Colony morphology of *Xylella fastidiosa* almond leaf scorch strains

Jianchi Chen, Russell Groves, Yiwei Zheng, Edwin L. Civerolo, Mario Viveros, and Mark Freeman

Abstract: *Xylella fastidiosa* is the causal agent of almond leaf scorch disease (ALSD), which is currently reemerging in California as a potential threat to almond (*Prunus dulcis*) production. We previously reported the presence of different colony morphotypes of *X. fastidiosa* ALSD strains on periwinkle wilt medium solidified with Gelrite and their association with genotypes or pathotypes after a low number of serial transfers. The morphotypes could be differentiated by single nucleotide polymorphisms and were classified as A- and G-genotypes. The stability of colony morphology was not studied. Yet, it is an important issue in the bacterial characterization. In this project, we evaluated the variations of colony morphology through 14 subculture passages. The G-genotype strains were consistently (>99%) associated with smooth colony morphotypes. Similarly, A-genotype strains were consistently associated with rough colony morphotypes. Rough A-genotype strains reversed to smooth colony variants at a low frequency suggesting phase variation in *X. fastidiosa*. In an ALSD survey, we successfully used colony morphology to discern *X. fastidiosa* genotypes with >95% accuracy. The phenotypic traits described in this study are of value for further biological and genetic studies of *X. fastidiosa*.

Key words: Xylella fastidiosa, almond leaf scorch disease, colony morphology, morphotyping, genotyping, epidemiology.

Résumé : Le *Xylella fastidiosa* est l'agent causal du leaf scorch de l'amandier (almond leaf scorch disease; ALSD) actuellement en résurgence en Californie et qui présente une menace potentielle pour la production d'amandes (*Prunus dulcis*). Nous avions signalé précédemment la présence de différents morphotypes de colonie parmi les souches ALSD du *X. fastidiosa* sur le milieu *periwinkle wilt* solidifié avec du Gelrite ainsi que leurs liens avec les génotypes ou pathotypes à la suite d'un nombre réduit de transferts en série. Les types morphologiques avaient pu être différenciés par le polymorphisme au niveau d'un seul nucléotide et avaient été classés comme génotypes A et G. La stabilité de la morphologie des colonies n'avait pas été étudiée. Pourtant, c'est un élément important de la caractérisation des bactéries. Dans le présent projet, nous avons étudié les variations de la morphologie de la colonie au travers d'une succession de 14 repiquages. Les souches de génotype G étaient invariablement (>99 %) associées aux morphotypes « colonie lisse ». De même, les souches de génotype A étaient invariablement associées aux morphotypes « colonie irrégulière ». Un faible pourcentage des souches de génotype A (colonie irrégulière) changea en variants à colonies lisses, ce qui suggère une variation de phase chez le *X. fastidiosa*. Dans un relevé de l'ALSD, nous avons pu utilizer la morphologie des colonies pour discerner les génotypes de *X. fastidiosa* avec une précision de plus de 95 %. Les caractères phénotypiques décrits dans la présente étude sont importants pour les futures études sur la biologie et la génétique du *X. fastidiosa*.

Mots-clés : Xylella fastidiosa, leaf scorch de l'amandier, morphologie des colonies, détermination morphotypique, détermination génotypique, épidémiologie.

Accepted 14 April 2007.

J. Chen,[1] R. Groves, and E.L. Civerolo. United States Department of Agriculture, Agriculture Research Service, San Joaquin Valley Agricultural Sciences Center, 9611 South Riverbend Avenue, Parlier, CA 93648, USA.
Y. Zheng. Your-Way Consulting, Fresno, CA 93720, USA.
M. Viveros. University of California Cooperative Extension, 1031 South Mount Vernon Avenue, Bakersfield, CA 93307, USA.
M. Freeman. University of California Cooperative Extension, 1720 South Maple Avenue, Fresno, CA 93702, USA.

[1]Corresponding author (e-mail: jichen@fresno.ars.usda.gov).

Introduction

Xylella fastidiosa Wells et al. is the causal agent of almond leaf scorch disease (ALSD), which is currently reemerging in California as a potential threat to almond (*Prunus dulcis* (Mill.) D.A. Webb) production. The whole genome sequencing of four *X. fastidiosa* strains has generated a large amount of genotypic data (Bhattacharyya et al. 2002; Simpson et al. 2000; Van Sluys et al. 2003). In contrast, there is limited information regarding phenotypic characteristics of *X. fastidiosa* because of the difficulty growing the pathogen in culture. Variations in colony morphology were first reported for phony peach and plum leaf scald strains isolated in Florida on periwinkle wilt (PW) agar medium (Davis et al. 1981). When the species of *X. fastidiosa* was proposed, two colony morphotypes, smooth and rough, were described (Wells et al. 1987). However, there has been no further characterization of these colony morphology variants with respect to pathogen biology and research applications.

Unique cultural characters, such as slow growth, nonpigmentation, and opalescent colony, are critical criteria for the species determination of *X. fastidiosa*. Growth preference in different media has been used for strain differentiation (Hopkins 2001). Colony morphology is valuable for epidemiological studies, because it is a phenotypic trait that is easy to observe. Differences in colony morphology also imply variations in bacterial cell surfaces covered with molecules, such as extracellular polysaccharide (EPS), lipopolysaccharide, and other cell-surface antigens. Cell-surface molecules are involved with environment adaptation and pathogenicity (Denny 1995; Kelman 1954; Leigh and Coplin 1992). Therefore, characterization of colony morphology and the related phenotypic traits can serve as an initial step towards understanding host–pathogen interactions.

We recently reported that *X. fastidiosa* strains isolated from ALSD samples had smooth and "pitlike" colony morphotypes when initially isolated and in subsequent transfers (Chen et al. 2005). The two colony morphotypes were associated with A- and G-genotypes, defined by single nucleotide polymorphisms (SNPs) in the 16S rDNA sequences (Chen et al. 2005). Further study found that the pitlike strains could be grouped with the rough morphotype described earlier (Davis et al. 1981). The purpose of the present study was to further characterize the colony morphology of *X. fastidiosa* ALSD strains in continuous subcultures to determine if the morphological features were stable. Efforts were made to evaluate the use of colony morphology as a simple tool to assist genotype or pathotype identification.

Materials and methods

Xylella fastidiosa strains

Xylella fastidiosa strain M12 (A-genotype and rough morphotype) and M23 (G-genotype and smooth morphotype) were isolated from two different almond orchards in Kern County, California, in September 2003. The two strains were cloned three times consecutively from a single colony (triple cloned) before further subculturing. Reference strains were Temecula-1 (G-genotype), isolated from grape (*Vitis vinifera* L.) in Temecula, California, and Dixon (ATCC 700965) (A-genotype), isolated from an infected almond near Dixon, California. At the beginning of this study, colonies of Temecula-1 and Dixon did not fit into either rough or typical smooth morphotypes. They were convex with undulate margins. A set of 65 *X. fastidiosa* strains (47 A-genotypes and 18 G-genotypes) isolated from ALSD trees in an orchard in Kern County in November 2003 were added to expand the colony morphology observations. Each strain was triple cloned before subculturing.

Evaluation of colony morphotypes and imprints

All bacterial strains were cultured on PW-G medium, a PW medium (Davis et al. 1981) solidified with Gelrite® (Sigma–Aldrich, Inc., St. Louis, Mo.; Hill and Purcell 1995). Bacterial cultures were streaked to single colonies. The culture plates were sealed with Parafilm and incubated at 28 °C. Bacterial colony morphology was examined from the underside of the colonies in inverted culture plates using a stereo binocular microscope illuminated by fiber-optic bifurcated lights following the scheme outlined by Smibert and Krieg (1981). This included elevation, form, margin, opacity, pigmentation, size, surface appearance, and texture of the bacterial colony (Table 1). Images were recorded using a DP12 microscopic digital camera (Olympus Optical, Ltd., Tokyo, Japan), or a LEICA DFC 480 digital camera (Leica Microsystems Inc., Bannockburn, Ill.). Bacterial strains were subcultured monthly for 14 months.

Colony imprints were described as the etchings of the medium surface covered by the bacterial colony. Bacterial colonies or cell masses on PW-G plates were removed using a sterile inoculation loop. Colony imprints were observed under a stereo binocular microscope. For comparison purposes, *X. fastidiosa* strains were also grown on PW agar medium. The colony morphology together with imprints was observed in the same manner as those in PW-G medium.

Pathogenicity in grapevine

To confirm that G-genotypes, but not A-genotypes, of *X. fastidiosa* strains caused Pierce's disease (PD) to grapevine, *X. fastdiosa* strains of M12, M23, Temecula-1, and Dixon were cultured on PW-G medium at 28 °C for 10 days. Bacterial cells were scraped from the medium and suspended in sterile deionized water. The suspension was adjusted to an optical density of 0.2 at 620 nm (~10^6 colony forming units). Two 10 µL aliquots of cell suspension were inoculated into two separate locations at the lower portion of the stem of grape cultivar 'Cabernet Sauvignon' by the pin-prick method (Hopkins 2001) in a greenhouse. Four replicate plants were set up for each bacterial strain, and sterile water was used as a control. Plants were held for 4–8 weeks postinoculation at 31 °C and a 14 h light : 10 h dark photoperiod. Pathogenicity was indicated by the presence of leaf marginal necrosis and isolation of *X. fastidiosa* cells from leaf petioles.

Colony morphotyping and genotyping

Colony morphotyping was performed on the initial isolation cultures. Two sample collection designs were used. The first was for an orchardwide distribution study (Table 2). Three orchards in Fresno County and two orchards in Kern

Table 1. Colony morphology of *Xylella fastidiosa* almond leaf scorch strains that correspond to 16S rDNA single nucleotide polymorphism genotypes and observed variations during 14 serial passages.

Colony morphology	A-genotype	A-genotype variations	G-genotype	G-genotype variations
Elevation	Umbonate, pitlike	Convex (<1%)*	Convex	Umbonate (<1%)*
Form	Circular	Irregular	Circular	Irregular (unstable)
Margin	Erose or nonentire	Entire, undulate (<1%)*	Entire	Lobate, undulate (<1%)*
Size (mm)	0.1–1.0	0.1–1.0	0.1–1.0	≤ 4.0 (unstable)
Surface appearance	Rough	Smooth (<1%)*	Smooth	Same
Texture	Viscous	Same	Viscous	Same
Opacity	Opalescent	Same	Opalescent	Same
Pigmentation	None	None	None	None

*Percentages in parentheses are estimate frequencies from counting of at least 100 randomly selected bacterial colonies.

Table 2. Results of phenotyping and genotying of *Xylella fastidiosa* strains from samples of five almond orchards in the central and southern San Joaquin Valley of California.

	Isolation*		Colony morphotype			16S rDNA SNP type†	
Orchard	Total sample	Positive sample	Smooth	Rough	Smooth + rough	G	A
Fresno A	44	8	8	0	0	8	0
Fresno B	128	14	14	0	0	14	0
Fresno C	113	100	99	0	1	99+1	0
Kern D	36	13	12	1	0	12	1
Kern E	57	29	14	13	2	14+2	13+2

*Number of symptomatic samples collected and from which isolations were attempted (total) and from which isolations were successful (positive).
†SNP, single nucleotide poylmorphism.

County were identified based on the presence of characteristic ALSD symptoms between August and October 2004. Each orchard was visually surveyed for ALSD symptoms on a tree by tree basis as previously described (Groves et al. 2005). Samples were collected from every symptomatic tree and petiole tissues were used for *X. fastidiosa* isolation (Chen et al. 2005). The second sample collection design was for an area-wide distribution study (Table 3). Eleven orchards in Fresno, Tulare, and Kern counties were selected based on the presence of ALSD symptoms. Samples were collected between 13 September and 16 September 2004 when symptoms were obvious. Leaf samples were collected from a total of 43 ALSD symptomatic trees and cultured for presence of *X. fastidiosa*.

Colonies of *X. fastidiosa* were divided into pitlike (more descriptive than rough) and smooth categories. Their genotypes were confirmed by multiplex SNP polymerase chain reaction (PCR) (Chen et al. 2005). Briefly, primer set Teme150fc (5′-TCTACCTTATCGTGGGGGAC-3′) – Teme454rg (5′-AACAACTAGGTATTAACCAATTGCC-3′) amplified a distinct 348 bp amplicon indicative of a G-genotype. Primer set Dixon454fa (5′-CCTTTTGTTGGGGAAGAAAA-3′) – Dixon1261rg (5′-TAGCTCACCCTCGCGAGATC-3′) generated a distinct 847 bp amplicon indicative of an A-genotype strain. DNA templates were prepared by suspending a loopful of cell culture into 100 µL of sterile water from PW-G media. Each PCR reaction (25 µL) was carried out using the TaKaRa taq™ (Hot Start Version) kit (Takara Bio Inc., Seta 3-4-1, Otsu, Shiga, 520-2193, Japan). Amplification was conducted in an MJ Research Thermocycler (Model PTC-100; MJ Research, Inc., Waltham, Mass.) with an initial denature at 96 °C for 10 min, followed by 30 cycles consisting of: denaturing at 96 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. The amplified DNAs was resolved by electrophoresis in 1.5% agarose gels and visualized by using ethidium bromide staining and ultraviolet light.

Results

Colony variations

Through 14 serial passages over 14 months, all bacterial colonies remained consistently opalescent and nonpigmented as previously reported (Davis et al. 1981; Wells et al. 1987). Over 99% of the well-separated colonies were in circular or nearly circular form (Figs. 1A, 1C, 1D, 1E, 1F, and 1G). Irregular form colonies ranged from nongeometrical shapes (Figs. 1B and 1H) to near circular but with undulate margins (Figs. 1I and 1J). All bacterial colonies were similarly viscous when tested by touching with an inoculation loop (Smibert and Krieg 1981). Well-separated colonies of both genotype strains ranged between 0.1 to 1.0 mm in diameter, but G-genotype strains also developed much larger colonies (up to 4 mm) than A-genotype strains.

Differences between the A- and G-genotypes were most noticeable in colony elevation (umbonate vs. convex), margin (erose or nonentire vs. entire), and surface appearance (rough vs. smooth) and representatively shown in Figs. 1A and 1E. The optimal time to differentiate the smooth and rough colony morphotypes was in the initial isolation culture. The rough character of A-genotype colonies could be seen as early as 5 days following isolation. As culture pas-

Table 3. Distribution of colony morphotypes of *Xylella fastidiosa* associated with almond leaf scorch disease in the central and southern San Joaquin Valley of California.

County and orchard*	Cultivar	Colony morphology		Isolation negative
		Smooth	Rough	
Fresno				
1	'Nonpareil'			4
2	'Nonpareil'	1		1
3	'Sonora'	3		
	'Nonpareil'	1		1
4	'Mission'	1		
	'Fritz'			1
	'NePlus'			1
5	'Price'	2		
6		1		
7		1		
Tulare				
8	'Sonora'	1	2	1
Kern				
9	'Sonora'			3
	'Padre'			4
	'Butte'			1
	'Nonpareil'			2
	'Sonora'		2	1
	'Nonpareil'		1	
10	'Sonora'		3	1
	'Nonpareil'		1	
11	'Sonora'		2†	
	Total	11	11	21

*The geographical locations of the three counties in San Joaquin Valley of California are Fresno, Tulare and Kern from north to south.
†Initially recognized as smooth colony morphotype because of the weak pitlike appearance.

sages increased, variations of colony morphology appeared at a very low frequency (<1%).

Variations within A- and G-genotype strains were summarized in Table 1. One interesting colony variation was the conversion of rough morphotype to smooth morphotype in some A-genotype strains. With strain M12, a smooth type colony (at a frequency of <1%) was identified at passage 12 and triple cloned (Fig. 1F). The smooth morphotype was heritable for more than 10 successive generations. The reversion from smooth to rough colony morphotype had not been observed. Along this line, colonies of strain Dixon were never observed as pitlike but an undulate margin type (Fig. 1J). Also, one of the 65 strain sets, Kern 1a, an A-genotype strain, was consistently an undulate margin type (Table 1). A random amplified polymorphic DNA (RAPD) analysis using five primers reported previously (Albibi et al. 1998) could not detect the difference between the undulate and pitlike morphotypes (data not shown).

Second, the colony morphology of strain Temecula-1 remained consistent throughout the study (Fig. 1I). However, when this strain was inoculated into grapevine, resulting in PD symptoms, all of the reisolated colonies were typically circular with entire margin, identical to those of strain M23 shown in Fig. 1A. A third variation was the development of large colonies, up to 4 mm in diameter, from G-genotype strains. We observed this variation three times during the 14 transfer passages in strain M23. The colony appeared more fluidal, and the centre appeared cloudy (Fig. 1C). After extended incubation (e.g., 30 days), the colony elevation became depressed and showed a rough surface with an entire margin (Fig. 1D). Fourth, although the rough or pitlike colony appearance was a distinctive character of the A-genotype, we also found that a few pitlike colonies with smooth margins in the passage 10 subculture of strain M23 (data not shown). However, subculturing from single colonies reversed the pitlike character to the smooth type.

Colony imprints

Removal of colony mass revealed the presence of a true pit, or surface etching, under both A- and G-genotype bacterial colonies on both PW-G and PW-agar media (Fig. 2). However, weaker imprints were observed from PW agar than those on PW-G. Colony imprints were more obvious in older cultures (>10 days). Rough morphotype colonies produced rough imprints. Smooth morphotype colonies produced smooth imprints.

Grape pathogenicity test

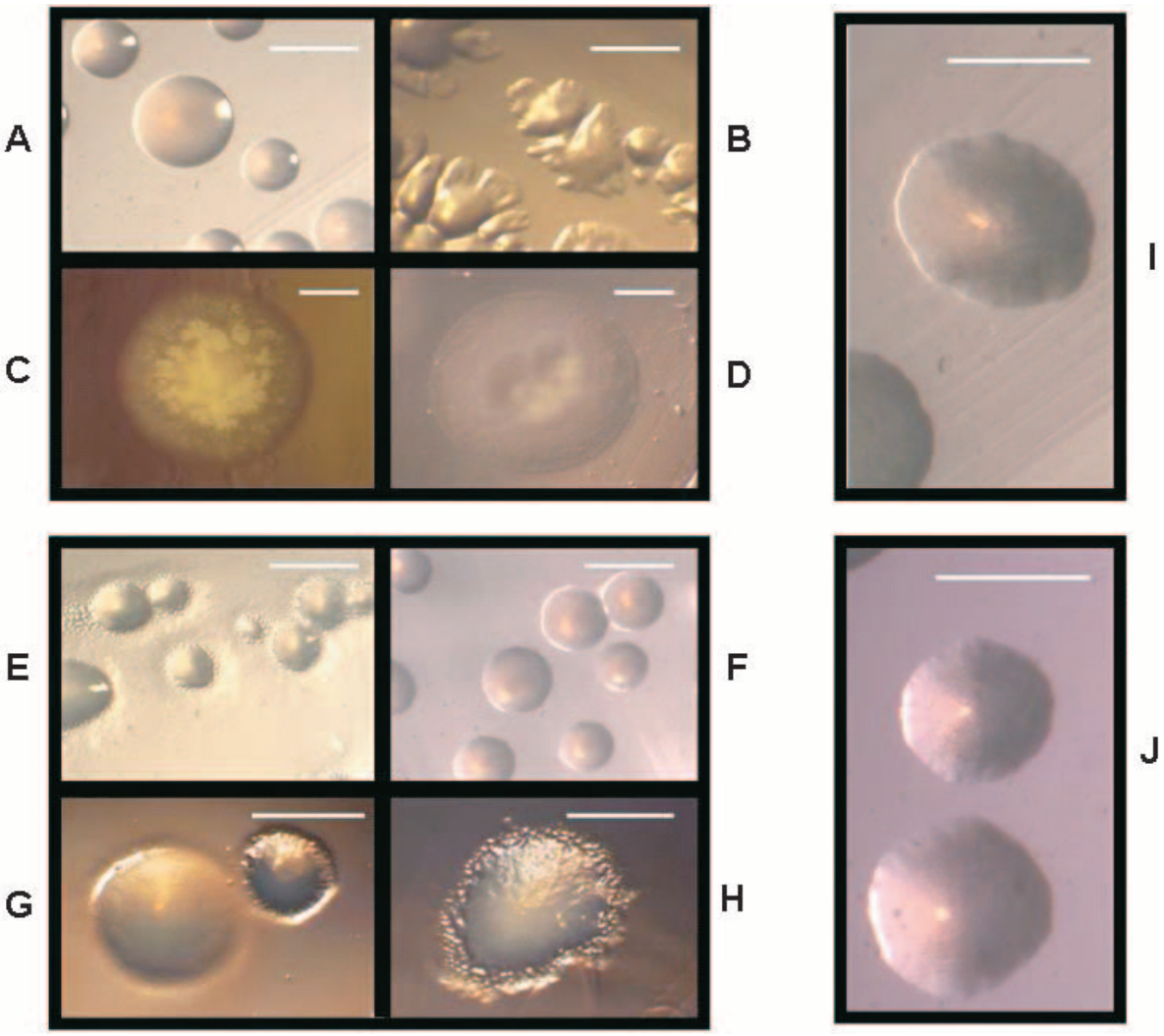
All of the grapevines inoculated with G-genotype strains M23 and Temecula-1, showed marginal necrosis symptoms. The pathogen was reisolated, and its identity was confirmed by colony morphology and PCR. As mentioned above, the colony morphotype of strain Temecula-1 directly isolated from grape tissue was a smooth type not the undulate type. No symptoms were observed on grapevines inoculated with A-genotype strains M12 or Dixon. No *X. fastidiosa* cells were isolated from asymptomatic grapevines.

Application of colony morphotyping

The results of *X. fastdiosa* isolation and colony morphotyping from five orchards in the central and southern San Joaquin Valley are shown in Table 2. Strains isolated from the three Fresno orchards (A, B, and C) were morphologically and genotypically much more homogenous than those isolated from Kern County orchards (D and E). One sample from Fresno County (orchard C) and one sample from Kern County (orchard E) yielded mixed morphotype or genotype strains. Colony morphotype was confirmed to correlate to colony genotype as determined by SNP analysis.

The results of the areawide survey are shown in Table 3. Among the 43 samples, 22 were isolation positive with 11 G-genotype strains and 11 A-genotype strains. Among the 11 G-genotype strains, 10 were from Fresno County, and one was from Tulare County. All of the G-genotype strains had the smooth colony morphotype. Among the 11 A-genotype strains, nine were from Kern County and two from Tulare County. They were all clearly pitlike morphotypes. An exception occurred in a sample from Kern County (orchard 11). The bacterial culture was initially grouped into the smooth colony morphotype, but later PCR results indicated it to be an A-genotype. Regardless of this disagreement, results of colony morphotyping were well matched (21 of 22 or 95%) with those of PCR genotyping.

Fig. 1. Examples of colony morphology variations in *Xylella fastidiosa* almond leaf scorch strains: (A, B, C, D, and I) G-genotypes; (E, F, G, H, and J) A-genotypes. Fig. 1I is strain Temecula-1, which causes Pierce's disease of grapevine, and Fig. 1J is strain Dixon, which causes almond leaf scorch disease. Scale bars = 0.5 mm.



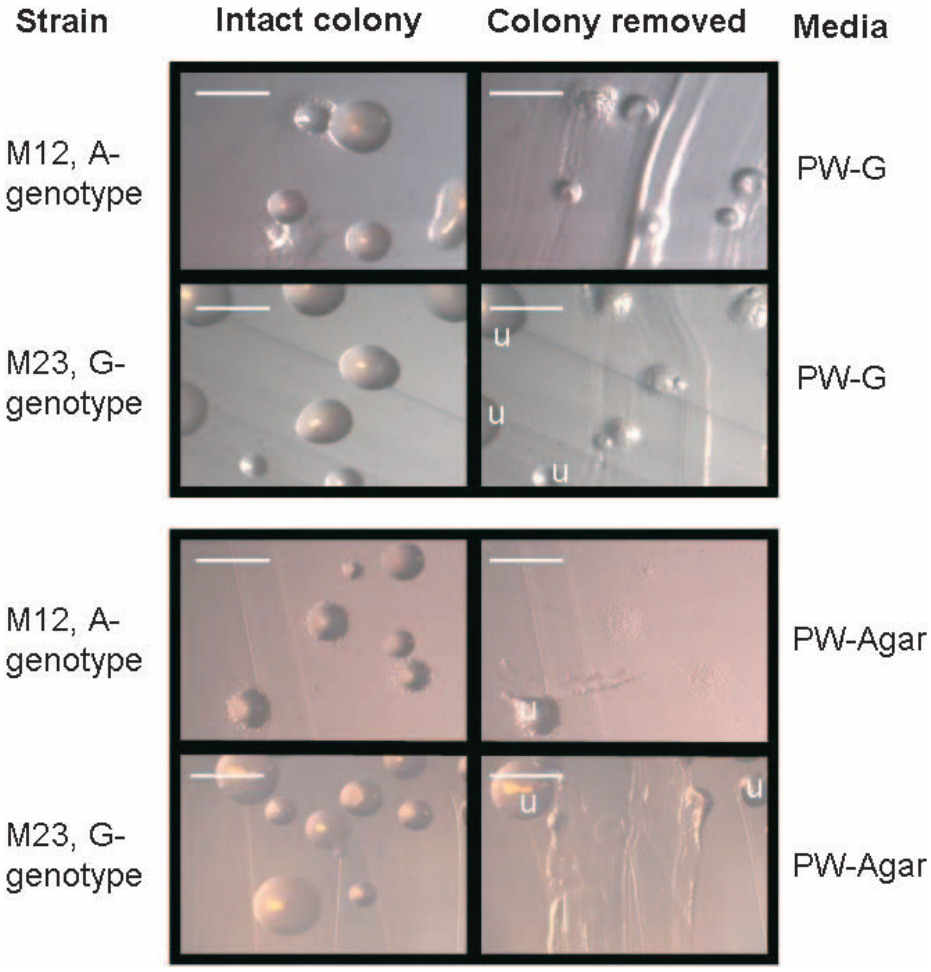
Discussion

Our observations of *X. fastidiosa* colonies on PW-G indicate that the colony morphology of A- and G-genotype strains isolated from ALSD-affected trees is a stable phenotypic trait with a very low frequency of variation. This suggests that colony morphology has the potential to be used as a diagnostic tool for strain characterization in ALSD studies. Morphological typing can quickly identify *X. fastidiosa* genotypes even under situations when the population is composed of mixed strains. In this regard, morphological typing is advantageous over the current PCR protocols. The correlation between colony morphotype and genotype or pathotype of *X. fastidiosa* strains from other hosts remains to be studied in the future. In a previous report (Davis et al. 1981), both smooth and rough morphotypes were reported in the plum leaf scald strain (A-genotype) of *X. fastidiosa* in PW agar medium. Further characterization of these colony morphotypes was not reported.

The biological and molecular basis for colony morphology of *X. fastidiosa* is an interesting issue. The lobate margin (Fig. 1B) and the formation of microcolonies around the margin (Fig. 1E, 1H) are suggestive of bacterial motility. Motility of *X. fastidiosa* has been documented on media and in the xylem vascular system (Meng et al. 2005). A second factor influencing colony morphology is slime (presumably EPS) production. *Xylella fastidiosa* cells are capable of dramatically increasing EPS production, leading to large colonies (Fig. 1C). It is unknown what triggers the overproduction of the bacterial EPS.

The change in colony morphology is considered to be a type of phase variation (Van der Woude and Baumler 2004). The fact that the smooth variant of strain M12 (Fig. 1F) could be maintained for over 10 subcultures suggests that a genetic mechanism(s) was responsible for the phase variation. Phase variation has been described for many animal and plant bacterial pathogens, and it enables bacteria to undergo rapid microevolution and to adapt to different environments (Denny 1995; Kelman 1954; Kingsley et al. 1993; Lugtenberg et al. 2002). This is the first report of the presence of phase variation in *X. fastidiosa*. The biological role of phase variation in *X. fastidiosa* deserves future research attention.

Fig. 2. Colony morphotypes and imprints of *Xylella fastidiosa* almond leaf scorch strains M12 and M23 on PW-G and PW agar media after incubation at 28 °C for 20 days. The letter u marks the bacterial colonies where their slime was not removed. Scale bars = 0.5 mm.



To our knowledge, there has not been any report on the formation of colony imprints on solid media from *X. fastidiosa* strains. The etching appearance suggests the involvement of enzymatic depolymerization of polysaccharides found in Gelrite® and agar. Gelrite® is the commercial name of gellan, a high molecular mass polysaccharide gum, which is principally composed of a tetrasaccharide repeating unit of one rhamnose, one glucuronic acid, and two glucose units. Agar consists primarily of a linear polymer of galactopyranose. Orthologs of gellan lyase (BAA29068) and agarases (BAD29947) have been found in the genome of strain Temecula-1, PD1410, and PD1802 for gellan lyase and PD1611 and PD1691 for agarase, and the other three *X. fastidiosa* strains (Bhattacharyya et al. 2002; Simpson et al. 2000; Van Sluys et al. 2003). However, whether these genes are responsible for the polysaccharide depolymerization is unclear and awaits experimental proof.

Prior to this study, strain Temecula-1 had already been subjected to an unknown number of subcultures. We believe that the original colony morphology of this strain was a smooth type, similar to that isolated from the infected grapevine in this study. We speculate that continuous subculturing may eventually have caused the change in colony form, possibly because of the change in bacterial EPS production as well as bacterial motility. However, the change in colony morphology did not affect the pathogenicity. Data from this study also showed that A-genotype strains (M12 and Dixon) did not cause symptoms on grapevine, consistent with a previous report (Almeida and Purcell 2003).

In a separate experiment (data not shown), we mixed strain M12 and M23 in a 1:1 ratio and inoculated a single grapevine. PD symptoms were observed. Isolation on PW-G medium resulted in all smooth type colonies, and the G-genotype (strain M23) was confirmed by PCR. However, in the subculture in which cells were streaked to single colonies, three rough morphology colonies were recognized from the background of predominantly smooth colonies. These rough colonies were subsequently confirmed to be A-genotype (strain M12) by PCR, indicating that A-genotype strains were capable of multiplying in grapevines.

Because of the technical simplicity and the close correlation between the colony morphotypes and genotypes, colony morphotyping was used to assist a survey study of ALSD genotypes in the San Joaquin Valley. Our study showed that the distributions of *X. fastidiosa* A- and G-genotypes were not uniform (Table 3). The cause of the nonuniform distribution could be that the two genotypes (i) were introduced at different times, (ii) differ in their overwintering survival, (iii) differ in transmission efficien-

cies by insect vector(s), and (iv) differ in pathogenicities among different almond cultivars and cultivation practices.

In summary, *X. fastidiosa* A- and G-genotype strains isolated from ALSD-affected trees can be distinguished based on rough (pitlike) and smooth colony morphotypes with a 95% or higher level of accuracy. Colony morphotyping is more reliable in primary isolation culture and is a valuable tool for disease epidemiology studies. Colony morphotyping is particularly advantageous in the event of mixed-strain infections. We hypothesize that colony morphology of *X. fastidiosa* is a function of EPS production combined with bacterial motility. Putative genes for EPS and motility pili (Type IV pili) have been annotated in the four *X. fastidiosa* genomes (Bhattacharyya et al. 2002; Simpson et al. 2000; Van Sluys et al. 2003). The relationships between the function of EPS and pili genes and the changes in colony morphology, colony imprints, and pathogenicity remain to be determined.

Acknowledgements

Part of this research was supported by the University of California Division of Agriculture and Natural Resouces fund. We appreciate the technical support of R. Alvarez and G. Phillips.

References

Albibi, R., Chen, J., Lamikanra, O., Banks, D., Jarret, R.L., and Smith, B.J. 1998. RAPD fingerprinting *Xylella fastidiosa* Pierce's disease strains isolated from a vineyard in North Florida. FEMS Microbiol. Lett. 165: 347–352.

Almeida, R.P.P., and Purcell, A.H. 2003. Biological traits of *Xylella fastidiosa* strains from grapes and almonds. Appl. Environ. Microbiol. 69: 7447–7452.

Bhattacharyya, A., Stilwagen, S., Reznik, G., Feil, H., Feil, W.S., Anderson, I., Bernal, A., D'Souz, M., Ivanova, N., Kapatral, V., Larsen, N., Los, T., Lykidis, A., Selkov, E., Jr., Walunas, T.L., Purcell, A., Edwards, R.A., Hawkins, T., Haselkorn, R., Overbeek, R., Kyrpides, N.C., and Predki, P.F. 2002. Draft sequencing and comparative genomics of *Xylella fastidiosa* strains reveal novel biological insights. Genome Res. 12: 1556–1563.

Chen, J., Groves, R., Civerolo, E.L., Viveros, M., Freeman, M., and Zheng, Y. 2005. Two *Xylella fastidiosa* genotypes associated with almond leaf scorch disease on the same location in California. Phytopathology, 95: 708–714.

Davis, M.J., French, W.J., and Schaad, N.W. 1981. Axenic culture of the bacteria associated with phony disease of peach and plum leaf scald. Curr. Microbiol. 6: 309–314

Denny, T.P. 1995. Involvement of bacterial polysaccharides in plant pathogenesis. Annu. Rev. Phytopathol. 33: 173–179.

Groves, R.L., Chen, J., Civerolo, E.L., Freeman, M.W., and Viveros, M.A. 2005. Spatial analysis of almond leaf scorch disease in the San Joaquin Valley of California: factors affecting pathogen distribution and spread. Plant Dis. 89: 581–589.

Hill, B.L., and Purcell, A.H. 1995. Acquisition and retention of *Xylella fastidiosa* by an efficient vector, *Graphocephala atropunctata*. Phytopathology, 85: 209–212

Hopkins, D. 2001. *Xylella fastidiosa*. *In* Laboratory guide for identification of plant pathogenic bacteria. *Edited by* N.W. Schaad, J.B, Jones, and W. Chun. American Phytopathological Society Press, St. Paul, Minn. pp. 201–213.

Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology, 44: 693–695.

Kingsley, M.T., Gabriel, D.W., Marlow, G.C., and Roberts, P.D. 1993. The *opsX* locus of *Xanthomonas campestris* affects host range and biosynthesis of lipopolysaccharide and extracellular polysaccharide. J. Bacteriol. 175: 5839–5850.

Leigh, J.A., and Coplin, D.L. 1992. Exopolysaccharides in plant–bacterial interactions. Annu. Rev. Microbiol. 46: 307–346.

Lugtenberg, B.J., Chin-A-Woeng, T.F., and Bloemberg, G.V. 2002. Microbe–plant interactions: principles and mechanisms. Antonie Van Leeuwenhoek, 81: 373–383.

Meng, Y.Z., Li, Y.X., Galvani, C.D., Hao, G.X., Turner, J.N., Burr, T.J., and Hoch, H.C. 2005. Upstream migration of *Xylella fastidiosa* via pilus-driven twitching motility. J. Bacteriol. 187: 5560–5567.

Simpson, A.J.G., Reinach, F.C., Arruda, P., Abreu, F.A., Acencio, M., Alvarenga, R., et al. 2000. The genome sequence of the plant pathogen *Xylella fastidiosa*. Nature (London), 406: 151–157.

Smibert, R.M., and Krieg, N.R. 1981. General characterization. *In* Manual of methods for general bacteriology. *Edited by* P. Gerhardt. American Society for Microbiology, Washington, D.C. pp. 409–443.

Van Sluys, M.A., Oliveira, M.C., Monteiro-Vitorello, C.B., Miyaki, C.Y., Furlan, L.R., Camargo, L.E.A., et al. 2003. Comparative analyses of the complete genome sequences of Pierce's disease and citrus variegated chlorosis strains of *Xylella fastidiosa*. J. Bacteriol. 185: 1018–1026.

Van der Woude, M.W., and Baumler, A.J. 2004. Phase and antigenic variation in bacteria. Clin. Microbiol. Rev. 17: 581–611.

Wells, J.M., Raju, B.C., Hung, H.-Y., Weisburg, W.G., Mandelco-Paul, L., and Brenner, B.J. 1987. *Xylella fastidiosa* gen. nov., sp. nov.: gram-negative, xylem-limited, fastidious plant bacteria related to *Xanthomonas*-spp. Int. J. Syst. Bacteriol. 37: 136–143.