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Spiroplasmas infectious agents of plants

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ABSTRACT

The aim is to present a review of the main features that point to the spiroplasmas as plant pathogens. Spiroplasmas are most often found in association with plants and insects and plants flowers, and the interactions of spiroplasma/host can be classified as commensal, pathogenic or mutualistic. Some insect-derived spiroplasmas are entomopathogens. S. melliferum and S. apis are honey bee pathogens. They cross the insect-gut barrier and reach the hemolymph, where multiply abundantly and kill the bee. Many insects spiroplasmas are not pathogenic, are often restricted to the gut and may be regarded as mutualists or incidental commensals. Among the many components important for growth of spiroplasmas, lipids are some of the most significant. Like members of the genus Mycoplasma, the spiroplasmas so far examined are incapable of the biosynthesis of cholesterol and long-chain fatty acids. Spiroplasmas incorporate cholesterol and fatty acid into their membranes when these lipids are supplied in the culture medium. Because of insolubility of cholesterol in water and the toxicity of free fatty acids, the provision of cholesterol in an assimilable form and of fatty acids in nontoxic form for the growth of spiroplasmas presents a problem. Spiroplasmas are capable of chemotactic responses to certain chemical stimuli, motility and chemotaxis are clearly demonstrated by radial migration in soft agar. The existence of chemotaxis implies that spiroplasmas are able to bias random motility so as to produce net migration in a preferred direction. Recent studies have revealed that insect-microbe symbiotic systems often respond to environmental conditions like ambient temperature in an unpredictable manner, which results from complex interactions between host genotype, symbiont genotype, and environmental factors.

Key words: Mollicutes, spiroplasmas, infectious agent, insect, plant, agroecosystem.

INTRODUCTION

Spiroplasma spp. are helical, motile bacteria that lack cell wall and flagellum, and are enclosed within a single membrane with their genomes ranging from approximately 0.78-2.20 Mb in size, the smallest among known self-replicating prokaryotes. So they have been used as model organisms for studying movement, metabolisms and sex ratio. Currently, 34 serological groups are recognized; three of these groups encompass 15 subgroups of inter-related

stains. To date, 37 species among all serogroups and subgroups have been given binomial names. Complete characterization of a new species involves numerous phenotypic and genotypic tests as outlined in the minimal standards document, incluiding phylogenetic data and a reevaluated set of required phenotypic and genotypic tests. Spiroplasma spp., are most often found in association with plants and insects and plants flowers, and the interactions of spiroplasma/host can be classified as commensal, pathogenic or mutualistic [1].

Spiroplasma citri, the agent of citrus stubborn was discovered and cultured in 1970 and shown to be helical and motile (Figure 1), *S. kunkelii* is the causal agent of corn stunt and *S. phoeniceum*, responsible for periwinkle yellows, was discovered in Syria [2] (Figure 2). *S. citri* can infect members of many plant families, and diseases symptoms suggest that the organisms produce toxins. Phytotoxic substances have been detected in, and partially purified from spiroplasma cultures. The corn stunt spiroplasma does not produce toxins and probably affects plants by interfering with hormone metabolism [3]. The aim is to present a review of the main features that point to the spiroplasmas as plant pathogens.

EPIDEMIOLOGY OF SPIROPLASMAS

Epidemic of citrus stubborn disease have been known in the Mediterranean citrusgrowing areas as early as 1928. One such outbreak of stubborn disease occurred around 1980 in newly established citrus nurseries on the Syrian coast. Natural transmission of *S. citri* could be shown to be involved. Enzyme-linked immunosorbent assay (ELISA) and culture of *S. citri* was used to detect the presence of *S. citri* in leafhoppers. Over 50 leafhopper species were submitted to these analyses, not only in Syria but also in Morocco and France (Corsica) from 1978 to 1985. Neoaliturus (Circulifer) haematoceps was the only species found to harbor the spiroplasma in Morocco, Syria and France (Corsica). Leafhoppers of this species were collected in Corsica, raised in Bordeaux and shown to be vectors of *S. citri*. The search for *N. haematoceps* in nature has revealed that Salsola kali (Chenopodiacae) is a favored host plant of this leafhopper. This plant has a wide geographical distribution. In Iran, for instance, it is well known, and grows close to sugar beet and citrus-growing areas. In such areas, sugar beet is known to be infected by curly top virus, and citrus by citrus stubborn disease. *N. haematoceps* is a vector of both of these diseases. *N. tenellus*, the vector of *S. citri* in the USA, is present in the Mediterranean area, but in view of its paucity it does not seem to be a major vector there. In Iran, even though less abundant than *N. haematoceps*, it could play a role in *S. citri* transmission [4].

The citrus pathogen has been known as *S. citri* since 1973, the corn stunt agent was cultured in 1975 and fully characterized as *S. kunkelii* by 1986. The third and only other phytopathogenic spiroplasma is *S. phoeniceum*, cultured from naturally infected periwinkle plants in Syrian and described in 1986. The three spiroplasmas are restricted to the phloem sievetubes of the infected plants and are transmitted from plant by various phloem feeding leafhopper vectors in which the spiroplasmas multiply. Following the pioneering work on *S. citri* and *S. kunkelli*, closed to fifty other spiroplasma species or proposed species have been discovered. All spiroplasmas have been isolated from insects, ticks and plants, insects are particularly rich source of spiroplasmas (Figure 3). Some insect-derived spiroplasmas are entomopathogens. *S. melliferum* and *S. apis* are honey bee pathogens. They cross the insect-gut barrier and reach the hemolymph, where multiply abundantly and kill the bee. *S. floricola* is the agent of lethargy disease of *Melolontha melolontha* (cockchafer). *S. poulsonii* infects the neotropical species of Drosophila, is transmitted transovarially and kills the male progeny of an infected female fly, hence the name sex ratio spiroplasma. Some insect-derived spiroplasmas are also found on plant (flower) surfaces, for instance *S. apis* was cultured the surfaces of flowers growing in the vicinity of affected beehives. This suggests that the plant surface spiroplasmas are deposited on these surfaces by contaminated insects. Many insects spiroplasmas are not pathogenic, are often restricted to the gut and may be regarded as mutualists or incidental commensals. Of the three known tick spiroplasmas, only *S. mirum* obtained from rabbit ticks is pathogenic to the vertebrate animal (chick embryo, new-born rodents, adult rabbit), but only upon experimental inoculation of the spiroplasma. Transposon Tn 4001 mutagenesis has been applied for the first time to *S. citri*, and pathogenicity can now be studied at the genetic level. One Tn 4001 mutant does not multiply in the leafhoppers and is, therefore, not transmitted to the plant [5].

CULTIVATION PHYTOPATHOGENIC SPIROPLASMAS

Spiroplasmas are fastidious prokaryotes which, like mycoplasmas, can be cultivated only in rather complex and enriched media. Previously, these media contained several chemically undefined components, such as peptone, tryptone, beef heart infusion, yeast extract, and animal sera. Studies of the nutritional requirements and metabolic capabilities of sapiroplasmas have been impeded by the lack of chemically defined media. Recently, a chemically defined medium for the cultivation of some epiphytic spiroplasmas has been reported. This medium has been used in

nutritional and metabolic studies of these spiroplasmas, but it does not support the growth of the phytopathogenic species *Spiroplasma citri* [6,7]. The composition of the chemically defined medium LD82 is shown in table 1, some ingredients were prepared as concentrated stock solutions. tyrosine, nucleosides and bases, vitamins, and cofactors and nucleotides were prepared as four separate concentrated (100x) stock solutions in deionized water. The chemically defined medium LD82 supported good growth of the four strains of epiphytic and insect-pathogenic spiroplasmas and the two strains of the phytopathogenic spiroplasmas *S. citri* but failed to support growth of corn stunt spiroplasma I747. In LD82 broth, cell titers of the six cultured strains, ranging from 2.0 x 10^9 to 6.0 x 10^9 CFU/ml at the stationary phase of growth, were comparable to those attained in the undefined medium LD8A, although the growth rates in LD82 were somewhat slower than those observed in LD8A. Although six different spiroplasma strains grew in LD82, optimal concentrations of NaCl for growth in the medium may differ among the strains. For example, honeybee spiroplasma strain AS576 grew better with a higher concentration of NaCl in the medium than the other spiroplasma strains did [8].

All spiroplasma strains that grew in LD82 broth formed visible colonies within 5 to 9 days of incubation on LD82 agar medium. However, the morphology of colonies differed somewhat among the spiroplasma strains. None of the spiroplasmas formed fried-egg colonies. All formed diffuse colonies, but those formed by *S. floricola* 23-6T were more diffuse than those formed by the other strains [3].

Among the many components important for growth of spiroplasmas, lipids are some of the most significant. Like members of the genus *Mycoplasma*, the spiroplasmas so far examined are incapable of the biosynthesis of cholesterol and long-chain fatty acids. Spiroplasmas incorporate cholesterol and fatty acid into their membranes when these lipids are supplied in the culture medium. Because of insolubility of cholesterol in water and the toxicity of free fatty acids, the provision of cholesterol in an assimilable form and of fatty acids in nontoxic form for the growth of spiroplasmas presents a problem. Medium LD82 thus provides a valuable tool for the study of nutritional requirements and metabolic and biosynthetic pathways, not only of epiphytic and insect-pathogenic spiroplasmas but also of important phytopathogenic spiroplasmas [9].

Cell-free extracts from 10 strains of S*piroplasma* species were examined for 67 enzyme activities of the Embden-Meyerhof-Parnas pathway, pentose phosphate shunt, tricarboxylic acid cycle, and purine and pyrimidine pathways. The spiroplasmas were fermentative, possessing enzyme activities that converted glucose 6-phosphate to pyruvate and lactate by the Embden-Meyerhof-Parnas pathway. Substrate phosphorylation was found in all strains. A modified pentose phosphate shunt was present, which was characterized by a lack of detectable glucose 6-phosphate and 6-phosphogluconate dehydrogenase activities. Spiroplasmas could synthesize purine mononucleotides by using pyrophosphate (PP,) as the orthophosphate donor. All spiroplasmas except *Spiroplusmu floricolu* used adenosine triphosphate (ATP) to phosphorylate deoxyguanosine; no other nucleoside could be phosphorylated with ATP by any spiroplasma tested. These results contrast with those reported for other mollicutes, in which PP, serves as the orthophosphate donor in the nucleoside kinase reaction. The participation of ATP rather than PPi in this reaction is unknown in other mollicutes regardless of the nucleoside reactant. Deoxypyrimidine enzyme activities were similar but varied in the reactions involving deamination of deoxycytidine triphosphate and deoxycytidine. All *Spiroplasma* spp. strains had deoxyuridine triphosphatase activity. Uridine phosphorylase activity varied among strains and is possibly group dependent.

As in all other mollicutes, a tricarboxylic acid cycle is apparently absent in *Spiroplasma* spp. Reduced nicotinamide adenine dinucleotide oxidase activity was localized in the cytoplasmic fraction of all spiroplasma species tested. The members of the *Spiroplasrnataceae* are essentially metabolically homogeneous in the highly conserved pathways, but differ from other mollicutes in several important respects. These differences are of probable phylogenetic significance and may provide tools for recognition of higher taxonomic levels of mollicutes [10].

MOTILITY AND CHEMOTAXIS

Spiroplasmas are capable of chemotactic responses to certain chemical stimuli, motility and chemotaxis are clearly demonstrated by radial migration in soft agar. The spiroplasmas, initially confined to the central area of the plate, moved outwards, presumably responding to gradients of attractants, caused by utilization of nutrients in the medium, and repellents, caused by metabolism (e.g. lactic acid). At the same time the organisms were growing so that the rate of production of acidic metabolites was increasing. Motility diminishes as the pH value is lowered, and this phenomenon probably explains why the spiroplasmas stop swimming and form colonies, rather than continue moving to the edge of the plate. On a smaller scale, motility in agar **is** illustrated by the rough appearance of normal

spiroplasma colonies caused by the establishment of satellite microcolonies by spiroplasmas migrating from the parent colony, compared with the smooth colonies produced by non-motile strains It should be possible to use the radial migration phenomenon as an aid to isolating nonmotile and non-chemotactic mutants. The behaviour of spiroplasmas in viscous media is very similar to that of leptospiras, in that high viscosity favours translational motility. The response termed viscotaxis (i.e. migration in the direction of a viscosity gradient) follows as a necessary consequence of the dependence of swimming speed on viscosity, because the velocity component of random motion up the gradient will tend to increase in value, whereas that down the gradient will decrease. Thus random motion will be biased to that spiroplasmas accumulate in capillaries containing viscous media [11].

The natural habitats of spiroplasmas so far described are the interior of phloem sieve tubes, the nectar of flowers and the tissues of arthropods. All these environments are viscous, so it is likely that dispersal of spiroplasmas is facilitated by their ability to swim rapidly in viscous media. Complex nutrient mixtures, sugars and many amino acids tend to be attractants, whereas hydrophobic amino acids, acidic metabolites and heavy metals are repellents. The existence of chemotaxis implies that spiroplasmas are able to bias random motility so as to produce net migration in a preferred direction. The mechanism for viscotaxis suggests a possible analogous explanation for chemotaxis, namely that the speed of swimming of spiroplasmas is a function of the concentration of effector to which the cells are exposed. Increasing concentrations of attractants would induce faster swimming and repellents would reduce the speed. In flagellated bacteria, directional bias is accomplished by varying the relative frequency of runs and tumbles, by altering the direction of rotation of the flagella. The ability of spiroplasmas to respond to chemical gradients may be an important factor in the natural host cycle. In the phloem of plants there is a concentration gradient of photosynthetic products from roots to shoots, so that spiroplasmas may tend to migrate to the top of actively photosynthesizing plants. This may explain why spiroplasmas are readily detectable only in young shoots of citrus plants infected by graft inoculation. Young shoots are often preferred by feeding insects, and the concentration of spiroplasmas in these tissues may favour acquisition by insects. Finally, in leaf hopper vectors spiroplasmas appear to accumulate to high concentrations in salivary glands, whence they are discharged with saliva into plants. It may be that chemotaxis is responsible for the accumulation of spiroplasmas in the glands [11].

SPIROPLASMAS AND COCONUT PALMS

The initial isolations of spiroplasmas from palms affected by lethal yellowing disease raised great hopes of a breakthrough in the lethal yellowing problem, which has been the subject of intensive research in Jamaica for many years. The subsequent failure of attempts to repeat this work suggests that these organisms are not the causal agents and, indeed, casts doubt on the origin of the original isolates; we expect that these questions may be answered by the results of pathogenicity tests and ELISA experiments, now in progress. A possible explanation for these findings is that initial isolation of spiroplasmas from palms is very difficult, and the isolates obtained represent an unlikely and fortuitous event. In this case, the apparent isolation of spiroplasmas from healthy palms and control media may have been due to cross-contamination during subculturing. We consider it unlikely that these isolates derived from contaminated growth medium constituents, as stringent precautions were taken to eliminate likely sources such as sera, and the isolates were distributed in a non-random sequence of culture vials. No *Mycoplasma* or *Acholeplasma* spp., which are known contaminants of commercial sera [12].

Demonstration that lethal yellowing is caused by a spiroplasma would clearly support the view that non-cultivable MLO associated with other plant yellows diseases may be spiroplasmas. Such conclusions are not justified at present, and must also be reconciled with the in vivo morphology of lethal yellowing MLO described by Waters and Hunt [13]. These authors found no evidence for spiroplasmal morphology in a detailed analysis of serial ultrathin sections of MLO in sieve elements from lethal yellowing-diseased coconut roots. However, single ultrathin sections of spiroplasmas in infected insects and occasionally in plants have suggested that helical morphology is not always maintained in vivo [14].

Recent reports suggest that at least four serogroups can be distinguished on the basis of antigenic properties, and the two reference strains investigated represent only one of these groupings. Tests carried indicate that the coconut isolates show some relationships to the 277F spiroplasma (originally isolated from rabbit ticks) in metabolic inhibition and growth inhibition (but not deformation) tests, and to the BC3 (honeybee) and LB12 (green leaf bug) spiroplasmas in metabolic inhibition tests. These results strongly suggest that the palm spiroplasmas represent a new subgroup in the *S. citri* complex, but further work is needed to explore the relationship of the palm isolates to other spiroplasmas strains [12].

INTERACTION WITH INSECTS AND PLANT

Ultrastructural studies using scanning electron microscopy (SEM), negative-staining transmission electron microscopy (TEM), and thin-sectioning TEM on four species of *Spiroplasma*, in vitro and/or in vivo, indicated that their helices commonly possess one tapered end (tip structure) and one blunt or round end. These tip structures appeared morphologically different from the rest of the helix, exhibiting an electron-dense conical or rod-shaped core. In thin sections of the midgut of the leafhopper *Dalbulus elimatus*, the tip structures of *Spiroplasma kunkelii* in the midgut lumen were mostly aligned between microvilli, perpendicular to the apical plasma membrane of epithelial cells. These tip structures appeared frequently attached or closely apposed to the plasma membrane, in which cup-shaped invaginations close to the tips was observed. Pleomorphic forms of spiroplasma, enclosed in membranous vesicles. These findings suggest that the tip structure may be involved in the orientation and attachment of spiroplasma helices in relation to their host cells, and thus may be functionally comparable to the "attachment organelle" of mycoplasmas. Additionally, pili-like structures were observed by negative-staining TEM on the surface of *Spiroplasma melliferum*, and in thin sections of *S. kunkelii* infecting the leafhopper vector *Dalbulus gelbus* [15-17].

The temperature conditions reported 18°C, 25°C, and 28°C, are within the range of natural conditions wherein both *D. melanogaster* and *D. nebulosa* are able to grow and reproduce. Notwithstanding this, the spiroplasmas infection and transmission were significantly suppressed under the higher and lower, but ecologically realistic, temperatures. In natural *Drosophila* populations, infection frequencies of male-killing spiroplasmas were reported to be generally low, although the male-killing phenotype has been regarded as an evolutionarily adaptive trait for maternally transmitted symbionts on the grounds that infected females can gain an extra fitness due to reallocated resources from their killed brothers. For example, in a natural population of *D. nebulosa* in Campo Grande, Brazil, frequencies of the male-killing phenotype due to the spiroplasma NSRO were from 3 to 6% depending on the season. In Brazilian natural populations of *D. melanogaster*, the frequency of infection with the male-killing spiroplasmas MSRO was 2.3%. The temperature-dependent instability of vertical transmission may be relevant to the low infection frequencies of the male-killing spiroplasmas observed in natural *Drosophila* populations. In an attempt to gain further insights into the infection dynamics of the symbiont in nature, experiments under fluctuating-temperature conditions will be of interest. Recent studies have revealed that insect-microbe symbiotic systems often respond to environmental conditions like ambient temperature in an unpredictable manner, which results from complex interactions between host genotype, symbiont genotype, and environmental factors. The *Drosophila-Spiroplasma* symbiosis, wherein the host insect is genetically manipulatable, the host-symbiont combinations are experimentally exchangeable, and the symbiont infection is easily recognizable by the remarkable male-killing phenotype, would provide an ideal model system for experimentally dissecting the relevant factors underpinning the complex nature of the symbiotic interactions [18,19].

The Ciha-1 cell line is one of the very few established from *Cicadellidae* insects and the first, to our knowledge, from the *S. citri* natural vector *C. haematoceps*. These cells were infected ex vivo by *S. citri* and differences in adhesion/entry were observed between transmissible and non-insect-transmissible strains. In addition, the morphological changes of spiroplasmas that were observed in vivo in *S. citri*-infected leafhoppers were also found to occur ex vivo, upon infection of Ciha-1 cells [20,21].

Male-killing phenotypes are found in a variety of insects and are often associated with maternally inherited endosymbiotic bacteria. In several species of *Drosophila*, male-killing endosymbionts of the genus *Spiroplasma* have been found at low frequencies (0.1 to 3%). Spiroplasma infection without causing malekilling was shown to be prevalent (23 to 66%) in Japanese populations of *Drosophila hydei*. Molecular phylogenetic analyses showed that *D. hydei* was infected with a single strain of spiroplasma, which was closely related to male-killing spiroplasmas from other *Drosophila* species. Artificial-transfer experiments suggested that the spiroplasma genotype rather than the host genotype was responsible for the absence of the male-killing phenotype. Infection densities of the spiroplasma in the natural host, *D. hydei*, and in the artificial host, *Drosophila melanogaster*, were significantly lower than those of the male-killing spiroplasma NSRO, which was in accordance with the hypothesis that a threshold infection density is needed for the spiroplasma-induced male-killing expression [22,23].

In nature, *S. citri* is transmitted in a propagative manner by the phloem-feeding leafhoppers *C. haematoceps* and *C. tenellus*. For transmission to occur, the ingested spiroplasmas must infect gut epithelial cells, where they multiply beforecrossing into the hemocoel. Spiroplasmas continue to multiply in the hemolymph and then invade other organs, including the salivary glands, from which they are injected into the phloem via salivary secretions during

insect feeding. Therefore, the ability of *S. citri* to be transmitted relies on its ability to multiply in the insect tissues and to cross physical barriers such as the intestinal epithelium and the salivary gland membranes. Experimental transmission of GII3-9a2 to periwinkle through injection to the leafhopper vector showed that, despite its ability to multiply to a high titer in the insect, the spiralinless mutant was poorly transmitted. As further documented by insect feeding through Parafilm membranes, transmission of the spiralinless mutant was 100 times less efficient than that of the wild-type strain GII-3. Due to the small number of spiroplasmas introduced into the plants by the leafhoppers, symptoms were delayed for several weeks compared to infection with *S. citri* GII-3 or GII3-11c1, and some plants even failed to develop symptoms [24].

In pathogenic mycoplasmas, adhesion to host cell membranes is the first step in the initiation of infection and is mediated by surface proteins called adhesins. Transmission of *S. citri* by its leafhopper vector also involves adherence and invasion of insect host cells. Based on virology studies on transmission of luteovirus by aphids, it was hypothesized that leafhopper transmission is mediated by recognition of specific spiroplasma membrane proteins, which leads to a process of receptor-mediated endocytosis. Recently, an adhesion-related protein (SARP1) has been characterized. However, the involvement of SARP1 in transmission of *S. citri* by its insect vector has not been documented. In this respect, spiralin, which is the most abundant surface protein, might function as a ligand to interact with insect protein receptors, allowing the spiroplasma to cross cellular barriers. Therefore, it would be of interest to determine whether spiralin specifically interacts with proteins from the leafhopper vector *C. haematoceps*. Alternatively, spiralin might have a protecting role. Recently, a model in which spiralin could form a protein "carpet" covering most if not all lipids of the spiroplasmas outer membrane bilayer was proposed. Such a protection of the spiroplasma membrane by spiralin might be crucial for spiroplasma survival in the insect tissues [24-26].

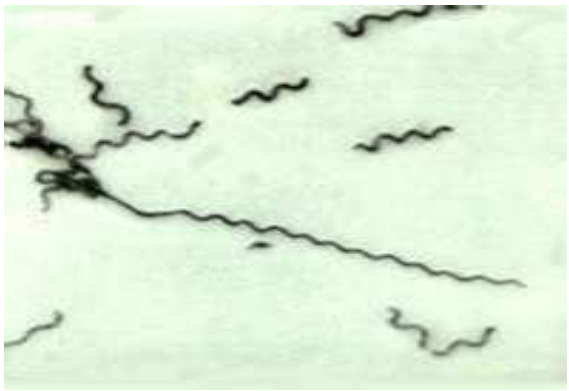

Fig. 1. Characteristic morphology of *Spiroplasma citri*

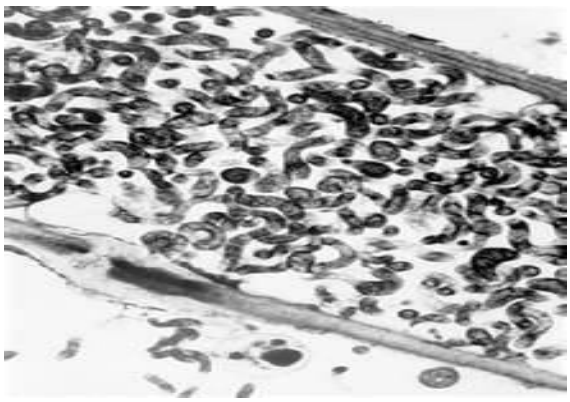

Fig. 2. *Spiroplasma kunkelii* showing its helical and membrane bound

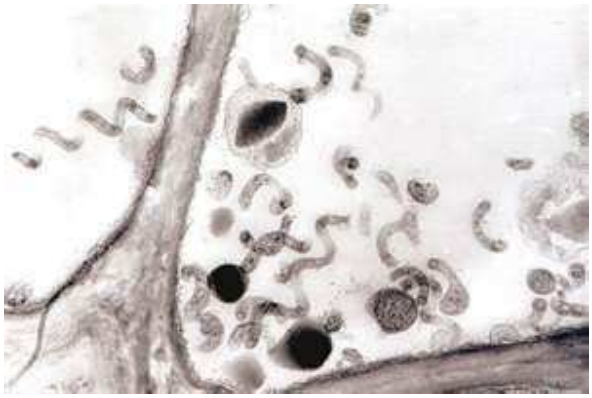

Fig. 3. Spiroplasmas in the phloem of an infected corn plant

Fig. 4. Corn stunt infected plant

Chlorosis of leaf margins is the first symptom of *S. kunkelii* infection, followed by reddening of tips of older leaves (some maize varieties do not redden). Small chlorotic spots appear 2-4 days later at the bases of newly developing leaves. In successive leaves above those bearing first symptoms, the chlorotic spots coalesce to form stripes that extend towards the leaf tips until entire leaves are affected. Later-emerging leaves may also develop chlorosis of the margins, yellowing or reddening, tearing, twisting, and are shortened. Plants are stunted and numerous ear shoots develop. Numerous tillers may also develop at the leaf axils and base of the plant, giving it a bushy appearance. Symptoms of corn stunt spiroplasma observed in Mexico varied with altitude. Some plants were consistently stunted with well-defined broad chlorotic streaking on the leaves. These symptoms are usually observed between 60 and 940 m above sea level. Plants that were not always stunted but whose leaf margins showed red to purple streaks, and plants that usually were not stunted but whose leaves showed either a diffuse yellow or a chlorotic stripe condition with or without red margins, were observed at all elevations surveyed and usually appeared around 7 days before or after anthesis. ELISA was better than DFM at detecting *S. kunkelii*, but both methods demonstrated that all samples with the first type of symptom, 51-70% of those with the second type, 43-46% of those with the third type and 3-11% of those without symptoms were infected by *S. kunkelii* [27,28]. The disease was more prevalent at lower than at higher elevations (Figure 4). The high prevalence and wide distribution of this spiroplasma in Mexico, and also confirm that maize plants having reddish or purplish leaves are often infected with *S. kunkelii*.

Table 1. Composition of the culture medium of Spiroplasmas

Vitamins (mg/liter)		Amino acids (mg/liter)		Inorganic salts (mg/liter)	
Biotin	1.0	L-alanine	400	KH_2PO_4	400
Calcium pantothenate	2.0	β-alanine	100	$MgSO_4$-$7H_2O$	800
Folic acid	2.0	L-aspartic acid	1000	NaCl	4640
i-Inositol	1.5	L-asparagine	600	Lipids (mg/liter)	
Niacin	2.0	L-arginine	600	Palmic acid	14.8
Nicotinamide	2.0	L-cysteine	600	Linoleic acid	16.5
Nucleosides (mg/liter)		L- glutamic acid	1800	Phosphatidic acid	4.9
Adenosine	45.0	L-glutamine	600	Phosphatidylcholine	7.0
Guanosine	30.0	L-isoleucine	400	Cholesterol	9.8
Cytidine	30.0	L-histidine	400	Organic acids (mg/liter)	
Thymidine	30.0	L-methionine	400	α-ketoglutaric acid	400
Uridine	30.0	L-proline	1000	Pyruvic acid	400
Inosine	30.0	L-serine	400	Cofactors and nucleotides (mg/liter)	
Carbohydrates (g/liter)		L-threonine	200	Coenzyme A	2.5
Fructose	4.0	L-tryptophan	200	NAD	2.5
Glucose	1.0	L-tyrosine	200	NADP	2.5
Sucrose	35.0	L-valine	400	FAD	3.0
Tween 80 (ml/liter)	0.148	Glycerol (ml/liter)	0.24	UTP	2.0
Bovine serum albumin (lipi-free) (g/liter)	9.0	Spermidine (mg/liter)	1000	Glutathione reduced	60.0

CONCLUSION

The taxonomy of plant pathogenic bacteria is currently in flux based on recent advances on how bacteria are classified. Most plant pathogenic bacteria belong to the following genera: *Erwinia, Pectobacterium, Pantoea, Agrobacterium, Pseudomonas, Ralstonia, Burkholderia, Acidovorax, Xanthomonas, Clavibacter, Streptomyces, Xylella and Spiroplasma*. Plant pathogenic bacteria cause many different kinds of symptoms that include galls and overgrowths, wilts, leaf spots, specks and blights, soft rots, as well as scabs and cankers. In contrast to viruses, which are inside host cells, walled bacteria grow in the spaces between cells and do not invade them. The means by which plant pathogenic bacteria cause disease is as varied as the types of symptoms they cause. Some plant pathogenic bacteria produce toxins or inject special proteins that lead to host cell death or they produce enzymes that break down key structural components of plant cells and their walls. An example is the production of enzymes by soft-rotting bacteria that degrade the pectin layer that holds plant cells together. Spiroplasmas are bacteria that lack rigid cell walls, and infect plants. As with viruses, many diseases caused by fastidious bacteria are named after the most important host plant or the one where the disease was first characterized, but some can also infect many other plants, such as gladiolus and phlox or tomato, spinach, onion, lettuce, celery, carrots, and strawberry, and many weeds.

REFERENCES

[1] Yu H, Liu S, Ruan K, Wang Z. *Wei Sheng Wu Xue Bao.*, **2009**, 49, 567-572.
[2] Garnier M, Foissac X, Gaurivaud P, Laigret F, Renaudin J, Saillard C, Bové JM. *CR Acad Sci III.*, **2001**, 324, 923-928.
[3] Daniels MJ. *Zentralbl. Bakteriol. Orig. A.*, **1979**, 245, 184-199.
[4] Bové JM, Fos A, Lellemand J. *Isr. J. Med. Sci*., **1987**, 23, 663-666.
[5] Bové JM (1997). *Wien Klin Wochenschr.*, **1997**, 8, 604-612.
[6] Chang, CJ, Chen TA. *Science*, **1981**, 215,1121-1122.
[7] Chan, CJ, Chen TA. *J. Bacteriol*., **1983**, 153, 452-457.
[8] Lee IM, Davis RE. *Appl. Environ. Microbiol*., **1983**, 46, 1247-1251.
[9] Freeman BA, Sissenstein R, McManus TT, Woodward JE, Lee IM, Mudd JB. *J. Bacteriol*., **1976**, 125, 946-954.
[10] Pollack JD, Mc Elwain, MC, Desantis D, Manolukas JT, Tully JG, Chang CJ, Whitcomb RF, Hackett KJ, Williams MV. *Int. J. Syst. Bacteriol*., **1989**, 39, 406-412.
[11] Daniels MJ, Longland JM, Gilbart J. *J. Gen.Microbiol.*, **1980**, 118, 429-436.
[12] Eden-Green SJ, Waters H. *J. Gen. Microbiol*., **1981**, 124, 263-270.
[13] Waters H, Hunt P. *J. Gen. Microbiol*., **1980**, 116, 111-113.
[14] Markham PG, Townsensr R, Plaskitt K, Saglio P. *Plant Dis. Report*., **1977**, 61, 342-345.

[15] Ammar ED, Fulton D, Bai X, Meulia T, Hogenhout SA. *Arch. Microbiol.*, **2004**, 181, 97-105.
[16] Wayadande AC, Fletcher J. *Phytopathology*, **1995,** 85, 256-1259.
[17] Gasparich GE. *Front. Biosci.*, **2002**, 7, 619–640.
[18] Anbutsu H, Goto S, Fukatsu T. *Appl. Environ. Microbiol.*, **2008**, 74, 6053-6059.
[19] Dunbar HE, Wilson AC, Ferguson NR, Moran NA. *PLoS Biol*., **2007**, 5, 1006-1015.
[20] Duret S, Batailler B, Danet JL, Beven L, Renaudin J, Arricau-Bouvery N. *Microbiology*, **2010**, 156, 1097-1107.
[21] Berg, M, Melcher U, Fletcher J. *Gene*, **2001**, 275, 57-64.
[22] Kageyama D, Anbutsu H, Watada M, Hosokawa T, Shimada M, Fukatsu T. *Appl. Environ. Microbiol*., **2006**, 72, 6667-6673.
[23] Anbutsu H, Fukatsu T. *Appl. Environ. Microbiol*., **2003**, 69, 1428-1434.
[24] Duret S, Berho N, Danet JL, Garnier M, Renaudin J. *Appl. Environ. Microbiol*, **2003**, 69, 6225-6234.
[25] Liu HY, Gumpf DJ, Oldfield GN, Calavan EC. *Phytopathology*, **1983**, 73, 585-590.
[26] Fletcher J, Wayadande A, Melcher U, Ye F. *Phytopathology*, **1998**, 88, 1351-1358.
[27] Oliveira E, Oliveira C, Souza I, Magalhães P, Andrade C, Hogenhout S. Maydica, **2005**, 50, 171-178.
[28] Nejat N, Vadamalai G, Dickinson M. *Spiroplasma citri*: A wide host range phytopathogen. *Plant Pathol J*., **2011**, 10, 46-56.