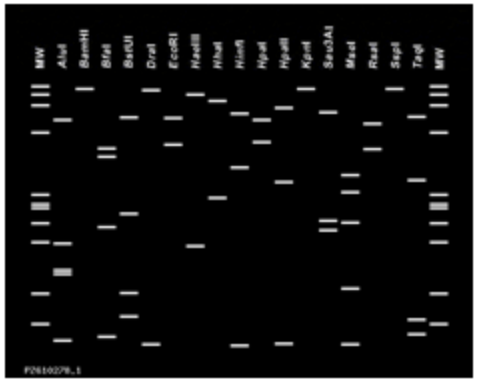
e. EL04 (PX909887)



f. EL05 (PX909888)



g. EL06 (PZ610277)



h. EL07 (PZ610278)

Fig. 5