

Govind Pratap Rao · Assunta Bertaccini  
Nicola Fiore · Lia W. Liefiting *Editors*

# Phytoplasmas: Plant Pathogenic Bacteria - I

Characterisation and Epidemiology of  
Phytoplasma - Associated Diseases

 Springer

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Characterisation and Epidemiology  
of Phytoplasma - Associated Diseases

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*Editors*

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## About the Editors



**Govind Pratap Rao** is working as a Principal Scientist (Plant Pathology) at Indian Agricultural Research Institute, New Delhi. He did his M.Sc (Botany) and Ph.D. in Plant Virology from Gorakhpur University. He did Post Doc at University of Urbana-Champaign, Illinois, USA, with Prof. R.E. Ford on Characterization of Sugarcane mosaic and maize dwarf mosaic viruses in 1994. Dr. Rao has 30 years of research experience on plant pathology especially on plant virology and phytoplasmas. He did significant contributions in characterization of viruses infecting cucurbits, sugarcane, maize and sorghum. He has published over 130 research publications and authored and edited nearly 17 books. He has also guided 3 M.Sc. and 12 Ph.D. students on different aspects of plant pathology. He has worked in different capacities as Scientific Officer, Sr. Scientific Officer (Plant Pathology), Head, Div. Plant Pathology and Officer-in-Charge at Research Stations of UP Council of Sugarcane Research centres at Seorahi, Gorakhpur and Shahjahanpur from 1987 to 2010. He has been awarded several prestigious awards. The most important ones are: National Biotechnology Associateship Award (1991–1992), DBT, Govt. of India; Young Scientist Award (1994–1995) from DST, Govt. of India; Overseas BOYSCAST Award (1996) from DST, Govt. of India; President Award, Society for General Microbiology, UK, 1998; Best U.P. Agriculture Scientist Award (UPCAR), Govt. of Uttar Pradesh in 2002; Vigyan Ratna Award by CST, Govt. of UP for the year 2003–2004; Jin Xiu Qiu Award in 2006 by People's Govt. of Guangxi Province, Nanning, China;

Global Award of Excellence, IS 2008, Al-Ahrish, Egypt; Dr. Ram Badan Singh Vishisht Krishi Vaigyanik Puraskar – 2014 by UPCAR, Lucknow, India and Leadership Excellence Award in Sugarcane Crop Protection by Thailand Society of Sugar Cane Technologists, Bangkok.

Dr. Rao is Editor-in-Chief of *Sugar Tech*, an international journal on sugar crops and related industries, and *Phytopathogenic Mollicutes*, an international journal on phloem limited microorganisms. Dr. Rao is also Secretary General of Indian Virological Society, New Delhi, and member of several prestigious scientific societies and organizations like, APS; ASM; ISSCT; IPWG; SSRP and IPS. Dr. Rao has visited over 30 countries as visiting scientists, for invited talk, post doc fellow, research training, panel discussion and for attending workshop and conferences. At present Dr. Rao is working on characterization, epidemiology and management of virus infected cereal crops, millets and maize and phytoplasmas infecting important agricultural and horticultural crops in India.



**Assunta Bertaccini** is a Plant Pathology Professor at the University of Bologna, invited speaker at many national and international meetings and seminars, member of scientific committees of several international meetings on biotechnology and virology/phytoplasmaology, and referee for numerous scientific international journals. Among others, she was awarded with the Emmy Klienenberger-Nobel award for distinguished research in mycoplasmaology. She is responsible for the phytobacteriology laboratories where she is leading a team mainly working on several aspects of plant bacteriology with emphasis on the plant diseases associated with phytoplasmas. Recently in her laboratory, phytoplasma cultivation in complex media was eventually achieved. She mentored a number of Ph.D. students, Plant Pathology specialization students, and undergraduate and master thesis students. She is author or coauthor of more than 800 publications, books and book chapters, Editor-in-Chief of *Phytopathogenic Mollicutes*, Senior Editor of *Phytopathologia Mediterranea*, and since 2007, she founded and is leading the International Phytoplasmaologist Working Group (IPWG) (<http://www.ipwgnet.org/>).





**Nicola Fiore** is a Plant Pathology Associate Professor at the University of Chile, Faculty of Agronomical Sciences, Department of Plant Health, Santiago, Chile. He is a member of several phytopathological societies. He is an invited speaker at many national and international meetings and seminars, member of scientific committees of several international meetings on virology/phytoplasmology, and referee for several scientific international journals. Award received: best poster in 22nd ICFV, Rome, 3–8 June, 2012. He is responsible for the Phytovirology Laboratory focusing on the diagnosis, epidemiology, and control of plant diseases caused by viruses, viroids, and bacteria (including phytoplasmas), applying biological approaches and molecular tools. He has elaborated and participated as a researcher or director in 20 research projects. He has mentored several students: 3 Ph.D., 6 Master's, and 6 undergraduate theses. He maintains constant professional contact with wine, table grape, and fruit producers, advising them on the prevention of diseases caused by viruses, viroids, and bacteria.



**Lia W. Liefing** is a Principal Scientist in the Virology team at the Plant Health and Environment Laboratory at the Ministry for Primary Industries (MPI) in New Zealand. The overall role of the team involves diagnosing virus and virus-like diseases including phytoplasmas and liberibacters of a wide range of host plants as well as providing technical advice on these diseases. Prior to her role at MPI, Lia completed a Ph.D. and two post-doctoral positions on phytoplasmas. The first post-doc at the University of California at Davis, USA, involved sequencing the genome of Western-X disease phytoplasma. On return to New Zealand, Lia's second post-doc was to sequence the genome of '*Candidatus Phytoplasma australiense*'. Both phytoplasma genome sequencing projects were performed by Sanger sequencing prior to the advent of next-generation sequencing. Lia was lead author on the IPPC diagnostic protocols for phytoplasmas and '*Candidatus Liberibacter solanacearum*' and is an Editor for *Plant Pathology*.

# Chapter 1

## Phytoplasmas: An Update



Assunta Bertaccini and Ing-Ming Lee

**Abstract** A summary of the research carried out on phytoplasma-associated diseases 50 years after their discovery is presented. The great majority of this research was devoted to classification and differentiation of these prokaryotes by molecular and bioinformatic tools applied to specific phytoplasma genes. The availability of a robust classification system has greatly facilitated phytoplasma identification leading to an increased knowledge of plant diseases worldwide. Phytoplasma biology study still needs to be improved to allow better management solutions to reduce the impact of these diseases in both agricultural and natural environments.

**Keywords** Taxonomy · Biology · Insect vector · Plant disease · Epidemiology

### 1.1 Introduction

Many yellows-type diseases, including aster yellows and paulownia witches' broom, were believed to be caused by viruses until 1967 when a group of Japanese scientists observed microorganisms resembling animal mycoplasmas in the phloem sieve tube elements of diseased plants by electron microscopy (Doi et al. 1967). These pleomorphic cell wall lacking bacteria were then named mycoplasma-like organisms (MLOs) (Fig. 1.1). They possess a unique lifestyle that allows them to live across plant and insect kingdoms. In the following decades, their detection was primarily based on electron microscopy images of diseased phloem tissue and biological properties, such as unique disease symptoms, specific insect vector, and plant host range. In subsequent years, the development of serological and molecular

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**Fig. 1.1** On the right electron microscopy picture of cross section of sieve tubes with phytoplasmas (X 6,000), and on the left symptoms associated with phytoplasma presence in aster (*Callistephus chinensis*)

tools such as monoclonal antibodies and cloned DNA probes greatly improved their detection.

DNA-specific amplification and sequencing provided evidence that MLOs constitute a large monophyletic group within the class *Mollicutes*, and the trivial name of “phytoplasma” was adopted followed by designation of the ‘*Candidatus* Phytoplasma’ genus (IRPCM 2004). Phytoplasmas have variable sizes and shapes (Fig. 1.1) and survive and multiply in the isotonic environments provided by plant phloem and insect hemolymph. The full genome sequence has been completed for two strains of aster yellows (‘*Candidatus* Phytoplasma asteris’), two strains of ‘*Ca. P. australiense*’, and a strain of ‘*Ca. P. mali*’ (Oshima et al. 2004; Bai et al. 2006; Kube et al. 2008; Tran-Nguyen et al. 2008; Andersen et al. 2013). A number of draft genome sequences are also reported (Saccardo et al. 2012; Mitrović et al. 2014; Zamorano and Fiore 2016). Phytoplasmas possess one of the smallest genomes among living organisms (Marcone et al. 1999) yet code complex metabolic pathways that allow them to interact with both their plant and insect hosts (Hogenhout et al. 2008). Phytoplasmas are quite often associated with severe and rapidly spreading plant diseases, they are also able to increase the metabolic activity of their hosts, modify insect fitness, increase plant shoot production, and change flower shape and color (Bertaccini et al. 2014); in other cases they are associated with severe decline and death of the infected plants. It is however not uncommon to detect phytoplasmas in asymptomatic plants, and this leaves open questions related to their pathogenicity and biology as relevant characteristics that must be provided together with phytoplasma identification when describing a new phytoplasma-associated disease.

## 1.2 Biological and Molecular Basis for Classification

Fifty years after their discovery, the role of phytoplasmas as plant pathogens is still only based on indirect biological proof, such as electron microscopy observation, specific DNA amplification, and symptom elimination after tetracycline treatments (Ishii et al. 1967). Insect and dodder transmission are the main tools available to confirm phytoplasma association with plant disease. Plants infected by phytoplasmas very often exhibit symptoms indicative of profound unbalance of growth regulators. Symptoms include virescence and phyllody of flowers, sterility, loss of apical dominance generating the proliferation of axillary buds with witches' broom formation (Fig. 1.1), abnormal internode elongation, and generalized stunting. The characteristic symptomatology is very useful for preliminary indication of the possible phytoplasma involvement in a disease. Some phytoplasmas also confer desirable features such as for poinsettia, for which the presence of a specific strain allowed it to be grown as an ornamental pot plant (Bertaccini et al. 1996; Lee et al. 1997).

Serological diagnostic techniques for detection of phytoplasmas began to emerge in the 1980s when polyclonal and monoclonal antisera were produced and tested (Lee et al. 1993b; Chen et al. 1993, 1994; Saeed et al. 1994) in plant tissues as well as in leafhopper vectors or potential vectors using immunofluorescence (Lherminier et al. 1990) and immunosorbent electron microscopy (Sinha 1979; Sinha and Benhamou 1983), dot blot, or ELISA (Boudon-Padieu et al. 1989). In other approaches, tissue blotting with direct or indirect antigen detection has been used for specific phytoplasma detection (Lin and Chen 1985). In more recent years, antibodies have been prepared to partial sequences of the major immunodominant proteins of some phytoplasmas (Berg et al. 1999; Blomquist et al. 2001; Hong et al. 2001; Mergenthaler et al. 2001; Kakizawa et al. 2001; Barbara et al. 2002; Wei et al. 2004; Arashida et al. 2008; Siampour et al. 2012). Starting from the 1990s, the application of molecular probes (Kirkpatrick et al. 1987; Lee and Davis 1988; Lee et al. 1992; Bertaccini et al. 1993), PCR (Ahrens and Seemüller 1992; Namba et al. 1993; Lee et al. 1993a; Schneider et al. 1993), and nested-PCR (Lee et al. 1994, 1995) together with restriction fragment length polymorphism (RFLP) analyses or sequencing allowed the broad detection of phytoplasmas (Lee et al. 1998a). The introduction of quantitative PCR assays (qPCR) has shown these assays to be sensitive with reduced risk of contamination making this technique a reliable alternative to nested PCR assays in routine testing (Bianco et al. 2004; Torres et al. 2005; Crosslin et al. 2006; Angelini et al. 2007; Baric et al. 2006; Hren et al. 2007; Hodgetts et al. 2009; Aldaghi et al. 2009; Berger et al. 2009; Pelletier et al. 2009; Nejat et al. 2010; Manimekalai et al. 2011; Monti et al. 2013; Córdova et al. 2014; Ikten et al. 2016; Satta et al. 2017; Linck et al. 2017). Microarray (Nicolaisen and Bertaccini 2007; Lenz et al. 2015), deep amplicon sequencing (Nicolaisen et al. 2011), and LAMP (Tomlinson et al. 2010; Bekele et al. 2011; Obura et al. 2011; Sugawara et al. 2012; Kogovšek et al. 2015; Vu et al. 2016) are other techniques used for phytoplasma detection, but not yet fully exploited and not always adequate due to lack of specificity or sensitivity.

### 1.3 16S Ribosomal DNA-Based Classification

The development of a robust and quite exhaustive classification system based on 16S ribosomal gene sequence (Lee et al. 1998a) was followed by the multilocus typing on other genes, which are usually different according to the diverse ‘*Candidatus Phytoplasma*’ species (Bertaccini 2015). A consensus for naming novel phytoplasmas was reached in 2004 (IRPCM) for which “a ‘*Candidatus (Ca.) Phytoplasma*’ species description should refer to a single, unique 16S rRNA gene sequence (>1200 bp),” and “a strain can be recognized as a novel ‘*Ca. Phytoplasma*’ species if its 16S rRNA gene sequence has <97.5% similarity to that of any previously described ‘*Ca. Phytoplasma*’ species.” Phytoplasma taxonomy still relies on these rules due to the lack of phenotypic characteristics of these bacteria that are needed to classify them in formal genus and species. Some phytoplasma strains which may warrant designation of a new taxon, but fail to meet the requirement of sharing <97.5% sequence similarity with existing ‘*Ca. Phytoplasma*’, can be differentiated and classified using additional unique biological properties such as antibody specificity, host range, and vector transmission specificity (Seemüller and Schneider 2004).

Phytoplasma 16S rRNA genes are highly conserved; however, they possess sufficient diversity to be used for their classification. Phytoplasmas are classified either into a ‘*Candidatus Phytoplasma*’ species based on percent sequence identity (IRPCM 2004) or into ribosomal groups and subgroups based on the presence of restriction sites (Lee et al. 1998a; Wei et al. 2007; Zhao et al. 2009b). The number of ‘*Candidatus Phytoplasma*’ species and ribosomal groups and subgroups is continually growing due to increased awareness of the importance of these pathogens in agriculture and environment worldwide (Bertaccini et al. 2014; Maejima et al. 2014). A formal description remains to be published for phytoplasmas associated with some well-known diseases such as grapevine “flavescence dorée” and some of the lethal yellowing associated agents for which still the grouping and subgrouping are the only official taxonomy (Table 1.1). A number of new ‘*Candidatus*’ species were proposed in the last few years indicating the great biodiversity in these microorganisms intensified by the frequent report of interoperon heterogeneity and/or mixed phytoplasma infection in both insects and plants (Schneider and Seemüller 1994; Lee et al. 1995, 1998b; Ho et al. 2001). Very recently two other ‘*Candidatus*’ species were officially proposed ‘*Ca. P. wodyetiae*’ and ‘*Ca. P. noviguineense*’ (Naderali et al. 2017; Miyazaki et al. 2018). There are thousands of 16S rRNA gene sequences of phytoplasmas deposited in the public databases as well as sequences to other conserved genomic regions used as supplemental tools for finer phytoplasma differentiation (Duduk and Bertaccini 2011). A barcode screening system was also developed in agreement with the 16S rDNA classification for fast phytoplasma detection and identification (Makarova et al. 2012). The *rp*, *tuf*, and *secY* genes show more variation than the 16S rRNA gene (Gundersen et al. 1996; Schneider et al. 1997; Cimerman et al. 2009; Marccone et al. 2000; Lee et al. 2006b, 2010, 2012; Danet et al. 2007; Arnaud et al. 2007; Martini et al. 2002, 2007;

**Table 1.1** Phytoplasma classification based on RFLP analyses and/or sequencing of 16S rDNA

16Sr grouping	Strain (acronym)	' <i>Candidatus</i> ' sp.	GenBank accession number	References
<b>16SrI – aster yellows</b>				
I-A	Aster yellows witches' broom (AY-WB)		NC_007716	Bai et al. (2006)
I-B	Aster yellows (MAY)	' <i>Ca. P. asteris</i> '	M30790	Lee et al. (2004a)
I-C	Clover phyllody (CPh)		AF222065	Lee et al. (2004a)
I-D	Paulownia witches' broom (PaWB)		AY265206	Lee et al. (2004a)
I-E	Blueberry stunt (BBS3)		AY265213	Lee et al. 2004a
I-F	Aster yellows apricot Spain (A-AY)		AY265211	Lee et al. (2004a)
I-I	Strawberry witches' broom (STRAWB1)		U96614	Jomantiene et al. (1998)
I-K	Strawberry witches' broom (STRAWB2)		U96616	Jomantiene et al. (1998)
I-L	Aster yellows (AV2192)		AY180957	Lee et al. (2003)
I-M	Aster yellows (AVUT)		AY265209	Lee et al. (2004a)
I-N	Aster yellows (IoWB)		AY265205	Lee et al. (2004a)
I-O	Soybean purple stem (SPS)		AF268405	Lee et al. (2002)
I-P	Aster yellows from <i>Populus</i> (PopAY)		AF503568	Šeruga et al. (2003)
I-Q	Cherry little leaf (ChLL)		AY034089	Valiunas et al. (2005)
I-R	Strawberry phylloid fruit (StrawbPhF)		AY102275	Jomantiene et al. (2002)
I-S	Pepper little leaf (PeLL)		DQ092321	Santos-Cervantes et al. (2008)
I-T	Tomato little leaf (ToLL)		DQ375238	Santos-Cervantes et al. (2008)
I-U	Mexican potato purple top (JAL8)		FJ914650	Santos-Cervantes et al. (2010)
I-V	Mexican potato purple top (SON18)		FJ914642	Santos-Cervantes et al. (2010)
I-W	Peach rosette-like disease (PRU0382)		HQ450211	Arocha-Rosete et al. (2011)
I-X	Papaya bunchy top (BTS)		JF781308	Acosta et al. (2013)
I-Y	Tomato "brote grande"	' <i>Ca. P. lycopersici</i> '	EF199549	Arocha et al. (2007)
I-Z	Papaya bunchy top (BTS)		JF781311	Acosta-Pérez et al. (2017)

(continued)

**Table 1.1** (continued)

16Sr grouping	Strain (acronym)	' <i>Candidatus</i> ' sp.	GenBank accession number	References
<b>16SrII – peanut witches' broom</b>				
II-A	Peanut witches' broom (PnWB)		L33765	Gundersen et al. (1994)
II-B	Lime witches' broom (WBDL)	' <i>Ca. P. aurantifolia</i> '	U15442	Zreik et al. (1995)
II-C	Faba bean phyllody (FBP)		X83432	Seemüller et al. (1998)
II-D	Papaya mosaic (PpM)	' <i>Ca. P. australasia</i> '	Y10096	White et al. (1998)
II-E	Picris echioides phyllody (PEY)		Y16393	Seemüller et al. (1998)
II-F	Cotton phyllody (CoP)		EF186827	Martini et al. (2007)
II-G	Cactus witches' broom (CWB)		EU099568	Cai et al. (2008)
II-J	Cactus witches' broom (CWB)		EU099552	Cai et al. (2008)
II-H	Cactus witches' broom (CWB)		EU099569	Cai et al. (2008)
II-K	Cactus witches' broom (CWB)		EU099572	Cai et al. (2008)
II-I	Cactus witches' broom (CWB)		EU099551	Cai et al. (2008)
II-L	Cactus witches' broom (CWB)		EU099546	Cai et al. (2008)
II-M	Potato purple top		FJ914643	Yadav et al. (2014)
II-N	Papaya BTSp		JF781309	Acosta et al. (2013)
II-O	Tabebuia witches' broom		EF647744	Mafia et al. (2007)
II-P	Cuban papaya phytoplasma		DQ286948	Perez-López et al. (2016)
II-Q	Papaya bunchy top (TSpHav02-IIA)		JF78131	Perez-López et al. (2016)
II-R	<i>Echinopsis</i> yellow patch		DQ535900	Perez-López et al. (2016)
II-S	<i>Amaranthus hypochondriacus</i> strain 52A		FJ357164	Perez-López et al. (2016)
II-T	Tomatillo witches' broom		U125185	Perez-López et al. (2016)
II-U	Papaya little leaf		KP057205	Yang et al. (2016)

(continued)

**Table 1.1** (continued)

16Sr grouping	Strain (acronym)	' <i>Candidatus</i> ' sp.	GenBank accession number	References
<b>16SrIII – X-disease</b>				
III-A	Peach X-disease (PX11CT1)	' <i>Ca. P. pruni</i> '	JQ044393	Davis et al. (2013)
III-B	Clover yellow edge (CYE)		AF173558	Davis et al. (2013)
III-C	Pecan bunch (PB)		GU004371	Davis et al. (2013)
III-D	Goldenrod yellows (GR1)		GU004372	Davis et al. (2013)
III-E	Spiraea stunt (SP1)		AF190228	Davis et al. (2013)
III-F	Milkweed yellows (MW1)		AF510724	Davis et al. (2013)
III-G	Walnut witches' broom (WWB)		AF190226/ AF190227	Davis et al. (2013)
III-J	Chayote witches' broom (ChWBIII)		AF147706	Montano et al. (2000)
III-K	Strawberry leafy fruit (SLF)		AF274876	Jomantiene et al. (1998)
III-H	Poinsettia branch-inducing (PoiBI)		AF190223	Davis et al. (2013)
III-I	Virginia grapevine yellows (VGYIII)		AF060875	Davis et al. 1998
III-L	Cassava frog skin disease (CFSD)		EU346761	Alvarez et al. (2009)
III-M	Potato purple top (MT117)		FJ226074	Davis et al. (2013)
III-N	Potato purple top (AKpot6)		GU004365	Davis et al. (2013)
III-O	Dandelion virescence (DanVir)		AF370120	Jomantiene et al. (2002)
III-P	Dandelion virescence (DanVir)		AF370119/ AF370120	Jomantiene et al. (2002)
III-Q	Black raspberry witches' broom (BRWB7)		AF302841	Davis et al. (2001)
III-R	Cirsium white leaf (CirWL)		AF373105	Zhao et al. (2009b)
III-S	Western peach X-disease (WX)		L04682	Zhao et al. (2009b)
III-T	Sweet and sour cherry (ChD)		FJ231728	Valiunas et al. (2009)
III-U	Cirsium white leaf (CWL)		AF373105/ AF373106	Jomantiene et al. (2002)

(continued)



**Table 1.1** (continued)

16Sr grouping	Strain (acronym)	' <i>Candidatus</i> ' sp.	GenBank accession number	References
III-V	Passion fruit phytoplasma (PassWB-Br4)		GU292082	Davis et al. (2012)
III-W	<i>Heterothalamus</i> little leaf (HetLL)		KC412029	Galdeano et al. (2013)
III-X	<i>Conyza</i> witches' broom		KC412026	Galdeano et al. (2013)
III-Y	Cranberry false blossom		KF62652	Lee et al. (2014)
III-Z	Broccoli stunt strain BSP-21		JX626327	Perez-López et al. (2016)
<b>16SrIV – coconut lethal yellowing</b>				
IV-A	Coconut lethal yellowing (LYJ-C8)		AF498307	Harrison et al. (2002)
IV-B	Yucatan coconut lethal decline (LDY)		U18753	Harrison et al. (1994)
IV-C	Tanzanian coconut lethal decline (LDT)		X80117	Harrison et al. (1994)
IV-D	Texas phoenix decline (TPD)		AF434969	Harrison et al. (2008)
IV-E	Coconut lethal yellowing (LYDR-B5)		DQ631639	Martinez et al. (2008)
IV-F	<i>Washingtonia robusta</i> decline		EU241512	Harrison et al. (2008)
<b>16SrV – elm yellows</b>				
V-A	Elm yellows (EY)	' <i>Ca. P. ulmi</i> '	AY197655	Lee et al. (2004b)
V-B	Jujube witches' broom (JWB-G1)	' <i>Ca. P. ziziphi</i> '	AB052876	Jung et al. (2003a)
V-C	"Flavescence dorée" (FD-C)		X76560	Martini et al. (1999)
V-D	"Flavescence dorée" (FD-D)		AJ548787	Martini et al. 1999
V-E	Rubus stunt (RuS)	' <i>Ca. P. rubi</i> '	AY197648	Malembic-Maher et al. (2011)
V-F	Balanite witches' broom (BltWB)	' <i>Ca. P. balanitae</i> '	AB689678	Win et al. (2013)
V-G	Korean jujube witches' broom		AB052879	Jung et al. (2003a)
V-H	<i>Bischofia polycarpa</i> witches' broom		KJ452547	Lai et al. (2014)
V-I	Blackberry witches' broom		KR233473	Fránová et al. (2016)

(continued)

**Table 1.1** (continued)

16Sr grouping	Strain (acronym)	' <i>Candidatus</i> ' sp.	GenBank accession number	References
<b>16SrVI – clover proliferation</b>				
VI-A	Clover proliferation (CP)	' <i>Ca. P. trifolii</i> '	AY390261	Hiruki and Wang (2004)
VI-B	Strawberry multiplier disease (MD)		AF190224	Jomantiene et al. (1998)
VI-C	Illinois elm yellows (EY-IL1)		AF409069	Jacobs et al. (2003)
VI-D	Periwinkle little leaf (PLL-Bd)		AF228053	Siddique et al. (2001)
VI-E	<i>Centaurea solstitialis</i> virescence (CSVI)		AY270156	Faggioli et al. (2004)
VI-F	Catharanthus phyllody (CPS)		EF186819	Martini et al. (2007)
VI-H	Portulaca little leaf phytoplasma (PLL-Ind)		EF651786	Samad et al. (2008)
VI-I	Passionfruit (WB-Br4)	' <i>Ca. P. sudamericanum</i> '	GU292081	Davis et al. (2012)
<b>16SrVII – ash yellows</b>				
VII-A	Ash yellows (AshY)	' <i>Ca. P. fraxini</i> '	AF092209	Griffiths et al. (1999)
VII-B	Erigeron witches' broom (ErWB)		AY034608	Barros et al. (2002)
VII-C	Argentinian alfalfa witches' broom (ArAWB)		AY147038	Conci et al. (2005)
VII-D	Erigeron witches' broom (EboWB-Br0)		KJ831066	Flôres et al. (2015)
<b>16SrVIII – loofah witches' broom</b>				
VIII-A	Loofah witches' broom (LufWB)	' <i>Ca. P. luffae</i> '	AF086621	Davis et al. (2017)
<b>16SrIX – pigeon pea witches' broom</b>				
IX-A	Pigeon pea witches' broom (PPWB)		AF248957	Gundersen et al. (1996)
IX-B	Almond witches' broom (AIWB)	' <i>Ca. P. phoenicium</i> '	AF515636	Verdin et al. (2003)
IX-C	Naxos periwinkle virescence (NAXOS)		HQ589191	Duduk et al. (2008)
IX-D	Almond witches' broom (AIWB)		AF515637	Verdin et al. (2003)
IX-E	<i>Juniperus</i> witches' broom		GQ925918	Davis et al. (2010)

(continued)

**Table 1.1** (continued)

16Sr grouping	Strain (acronym)	' <i>Candidatus</i> ' sp.	GenBank accession number	References
IX-F	Almond and stone fruit witches' broom (N27-2)		HQ407532	Molino Lova et al. (2011)
IX-G	Almond and stone fruit witches' broom (A1-1)		HQ407514	Molino Lova et al. (2011)
IX-H	Sarson phyllody		KU892213	Ahmad et al. (2017)
<b>16SrX – apple proliferation</b>				
X-A	Apple proliferation (AP)	' <i>Ca. P. mali</i> '	AJ542541	Seemüller and Schneider (2004)
X-B	European stone fruit yellows (ESFY)	' <i>Ca. P. prunorum</i> '	AJ542544	Seemüller and Schneider (2004)
X-C	Pear decline (PD)	' <i>Ca. P. pyri</i> '	AJ542543	Seemüller and Schneider (2004)
X-D	Spartium witches' broom (SpaWB)	' <i>Ca. P. spartii</i> '	X92869	Marcone et al. (2003a)
X-E	Black alder witches' broom (BAWB(BWB))		X76431	Seemüller et al. (1994)
<b>16SrXI – rice yellow dwarf</b>				
XI-A	Rice yellow dwarf (RYD)	' <i>Ca. P. oryzae</i> '	AB052873	Jung et al. (2003b)
XI-B	Sugarcane white leaf (SCWL)		X76432	Lee et al. (1998a, b)
XI-C	Leafhopper-borne (BVK)		X76429	Lee et al. (1998a, b)
XI-D	Sugarcane white leaf (SCWL)		KR020685	Zhang et al. (2016)
XI-E	Cirsium phytoplasma	' <i>Ca. P. cirsii</i> '	KR869146	Šafářová et al. (2016)
XI-F	Sugarcane grassy shoot (SCGS)		HF586636	Yadav et al. (2017)
<b>16SrXII – “stolbur”</b>				
XII-A	“Stolbur” (STOL11)	' <i>Ca. P. solani</i> '	AF248959	Quaglino et al. (2013)
XII-B	Australian grapevine yellows (AUSGY)	' <i>Ca. P. australiense</i> '	L76865	Davis et al. (1997)
XII-C	Strawberry lethal yellows (StrawLY)		AJ243045	Padovan et al. (2000)
XII-D	Japanese hydrangea phyllody	' <i>Ca. P. japonicum</i> '	AB010425	Sawayanagi et al. (1999)
XII-E	Yellows diseased strawberry (StrawY)	' <i>Ca. P. fragariae</i> '	DQ086423	Valiunas et al. (2006)
XII-F	“Bois noir” (BN-Op30)		EU836630	Quaglino et al. (2009)

(continued)

**Table 1.1** (continued)

16Sr grouping	Strain (acronym)	' <i>Candidatus</i> ' sp.	GenBank accession number	References
XII-G	"Bois noir" (BN-Fc3)		47EU8366	Quaglino et al. (2009)
XII-H	Bindweed yellows (BY-S57/11)	' <i>Ca. P. convolvuli</i> '	JN833705	Martini et al. (2012)
XII-I	Potato strain from China (169/Hezuo 88)		EU338445	Cheng et al. (2015)
<b>16SrXIII – Mexican periwinkle virescence</b>				
XIII-A	Mexican periwinkle virescence (MPV)	' <i>Ca. P. hispanicum</i> '	AF248960	Davis et al. (2016)
XIII-B	Strawberry green petal (STRAWB2)		U96616	Jomantiene et al. (1998)
XIII-C	Chinaberry yellows (CBY1)		AF495882	Harrison et al. (2002)
XIII-D	Mexican potato purple top (SINPV)		FJ914647	Santos-Cervantes et al. (2010)
XIII-E	Papaya apical curl necrosis (PACN)		EU719111	Melo et al. (2013)
XIII-F	Strawberry red leaf		KJ921641	Fernández et al. 2015
XIII-G	Chinaberry yellowing (ChTY)	' <i>Ca. P. meliae</i> '	KU850940	Fernández et al. (2016)
<b>16SrXIV – Bermuda grass white leaf</b>				
XIV-A	Bermuda grass white leaf (BGWL)	' <i>Ca. P. cynodontis</i> '	AJ550984	Marcone et al. (2003b)
XIV-B	Bermuda grass white leaf Iran strain		EF444485	Salehi et al. (2009)
XIV-C	Bermuda grass white leaf (RS304/13)		KP019339	Mitrovic et al. (2015)
<b>16SrXV – hibiscus witches' broom</b>				
XV-A	Hibiscus witches' broom (HibWB)	' <i>Ca. P. brasiliense</i> '	AF147708	Montano et al. (2001)
XV-B	Guazuma witches' broom (GWB)		HQ258882	Villalobos et al. (2011)
<b>16SrXVI – sugarcane yellow leaf</b>				
XVI-A	Sugarcane yellow leaf	' <i>Ca. P. graminis</i> '	AY725228	Arocha et al. (2005)
<b>16SrXVII – papaya bunchy top</b>				
XVII-A	Papaya bunchy top	' <i>Ca. P. caricae</i> '	AY725234	Arocha et al. (2005)
<b>16SrXVIII – American potato purple top wilt</b>				
XVIII-A	American potato purple top wilt	' <i>Ca. P. americanum</i> '	DQ174122	Lee et al. (2006a, b)

(continued)

**Table 1.1** (continued)

16Sr grouping	Strain (acronym)	' <i>Candidatus</i> ' sp.	GenBank accession number	References
<b>16SrXIX – chestnut witches' broom</b>				
XIX-A	Chestnut witches' broom	' <i>Ca. P. castaneae</i> '	AB054986	Jung et al. (2002)
<b>16SrXX – Rhamnus witches' broom</b>				
XX-A	Rhamnus witches' broom	' <i>Ca. P. rhamni</i> '	AJ583009	Marcone et al. (2003a)
<b>16SrXXI – Pinus phytoplasma</b>				
XXI-A	Pinus phytoplasma (PinP)	' <i>Ca. P. pini</i> '	AJ310849	Schneider et al. (2005)
<b>16SrXXII – lethal yellowing-type</b>				
XXII-A	Lethal yellowing Mozambique (LYDM 178)	' <i>Ca. P. palmicola</i> '	KF751387	Harrison et al. (2014)
XXII-B	Cape Saint Paul Wilt disease Ghana (LDG)		Y13912	Tymon et al. (1998)
<b>16SrXXIII<sup>a</sup></b>				
XXIII-A	Buckland valley grapevine yellows		AY083605	Wei et al. (2007)
<b>16SrXXIV<sup>a</sup></b>				
XXIV-A	Sorghum bunchy shoot		AF509322	Wei et al. (2007)
<b>16SrXXV<sup>a</sup></b>				
XXV-A	Weeping tea witches' broom		AF521672	Wei et al. (2007)
<b>16SrXXVI<sup>a</sup></b>				
XXVI-A	Sugarcane phytoplasma D3T1		AJ539179	Wei et al. (2007)
<b>16SrXXVII<sup>a</sup></b>				
XXVII-A	Sugarcane phytoplasma D3T2		AY539180	Wei et al. (2007)
<b>16SrXXVIII<sup>a</sup></b>				
XXVIII-A	Derbid phytoplasma		AY744945	Wei et al. (2007)
<b>16SrXXIX – cassia witches' broom</b>				
XXIX-A	Cassia witches' broom (CaWB)	' <i>Ca. P. omanense</i> '	EF666051	Al-Saady et al. (2008)
XXIX-B	Bindweed witches' broom (RBiWB)		KY047493	Esmailzadeh Hosseini et al. (2016)
<b>16SrXXX – salt cedar witches' broom</b>				
XXX-A	Salt cedar witches' broom	' <i>Ca. P. tamaricis</i> '	FJ432664	Zhao et al. (2009a)
<b>16SrXXXI – soybean stunt</b>				
XXXI-A	Soybean stunt (SoyST1c1)	' <i>Ca. P. costaricanum</i> '	HQ225630	Lee et al. (2011)

(continued)

**Table 1.1** (continued)

16Sr grouping	Strain (acronym)	' <i>Candidatus</i> ' sp.	GenBank accession number	References
<b>16SrXXXII – Malaysian periwinkle virescence and phylloidy</b>				
XXXII-A	Malaysian periwinkle virescence (MaPV)	' <i>Ca. P. malaysianum</i> '	EU371934	Nejat et al. (2013)
XXXII-B	Malayan yellow dwarf (MYD)		EU498727	Nejat et al. (2013)
XXXII-C	Malayan oil palm (MOP)		EU498728	Nejat et al. (2013)
<b>16SrXXXIII – <i>Allocasuarina muelleriana</i> phytoplasma</b>				
XXXIII-A	Allocasuarina phytoplasma	' <i>Ca. P. allocasuarinae</i> '	AY135523	Marcone et al. (2003a)

<sup>a</sup>Groups designed only on the basis of GenBank deposited sequences

Hodgetts et al. 2008; Mitrović et al. 2011, 2015) and are useful for epidemiological studies. However using only genetic differentiation to characterize phytoplasmas could end in producing just lists of genotypes if the knowledge of phytoplasma biology is not accompanying the appropriate taxonomy. Biological characteristics of phytoplasmas are needed in particular to help finding management solutions to reduce phytoplasma disease impact on worldwide agriculture.

## 1.4 Transmission and Epidemiology

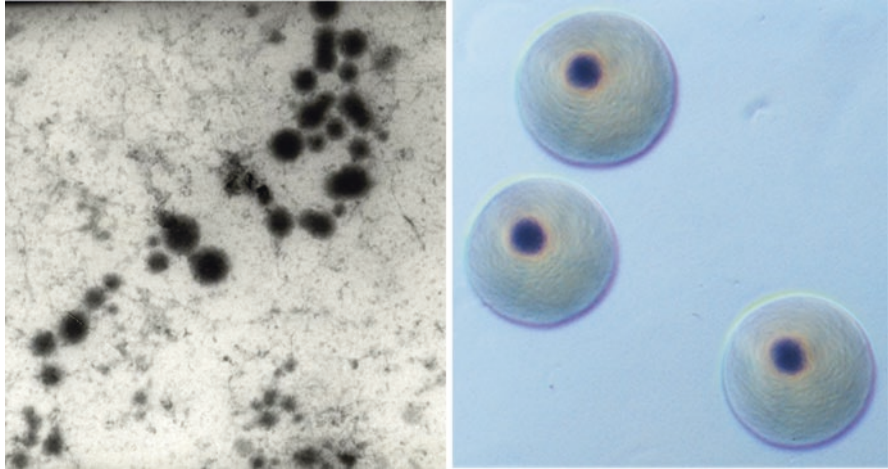
In nature phytoplasmas infect numerous plant species and as equally numerous insects that serve as their vectors in a successful three-way interaction. Phytoplasmas are mainly spread between plants by insects in the families Cicadellidae (leafhoppers) and Psyllidae (psyllids) and superfamily Fulgoroidea (plant hoppers), which feed on the phloem sap of infected plants; therefore their host range is dependent upon feeding habits of their insect vectors (Bertaccini 2007). Phytoplasmas overwinter in insect vectors or in perennial plant hosts and interact with insect hosts also reducing or enhancing their fitness (Sugio et al. 2011a). Transovarial transmission (Alma et al. 1997; Kawakita et al. 2000; Hanboonsong et al. 2002; Tedeschi et al. 2006) and seed transmission (Khan et al. 2002; Botti and Bertaccini 2006; Calari et al. 2011; Chung and Jeong 2014; Satta et al. 2016) were also demonstrated in some plant species/insect-phytoplasma combinations. Phytoplasmas are also efficiently spread via vegetative propagation such as cuttings, grafting, and micropropagation practices (Bertaccini et al. 1992; Jarausch et al. 1996; Bertaccini 2007).

## 1.5 Genomic Sequencing and Metabolic Features

A lot of information has been achieved by full genome sequencing especially related to putative biochemical pathways showing that phytoplasmas are very special microorganisms because they lack a lot of relevant features such as cell wall, mobility, key enzymes, and pathways. Phytoplasmas possess the smallest genome among bacteria; however, gene duplication and redundancy are well represented (Oshima et al. 2004). Moreover, extrachromosomal DNA or plasmids of various sizes have also been found in several phytoplasma groups together with sequences of variable mosaic (SVM) termed potential mobile units or PMUs (Schneider et al. 1992; Kuboyama et al. 1998; Rekab et al. 1999; Oshima et al. 2001; Jomantiene and Davis 2006; Jomantiene et al. 2007; Wei et al. 2008; Ishii et al. 2009; Toruno et al. 2010). Microarray analysis of ‘*Ca. P. asteris*’ strain OY-M revealed that expression of approximately 33% of the genes change during host switching between plant and insect (Oshima et al. 2011) and the phytoplasma may use transporters, secreted proteins, and metabolic enzymes in a host-specific manner. Several factors, namely, *tengu*, *SAP11*, *SAP54*, and *P38*, that could modulate possible phytoplasma pathogenicity were reported to induce symptoms similar to those observed in phytoplasma-infected plants when inserted into transgenic plants (Hoshi et al. 2009; Sugio et al. 2011a, b; MacLean et al. 2011; Neriya et al. 2014). Phytoplasmas possess two secretion systems, *YidC* and *Sec*, the latter seems to be common to most or all phytoplasmas (Kakizawa et al. 2004, 2009). It was also demonstrated that phytoplasmas lack ATP synthase genes suggesting a metabolism strongly dependent on glycolysis. While two sets of glycolytic enzymes were encoded in a duplicated genomic region of a strongly pathogenic strain of ‘*Ca. P. asteris*’, mild strains do not possess this duplication, suggesting this as a possible pathogenicity mechanism (Oshima et al. 2007). The glycolysis genes are completely absent in the full genome of ‘*Ca. P. mali*’ in which a gene-encoding 2-dehydro-3-deoxy-phosphogluconate aldolase was retrieved leading to the hypothesis that in this phytoplasma pyruvate is formed independently from glycolysis (Kube et al. 2012). It is possible that pathogenic mechanisms may differ according to the strain genomic content and/or the diverse environmental conditions such as different host species. The occurrence of major surface epitopes that are unique to each phytoplasma group or ‘*Candidatus* species’, suggests their role in specific interactions with host/insect cells. The Amp protein of the OY phytoplasma forms a complex with insect microfilaments correlated with their phytoplasma-transmitting capacity (Suzuki et al. 2006; Galetto et al. 2011).

## 1.6 Cultivation in Artificial Media

After preliminary evidence that phytoplasmas can be grown in cell-free media (Bertaccini et al. 2010; Contaldo et al. 2012, 2013), a recent description of a suitable and flexible medium was published (Contaldo et al. 2016). This is important information to enable the study of their biology: substantially pure cultures can be



**Fig. 1.2** Left. Ultrathin section of phytoplasma-like cells in agar medium embedded in Spurr resin and observed under transmission electron microscope at 5,000 X magnification (A. Bertaccini and A. Calzolari, 1984, unpublished). Right. Phytoplasma colonies growing in CB medium photographed under bifocal microscope 40 X (courtesy of N. Contaldo)

obtained to verify predicted metabolisms and biological properties of phytoplasmas outside their hosts. Colonies with identical morphology and positive for phytoplasma DNA presence (Fig. 1.2) confirm that despite the reduced genome size, phytoplasmas retain an independent metabolism that allows them to survive as parasites in environments as diverse as plant phloem and insect hemolymph and also in cell-free media.

Although further research is needed in order to optimize the culture system, the prospect of phytoplasma cultivation is now a real option. It will facilitate genome sequencing of further phytoplasma species and strains to allow comparative genomics which has been hampered by their intimate association with plant and insect hosts. Moreover, along with genomics, biochemical and physiological studies, phytoplasma cultivation will define their taxonomy. Selection and screening of plants resistant to phytoplasma infection as well as the study of the modes of colonization by phytoplasmas of plant and insect vectors will also be possible. As a consequence, strategies to manage and/or prevent phytoplasma-associated plant diseases more efficiently will be prepared and employed.

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