Using Ecology, Physiology, and Genomics to Understand Host Specificity in *Xanthomonas*†

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type III effectors, emergence, host jump, adaptation, habitat

Abstract

How pathogens coevolve with and adapt to their hosts are critical to understanding how host jumps and/or acquisition of novel traits can lead to new disease emergences. The *Xanthomonas* genus includes Gram-negative plant-pathogenic bacteria that collectively infect a broad range of crops and wild plant species. However, individual *Xanthomonas* strains usually cause disease on only a few plant species and are highly adapted to their hosts, making them pertinent models to study host specificity. This review summarizes our current understanding of the molecular basis of host specificity in the *Xanthomonas* genus, with a particular focus on the ecology, physiology, and pathogenicity of the bacterium. Despite our limited understanding of the basis of host specificity, type III effectors, microbe-associated molecular patterns, lipopolysaccharides, transcriptional regulators, and chemotactic sensors emerge as key determinants for shaping host specificity.

CONCEPTS UNDERLYING HOST SPECIFICITY AND EXAMPLES OF HOST JUMPS IN *XANTHOMONAS* SPP.

Definition of Host Specificity and its Importance in Agroecosystems

Host specificity is a tropism of an organism defined by its ability to colonize a host organism (101). This includes the entire spectrum of interactions between organisms, i.e., parasitism, mutualism, and commensalism. Plant-associated bacteria such as xanthomonads are mostly known through their pathogenic members and mainly for the part of their life cycle spent as pathogens. This means that their putative association as commensals on some host plants has been mostly overlooked. As a consequence, the host range of those pathogens can be imprecisely defined and generally lacks any potential asymptomatic hosts.

Host specificity: a tropism of an organism defined by its ability to colonize a host organism

Host range: the different host species with which an organism interacts

Host jumps: the ability of an organism to colonize a new host that is phylogenetically distant from its original hosts

The host range of plant pathogens is based on the completion of Koch's postulates. It is thus difficult to delimit because (*a*) uncultivated plant species are mostly untested and (*b*) depending on the inoculation method and the conditions, the assays could lead to nonspecific plant reactions difficult to distinguish from disease symptoms. As a consequence, there is differentiation between the natural (i.e., disease confirmed in environmental settings) and the experimental (i.e., determined after artificial inoculation) host ranges (23). The experimental host range reflects a potential of natural plant-bacteria interactions, which should be taken into account in the frame of climate change, global trade and transport, and modifications of agricultural practices that reshape the crop environment.

Agroecosystems are usually high-density, genetically homogeneous environments favoring the development of highly adapted and coevolved pathogens (127). Shifts in host specificity may broaden or limit host range and, in extreme scenarios, result in host jumps, in which a benign pathogen or a commensal organism may turn into a dangerous crop pathogen. A good example is *Pantoea agglomerans*, a commensal bacterium widely spread in natural and agricultural habitats that evolved into a tumorigenic bacterium on *Gypsophila* and beet after acquisition of a unique

plasmid bearing all genes required for gall formation and host specificity (10). In this context, determining the molecular bases of host specificity of pathogens is of fundamental importance, as it directly conditions the risk of disease outbreak and the potential emergence of novel diseases in crops. Because host specificity is not monogenic, very little is known about the molecular and genetic bases of host specificity in plant-pathogenic bacteria.

High Levels of Host Specificity Displayed in Pathogenic *Xanthomonas* Strains

The genus *Xanthomonas* represents a large group of Gram-negative bacterial plant pathogens that can cause diseases on at least 124 monocots and 268 dicots (60). Yet, the host range of each individual strain is typically restricted to a single plant or a few plants, generally from the same botanical family (**Figure 1**). Because of the narrow host range of individual *Xanthomonas* at the subspecies level, pathovars were defined as an infra-subspecific group of strains causing the same disease on the same host range (40). Thus, the pathovar concept does not solely rely on host range but also on tissue specificity. For example, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*; see **Table 1** for pathovar abbreviations) is a vascular pathogen of rice, whereas *X. oryzae* pv. *oryzicola* (*Xoc*) infects rice leaf parenchyma. Few pathovars present as broad a host range as *Xanthomonas campestris* pv. *campestris* (*Xcc*), which infects cultivated, ornamental, and wild species of the entire Brassicaceae family (136). Interestingly, this host specialization that yielded the contemporary pathovars likely occurred over the past few centuries, concomitant with the intensification of agriculture (97). *Xanthomonas* species are thus ideal models to investigate the mechanisms underlying host specificity.

Along with host range being defined at the plant species or genus level, several *Xanthomonas* intrapathovar groups of strains interact with intraspecies variants of hosts. Races have been described within several pathovars, such as *campestris*, *glycines*, *malvacearum*, *vesicatoria*, and *oryzae*, to group strains that interact specifically with some host cultivars near isogenic lines carrying specific resistance genes or varieties, i.e., intraspecies host variants (7, 35, 75, 83, 136, 151). The genetic determinants of this level of confined host-specific interactions were first designated as avirulence genes (48). Typically, *avr* genes encode proteins that interact with corresponding plant resistance proteins, thus leading to localized plant cell death limiting the spread and multiplication of the pathogen, characteristic of a hypersensitive response (HR). The interaction between *avr* and resistance genes was first described in the frame of the gene-for-gene hypothesis (48). Most bacterial *avr* genes, including those from *Xanthomonas* spp. (143), were identified as type III effectors (T3Es).

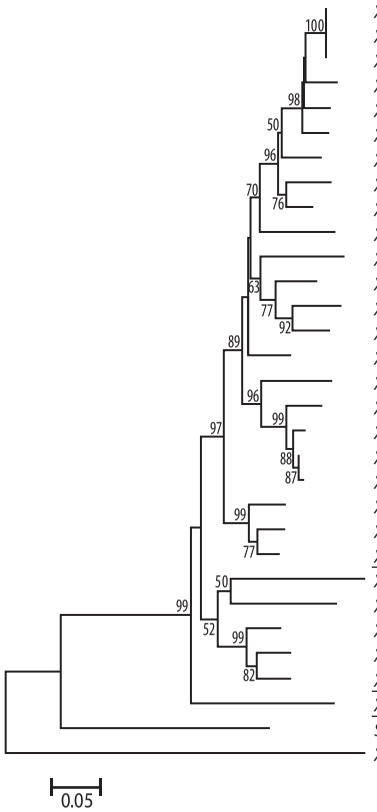
Sympatry: the coexistence of various microorganisms in the same environment that do not face an extrinsic barrier of genetic exchange

Allopatry: indicates that geographical barriers isolate biological populations that cannot exchange genetic material and diverge

Host shift: reflects the ability of an organism to colonize a new host that is phylogenetically closely related to its original host

Recent Reports of Host-Range Modifications in *Xanthomonas*

Most cases of disease emergence correspond to geographical expansion of pathologically preadapted lineages (not covered in this review; reviewed in 85) and do not rely on modifications of host range. Some others have likely involved sympatric (sympatry) or allopatric adaptation (allopatry), resulting in host shifts/jumps. Some recent cases of atypical host range suggest that we might also witness such host jumps. For instance, some strains of *X. campestris* pv. *raphani* (*Xcr*; causal agent of leaf spot on Brassicaceae) infecting solanaceous species such as tomato have been isolated in Canada and Russia (82, 106). Recently discovered *Xanthomonas* diseases, such as bacterial spot of ornamental asparagus and bacterial spot of rose, may also be examples of host jumps (64, 99). Two cases of emerging diseases due to *Xanthomonas vasicola* were linked to host shifts/jumps: (*a*) the emergence of *Xanthomonas* wilt on banana and plantain (*Musa* spp.) in the early 2000s in East Africa (132) and (*b*) the emergence of blight and dieback of *Eucalyptus* spp. (31). One evolutionary



a		b		c	d							e	f	g
***Xanthomonas* species**		Host		Tissue	Disease							TALEs	Gen.	Type strain
		Monocot	Dicot		BB	BC	BP	BR	BSp	BSt	BW			
X. alfalfae [2]	Clade II		*Fab, Rut*	NV					■			Y	3	3836
X. perforans			*Sol*	NV					■			N	34	7293
X. euvesicatoria			*Sol*	NV					■			Y	29	6864
X. melonis [2]			*Cuc, Ver*	NV				■	■					4644
X. citri [34]			*Ana, Ast, Fab, Lam, Lyt, Mal, Mar, Mel, Rut, Vit*	NV	■	■						Y	69	3369
X. axonopodis [56]		*Ama, Ara, Lil, Poa*	*Ana, Beg, Lyt, Eup, Fab, Mal, Oxa, Ped, Phy, Pip, Rub*	V/NV	■		■		■			Y	98	4924
X. bromi		*Poa*									■			1976
X. oryzae [3]		*Poa*		V/NV	■					■	■	Y	23	2532
X. vasicola [4]		*Are, Mus, Poa*	*Myr*	V					■		■	N	14	2543
X. 'cannabis' [4]			*Ast, Can, Fab, Mal, Sol*						■			N	2	*2877*
X. fragariae			*Ros*	NV					■			N	1	2157
X. pisi			*Fab*					■	■				1	4643
X. vesicatoria			*Sol*	NV					■			N	3	4645
X. dyei [3]			*Ath, Ebe, Mel, Mon, Myr, Ole, Rut*			■							1	7245
X. arboricola [8]		*Ara, Mus*	*Api, Ast, Bet, Jug, Ros, Sal*	NV	■	■						N	16	2528
X. campestris [5]			*Bra, Pap, Pla*	V/NV	■	■		■	■			Y	40	5251
X. populi			*Sal*	NV		■								1817
X. hortorum [5]			*Api, Ara, Ast, Ger*	V	■			■	■			Y	1	4935
X. cynarae			*Ast*	NV					■					4188
X. gardneri [2]			*Sol*	NV					■			Y	11	*19865*
X. cucurbitae			*Cuc*	NV					■					2542
X. codiaei			*Eup*						■		■			4690
X. cassavae			*Eup*	V	■				■			Y	1	4642
X. albilineans	Clade I	*Poa*		V						■		N	15	2523
X. sacchari [2]		*Can, Poa*										N	3	4641
X. theicola			*The*			■						Y		4691
X. hyacinthi		*Asp, Lil, Poa*		NV				■				Y	1	1156
X. translucens [11]		*Asp, Iri, Poa*	*Ana*	NV						■	■	Y	9	2054
X. 'maliensis'		*Poa*											1	7942
S. maltophilia														
X. fastidiosa														

Table 1 List of acronyms and former names of pathogens discussed in this review

Current names	Former names, synonyms	Acronyms	References
Xanthomonas alfalfae subsp. *citrumelo*	*X. axonopodis* pv. *citrumelo*, *X. campestris* pv. *citri* pathotype E	*Xac*	22
Xanthomonas axonopodis pv. *phaseoli*	*X. campestris* pv. *phaseoli*, *Xanthomonas phaseoli*	*Xap*	22
Xanthomonas campestris pv. *campestris*	None	*Xcc*	22
X. campestris pv. *raphani*	None	*Xcr*	22
Xanthomonas citri pv. *anacardii*	*X. axonopodis* pv. *anacardii*	*Xca*	2
X. citri pv. *citri*	*X. axonopodis* pv. *citri*, *X. campestris* pv. *citri* pathotype A	*Xcci*	2
X. citri pv. *malvacearum*	*X. axonopodis* pv. *malvacearum*	*Xcmal*	2
X. citri pv. *mangiferaeindicae*	*X. axonopodis* pv. *mangiferaeindicae*, *X. campestris* pv. *mangiferaeindicae*	*Xcm*	2
Xanthomonas euvesicatoria	*X. campestris* pv. *vesicatoria* type A	*Xe*	22
Xanthomonas oryzae pv. *oryzae*	*X. campestris* pv. *oryzae*	*Xoo*	2
X. oryzae pv. *oryzicola*	*X. campestris* pv. *oryzicola*	*Xoc*	2
Xanthomonas perforans	*X. campestris* pv. *vesicatoria* type C	*Xp*	22
Xanthomonas vasicola pv. *holcicola*	*X. campestris* pv. *holcicola*	*Xvh*	22
"*X. vasicola* pv. *musacearum*"	*X. campestris* pv. *musacearum*	*Xvm*	6
"*X. vasicola* pv. *vasculorum*"	*X. campestris* pv. *vasculorum* type B	*Xvv*	22

scenario suggests that "*X. vasicola* pv. *musacearum*" (*Xvm*) shifted from enset (*Ensete ventricosum*; a close relative of banana) to banana to cause *Xanthomonas* wilt in Ethiopia (121). However, another scenario was suggested for this emergent disease on banana in which a close bacterial relative, i.e., *X. vasicola* pv. *holcicola* (*Xvh*) or "*X. vasicola* pv. *vasculorum*" (*Xvv*), which are pathogenic to sorghum and sugarcane, respectively, jumped onto a *Musa* species (6). Historically, *X. vasicola* strains were known to cause disease exclusively on Poaceae, whereas recently isolated *X. vasicola* strain LMG

Figure 1

Phylogeny, host range, diseases, and genomic data currently available for *Xanthomonas* spp. phylogeny (neighbor joining tree, 1,000 replicates) of *Xanthomonas* spp. based on partial *gyrB* sequence of type strains. *Xylella fastidiosa* (strain 9a5c) and *Stenotrophomonas maltophilia* (strain ICMP 17,033) were used as outgroups. (*a*) Numbers in brackets indicate the number of subspecies or pathovars described within species as described by Parkinson et al. (103), Ah-You et al. (2), and Fischer-Le Saux et al. (47). The name *Xanthomonas cannabis* was proposed by Jacobs et al. (70), and the species *Xanthomonas maliensis* was described by Triplett et al. (131). (*b*) Main natural host plant families. Monocots: *Ama*, *Amatyllidaceae; Ara*, *Araceae; Are*, *Arecaceae; Asp*, *Asparagaceae; Can*, *Cannaceae; Iri*, *Iridaceae; Lil*, *Liliaceae; Mus*, *Musaceae; Poa*, *Poaceae*. Dicots: *Ana*, *Anacardiaceae; Api*, *Apiaceae; Ara*, *Araliceae; Ast*, *Asteraceae; Ath*, *Atherospermataceae; Beg*, *Begoniaceae; Bet*, *Betulaceae; Bra*, *Brassicaceae; Can*, *Cannabaceae; Cuc*, *Cucurbitaceae; Ebe*, *Ebenaceae; Eup*, *Euphorbiaceae; Fab*, *Fabaceae; Ger*, *Geraniaceae; Jug*, *Juglandaceae; Lam*, *Lamiaceae; Lyt*, *Lythraceae; Mal*, *Malvaceae; Mar*, *Martyniaceae; Mel*, *Meliaceae; Mon*, *Monimiaceae; Myr*, *Myrtaceae; Ole*, *Oleaceae; Oxa*, *Oxalidaceae; Pap*, *Papaveraceae; Ped*, *Pedaliaceae; Phy*, *Phyllanthaceae; Pip*, *Piperaceae; Pla*, *Plantaginaceae; Ros*, *Rosaceae; Rub*, *Rubiaceae; Rut*, *Rutaceae; Sal*, *Salicaceae; Sol*, *Solanaceae; The*, *Theaceae; Ver*, *Verbenaceae; Vit*, *Vitaceae*. (*c*) Tissue specificity, when known, is indicated by a colored box. A gray box indicates that no data is available. Abbreviations: V, vascular; NV, nonvascular. (*d*) Diseases: BB, bacterial blight; BC, bacterial canker; BP, bacterial pustule; BR, bacterial rot; BSp, bacterial spot; BSt, bacterial streak; BW, bacterial wilt. (*e*) Presence of TALEs (transcription activator-like effectors): Y, yes; N, no. If yes: at least one strain of the species carries at least one TALE. If no: no TALE found in the currently available genomic data. (*f*) The numbers of complete and draft genome sequences available on the National Center for Biotechnology Information website (**http://www.ncbi.nlm.nih.gov/**). (*g*) Numbers refer to strain codes in the Collection Française de Bactéries associées aux Plantes (**https://www6.inra.fr/cirm_eng/CFBP-Plant-Associated-Bacteria**], except for the *Xanthomonas gardneri* type strain, whose code is from American Type Culture Collection (**http://www.atcc.org/**), and the "*X. cannabis*" pathotype strain, which is from the National Collection of Plant Pathogenic Bacteria (**http://ncppb.fera.defra.gov.uk/**).

8711 of *Eucalyptus* causes symptoms on both *Eucalyptus* and Poaceae (31). Such emergences of adaptive clones within *Xanthomonas* species are likely the results of recombination and/or horizontal gene transfer (HGT). A better knowledge of the populations present in the nonhost or wild compartments would lead to the definition of possible reservoirs of adaptive genes.

HGT: horizontal gene transfer

MAMP: microbe-associated molecular pattern

ECOLOGY OF *XANTHOMONAS*: WHAT DO WE REALLY KNOW?

Plant Habitats

Xanthomonas spp. have traditionally been described as plant-associated bacteria that are not encountered in other environments (60). Within a plant, all aerial organs (i.e., stems, twigs, leaves, flowers, buds, fruits, and seeds) can be colonized by *Xanthomonas* and may express symptoms. The symptoms caused by *Xanthomonas* range from water-soaked spots on leaves to dieback and cankers and include wilting, rotting, hypertrophy, and hyperplasia (113) (**Figure 1**). Root colonization has been noted for vascular *Xanthomonas* but also for parenchyma colonizers such as *Xanthomonas citri* pv. *citri* (*Xcci*; see 122). Most *Xanthomonas* first colonize plant surfaces before gaining entry into the tissues through natural openings (i.e., stomata, hydathodes, lenticels, and nectaries) or wounds. The first stage of the molecular dialog between the bacterium and the plant occurs during this epiphytic life and/or directly at the entry point for those few *Xanthomonas* that do not colonize leaf surfaces (**Figure 2**). Thanks to various sensors, the bacterium perceives the environment as favorable or repulsive and eventually attaches to it. In turn, the plant may detect microbe-associated molecular patterns (MAMPs) and induce defense. Ingress through stomata and wounds leads to the invasion of the mesophyll. Penetration through hydathodes and wounds leads to the invasion of the plant vasculature and especially the xylem. For several *Xanthomonas* species such as *X. oryzae* or *X. campestris*, bacterial cells are found, depending on the pathovars, not only in xylem vessels but also between adjacent parenchyma cells (113). One of the few recent papers dealing with the histology of *Xanthomonas* is devoted to analysis of sugarcane colonization by the vascular pathogen *Xanthomonas albilineans*. As expected, the metaxylem and protoxylem are invaded by *X. albilineans* cells, but, surprisingly, this pathogen is also observed in the phloem, parenchyma, and bulliform cells of the infected leaves of sugarcane (94).

Xanthomonas spp. have so far been considered as pathogenic bacteria. As a consequence, their host range is mostly limited to susceptible host plants, and the asymptomatic hosts are mostly unknown. However, the occurrence of nonpathogenic *Xanthomonas* strains on various plants and tissues was reported in the late 1980s. Indeed, strains identified as *Xanthomonas* were isolated from symptomless plant tissues of rice, apple, and weeds sampled in bean fields and failed to induce symptoms on the host of isolation or any other plants (3, 36, 89). More recently, many nonpathogenic look-alikes of *Xanthomonas* pathogens have been reported from symptomatic or asymptomatic host tissues such as walnut buds, citrus or rice leaves, and bean seeds (21, 42, 52, 105, 131). These various reports demonstrate that both the diversity of *Xanthomonas* and the diversity of hosts of *Xanthomonas* have been underestimated.

Interactions Within the Plant-Associated Microbial Community

The composition of the plant-associated microbiota and the processes involved in its assembly are only beginning to be deciphered (78, 87). Microbiota composition and bacterial abundance can be host driven as a consequence of cuticle permeability or excretion of metabolites by plants (16, 138). Plants also release compounds in response to biotic and abiotic stresses that can selectively inhibit some microbes (37). The diverse microbial communities that colonize all

plant compartments interfere in various processes such as disease resistance (92) and metabolite production (8). Metabolite production by *Xanthomonas* could also modify the host microbiota composition, as illustrated by the broad-range antibiotic albicidin synthesized by the sugarcane pathogen *X. albilineans* (29). The study of the composition and dynamics of plant-associated microbiota, including *Xanthomonas*, has only recently been initiated (11). Hence, any direct or indirect influence of the microbiota on host specificity of pathogenic *Xanthomonas* is yet to be determined.

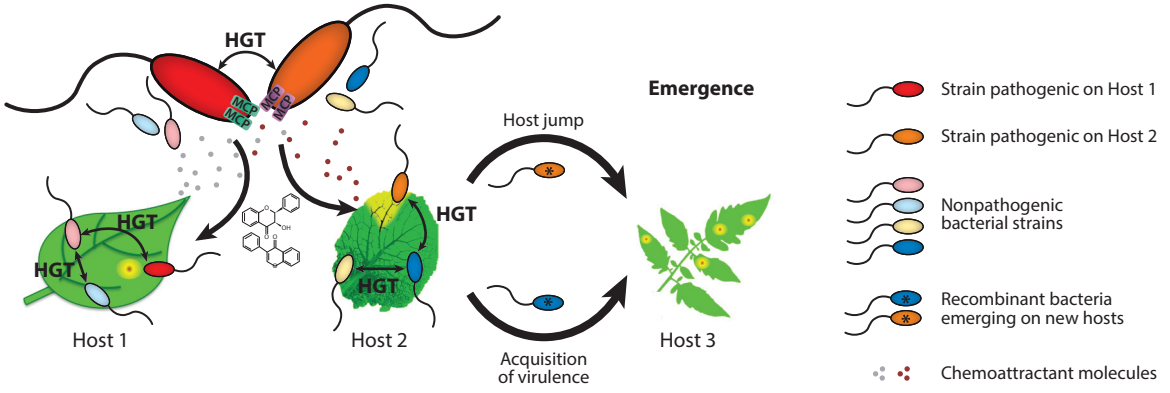
Heterogeneous populations of yellow-pigmented nonpathogenic strains from different plants and identified as *Xanthomonas* by various serological, protein profile, or sequence-based methods could not be assigned to existing pathovars, including those grouping strains from the same host species (42, 95, 131, 133). These strains do not cluster with the pathogenic strains of the host from which they were isolated. Interestingly, it was noticed that most of these strains belong to the *Xanthomonas arboricola* species (95). Some represent new separate lineages in known species (42, 47) and others form new clades such as the *Xanthomonas maliensis* species proposed to group the nonpathogenic strains isolated from disinfected rice leaves (131). These observations indicate that pathogenic and nonpathogenic *Xanthomonas* may evolve in sympatry, as they do with the other members of the natural microbiota.

Sympatry provides microorganisms with the opportunity to exchange genetic material, which can be done within highly diversified microbiota (**Figure 2**). Such promiscuous HGT is crucial for the adaptation to specific niches and is important in ecological diversification (144). It has been suggested that *Pseudomonas syringae* presents an epidemic structure, with ancestors of environmental strains forming the recombinant network from which new pathotypes emerge (137). The ancestor of virulent *Stenotrophomonas*, a Xanthomonadaceae, as well as virulence and resistance determinants, most likely originated in the environmental microbiota (91). It has been recently shown that a *X. arboricola* pv. *juglandis* strain representing an emergent clone in French walnut orchards harbors a 95-Kb integrative and conjugative element (ICE) that is identical to the one found in a *Stenotrophomonas maltophilia* strain and a *Pseudomonas aeruginosa* strain (26). Acquisition of this ICE, which encodes copper resistance genes, probably accounts for pathogen clonal expansion. Do these evolutionary patterns explain the evolution and epidemiology of diseases caused by *Xanthomonas*? Are the nonpathogenic xanthomonads and other members of the microbiota involved in the evolution of pathogenic lineages? These important questions remain to be answered and depend on the development of novel methodologies linking evolution, ecology, and plant pathology.

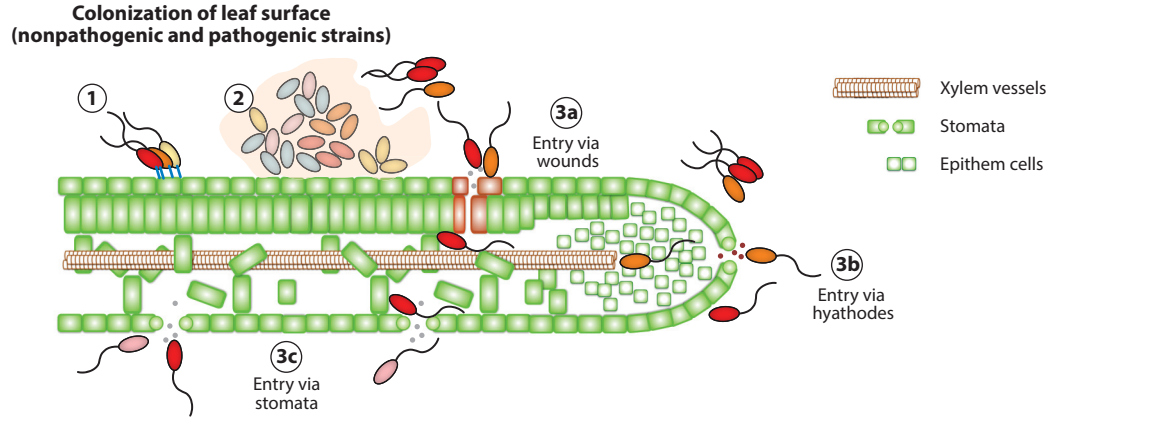
Nonplant Habitats

Only a few reports present data to support the idea that *Xanthomonas* can reside in nonplant environments. Strains of *X. campestris* were found associated with the micro-animals called tardigrades (81), and tardigrades are able to experimentally transmit *Xcr* to plants. However, the precise identity of the strains naturally associated with these animals and their phylogenetic proximity to plant pathogens were not determined, and nor was the frequency of such associations. Some transmissions of phytopathogenic *Xanthomonas*, such as *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) or *Xvm*, from plant to plant by leaf-feeding or pollinating insects are occasionally reported (74, 118). Various animals (as well as irrigation water) sampled in natural settings in Israel were contaminated by strains of *Xanthomonas* spp. that were virulent on pepper (13). Soil and permafrost were also mentioned as contaminated by xanthomonads (62, 119, 141). But the frequency and the biological relevance of such associations or contaminations are not yet known.

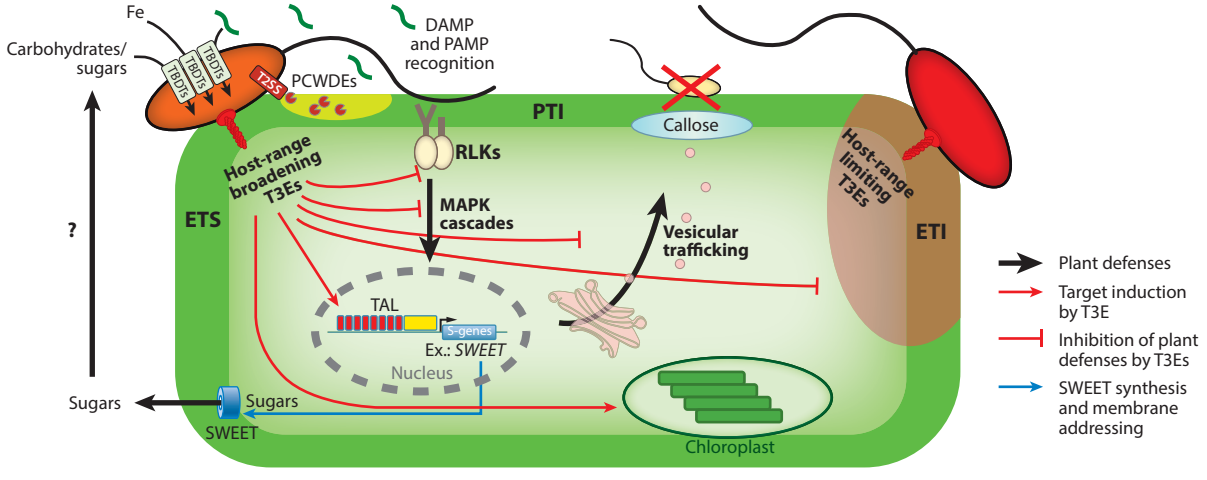
a **Differential sensing of host plants**



b **Differential entry inside host plant tissues**



c **Differential ability to block plant defenses and mobilize nutrients**



Some very exciting, soon to be published data reveal that strains belonging to the genus *Xanthomonas* were frequently identified and isolated in rain and snow in Virginia, representing between 7% and 19% of all cultivable bacteria (C.L. Monteil & B.A. Vinatzer, unpublished data). Such a phenomenon is thought to be related to previous observations of aerial transmission of *X. albilineans* recorded only days after tropical storms in the Caribbean (33). Should rain serve as a niche for plant-pathogenic *Xanthomonas* or strains representing yet unknown divergent lineages, the role of these rain-disseminated strains in the emergence of new strains with novel pathogenic properties should be evaluated in the future, as previously mentioned for the nonpathogenic *Xanthomonas* spp. isolated from plant environments.

MLSA: multilocus sequence analysis

MLVA: multilocus variable number of tandem repeat analysis

Population Biology as an Approach to Gather Insights into Host Range

The importance of host selection on population structure was assessed for many plant pathogens. Such data inform on the infection capabilities of outbreak populations and offer a means for improving strategies of host-resistance deployment. For xanthomonads, only a few studies have established an association between the observed genetic structure and host specificity. Different genetic lineages have been correlated with races or pathotypes (1, 41, 110). During *Xoo* field studies, mutations at the *avrXa7* gene produced virulent strains to rice with the resistance gene *Xa7*. A fitness penalty was associated with these adapted strains, suggesting that *Xa7* is a durable resistance gene (84, 134). Breakdown of resistance to bacterial spot in pepper (against race 1 and race 2) was found associated with the inactivation of the corresponding avirulence genes (*avrBs1* and *avrBs3*, respectively) by different means (e.g., mobile element insertion, plasmid loss) in outbreak populations (79). Multilocus sequence analysis (MLSA) and multilocus variable number of tandem repeat analysis (MLVA) revealed that *X. citri* pv. *mangiferaeindicae* (*Xcm*) (causal agent of mango bacterial canker), and not *X. citri* pv. *anacardii* as previously reported in Brazil, was involved in the emergence of a severe cashew disease in West Africa, thus illustrating a novel case of host jump (155). Molecular epidemiology analyses involving MLSA and MLVA have recently been used to decipher the genetic relatedness among pathogenic and nonpathogenic lineages of *X. arboricola* (42). Extensive sampling of both outbreak-causing and commensal strains would help in understanding the evolutionary bases of host adaptation of xanthomonads.

Figure 2

Models illustrating the concepts underlying host specificity for plant-pathogenic bacteria. Elements are not shown to scale. (*a*) Differential sensing of host plants: Bacterial sensors and methyl-accepting chemotaxis proteins (MCPs) detect attractant or repulsive chemicals produced by plants. Nonpathogenic and pathogenic strains of *Xanthomonas* reside in sympatry on leaf surfaces of various hosts and may exchange genes through horizontal gene transfer (HGT). (*b*) Differential capabilities to enter plant tissues: Bacterial cells (1) attach via their adhesins and (2) form biofilms on leaf surfaces. (3) Bacteria may enter leaf tissues via (3a) wounds. Vascular pathogens also enter via (3b) hydathodes, whereas foliar pathogens enter via (3c) stomata. (*c*) Differential ability to block plant defenses and to mobilize nutrients: Inside the leaf tissue of the host, bacterial cells trigger PTI [PAMP (pathogen-associated molecular pattern)-triggered immunity] through recognition of microbe-associated molecular patterns (MAMPs). The type III secretion system and type III effectors (T3Es) block the onset of PTI by acting on various pathways in the plant cell, abolishing the delivery of defense proteins and callose to the site of infection. Several T3Es are targeted to chloroplasts. TAL (transcription activator-like) effectors induce the transcription of susceptibility genes such as those coding SWEET sugar transporters. Bacterial cells must also efficiently mobilize substrates to multiply inside plant tissues. TonB-dependent transporters (TBDTs) may play a role in host specificity in that they mediate the assimilation of various substrates by bacterial cells. Abbreviations: DAMP, damage-associated molecular pattern; ETI, effector-triggered immunity; ETS, effector-triggered susceptibility; MAPK, mitogen-activated protein kinase; PCWDEs, plant cell wall–degrading enzymes; RLKs, receptor-like kinases.

ARE METABOLISM, CAPTURE, AND SENSING OF PLANT NUTRIENTS INVOLVED IN HOST SPECIFICITY?

CWDEs: cell wall–degrading enzymes

TBDTs: TonB-dependent transporters

Iron Uptake and Niche Adaptation

Iron is an essential component for life; although abundant in nature, its bioavailability is extremely low. Bacteria have developed specific pathways to take up and exploit either ferrous or ferric iron. However, the uptake of iron is tightly controlled because Fe^{2+} catalyzes the Fenton reaction, generating highly reactive hydroxyl radicals that can oxidize macromolecules (68). In *X. oryzae* species, xylem-inhabiting *Xoo* and mesophyll-colonizing *Xoc* share the *xsu-xss* gene cluster that is involved in the biosynthesis and the capture of a siderophore that binds ferric iron (102, 108). Both pathovars, *Xoo* and *Xoc*, also share the *feoABC* system involved in the uptake of free ferrous iron (102, 108). For *Xoo*, a *feoB* mutant is impaired in virulence, whereas *xss* mutants are not. However, in *Xoc* the infection of mesophyllic tissues depends on *xss* genes (108). Accordingly, expression of *Xoo feo* genes was detected in the xylem tissues of infected rice and no expression of *xss* operon could be detected in these tissues (102), whereas the *xsu-xss* operon is expressed during growth of *Xoc* in the mesophyll (108). These results suggest that *Xoo* and *Xoc* encounter different forms of iron in xylem and mesophyll tissues. Generally, iron exists as an Fe^{3+}-citrate complex in xylem vessels (100). However, it was shown that rice xylem sap contains both Fe^{2+} and Fe^{3+} at concentrations sufficient for bacterial growth (150). In contrast, mesophyll tissue might be iron limiting (43). Therefore, the Feo system may allow the tissue-specific adaptation of *Xoo* to rice xylem, but its role in host specificity is still unknown and needs to be explored.

A Diverse Enzymatic Arsenal to Degrade the Host Cell Wall

The plant cell wall is an intricate network of cellulose, noncellulosic polysaccharides, proteins, and aromatic substances, defining a battleground where plants and pathogenic microbes compete. *Xanthomonas* genomes display an extensive repertoire of plant cell wall–degrading enzymes (CWDEs) usually secreted by two type II secretion systems (T2SSs) encoded by *xps* and *xcs* gene clusters (32, 86, 104). Comparative genomics of eight *Xanthomonas* strains, representing vascular and nonvascular pathogens of brassica, citrus, pepper, rice, and tomato, revealed the presence of the *xps* cluster in all strains, whereas the *xcs* cluster was not detected in strains pathogenic on rice (86). One can envision a role, albeit not yet proven, of the *xcs* T2SS in the adaptation of *Xanthomonas* to a specific host. However, further investigations are needed to determine whether the *xcs* cluster could be negatively correlated to the adaptation of *Xanthomonas* to monocotyledon hosts.

Disparities in CWDE content occur within *Xanthomonas* species (32). The cellobiosidase CbhA was found only in xylem-inhabiting *Xanthomonas* and *Xylella fastidiosa*, illustrating tissue specificity (114). In *Xoo* KACC10331, strain mutations in a lipase/esterase (LipA) or a cellulase (ClsA) lead to partial loss of bacterial virulence on rice (73). Pretreatment of rice leaves with LipA or ClsA has allowed identification of rice genes, including transcription factors or jasmonic acid–associated genes, deregulated in both conditions (72, 109). Such a core set of genes may be critical for elicitation of plant immunity by the damage-associated molecular pattern (DAMP)-induced innate immunity of this host. Interestingly, several CWDEs are associated with TonB-dependent transporters (TBDTs) forming specific carbohydrate utilization (CUT) systems (14, 20, 34, 39). The pectate lyase activity of *Xcc* depends on the *exbD2* gene, which belongs to the TonB-ExbBD system and is required for the induction of HR on pepper through production of pectin-derived oligogalacturonides acting as DAMPs (139). This association may represent a specific adaptation of xanthomonads to their host plants.

Sensors of the Environment and Taxis

Bacteria have evolved a repertoire of sensory proteins that may detect environmental signals such as bacterial cell density or the presence of chemoattractants or chemorepellents, oxygen, and light (80, 90). The total number of sensory and signaling proteins encoded by a bacterial genome was proposed as a measure of the adaptive potential of the bacterium, i.e., its intelligence quotient (IQ) (49). Most of these sensory proteins are associated to response regulators to form two-component signal transduction systems (TCSTSs). Extracytoplasmic sigma factor (ECF) may also detect and transduce environmental signals (123). Sensory proteins of bacterial TCSTSs are usually transmembrane histidine kinases (HKs). Detection of a specific stimulus leads to the autophosphorylation of the HK and subsequent transfer of the phosphoryl group to the receiver domain of the response regulator (RR). The RR is then activated and initiates the response to the environmental stimulus through its C-terminal output domain. In *Xanthomonas*, approximately 100 genes encoding TCSTSs were identified per genome (107) and an IQ up to 123 was calculated, illustrating the ability of xanthomonads to adapt to various conditions and to actively sense the environmental parameters, defining them as extroverts (49). Recently, Xu et al. (147) showed that plant hormones, such as salicylic acid and abscissic acid, shape the outcome of rice-*Xoo* interactions through the regulator OryR (45). OryR has a structure similar to that of the regulator LuxR in the quorum-sensing system for N-acyl homoserine lactone (AHL) but missing the AHL synthase LuxI. Several other LuxR homologs, also called LuxR solos, such as XccR, XagR, and XocR, have been evidenced in *Xanthomonas* to sense host plant signals and consequently regulate pathogenicity factors (27, 146, 152).

Chemotaxis is one strategy used by bacteria to cope with changing environmental conditions. Chemotaxis allows motile bacteria to detect and move in response to the chemical composition of the environment. Attractive and repulsive molecules are detected by cell membrane–bound or cytosolic chemoreceptors, called methyl-accepting chemotaxis proteins (MCPs). Upon detection of a signal, a complex signalization pathway from MCPs to the flagellar system leads to a change of the flagellar rotation direction to alter swimming direction (61). Based on an analysis of the presence/absence of 70 candidate genes involved in chemotaxis, environment sensing, and adhesion in a collection of 173 strains representing a large diversity of *Xanthomonas* spp., most pathovars are characterized by unique repertoires of genes encoding sensors and adhesins (96). Furthermore, analysis of selection signatures in chemotactic-related genes from strains belonging to different pathovars has shown that adaptive divergence is acting on most of them, suggesting that these bacterial genes were shaped by the host plant. In *Xcci*, chemotaxis toward host grapefruit leaf extracts was evidenced. This phenotype results from several mechanisms, including the regulation of flagellar motility by XbmR through a regulatory pathway that involves FliA and c-di-GMP (149). Recently, the MCP gene *XCC0324* was found distributed in only one clade within the *Xanthomonas* genus. A group of alleles of this gene was confined to *X. campestris*, whereas groups of divergent alleles were found in phylogenetically close strains belonging to several species, none of which were pathogenic on *Brassicaceae* (69). Polymorphism accumulated in the Per-ARNT-Sim domain of these divergent alleles. The gene coding for the MCP XCC0324 is located in an *Xcc* chromosome in the cassette of an integron, indicating that it was certainly acquired by HGT. In planta experiments revealed that XCC0324 is required for an efficient internalization into leaf tissues of host plants, such as radish and *Arabidopsis*, and for the attraction of *Xcc* toward wounds on cabbage leaves. However, the attractive signal leading to ingress into host leaf tissues remains to be identified (69).

INVOLVEMENT OF ELICITORS OF PLANT IMMUNITY IN HOST SPECIFICITY

PTI: PAMP-triggered immunity

PAMP: pathogen-associated molecular pattern

T3SS: type III secretion system

MAMPs are evolutionarily conserved molecules of microbes whose presence is actively monitored by plant innate immunity to engage plant defenses. On its own, this PTI [PAMP (pathogen-associated molecular pattern)-triggered immunity] prevents infection by most microbes, suggesting that it could play a major role in the definition of host specificity.

Changes in Lipopolysaccharides Associated with Host Jumps

Lipopolysaccharides (LPSs) are the major component of the Gram-negative bacteria outer membrane, recognized as MAMPs in plants, and involved in several interactions with diverse organisms, from mammalian cells to bacteriophages. Comparative genomics of different xanthomonads revealed that the basis for LPSs and their association with host, disease, or host tissue preference was complex and may be different depending on the pathosystem. The first complete *Xanthomonas* genome sequences revealed that the LPS gene clusters of *Xoo* (vascular) and *Xoc* (nonvascular) pathovars, both pathogenic on rice, were very different, suggesting that LPSs can be involved in tissue specialization (86).

However, *Xanthomonas* spp. that infect the same plant species can also possess similar LPS clusters. Comparison of four *Xanthomonas* species infecting pepper and/or tomato revealed a common LPS cluster among the pepper pathogens (104). When two strains belonging to two phylogenetically close pathovars, *Xvm* (from banana) and *Xvv* (from sugarcane), were compared, both strains differed with respect to LPS synthesis and also type IV pili (125). Interestingly, the LPS cluster of the *Xvv* strain was similar to the LPS cluster from the distant sugarcane pathogen *X. albilineans*, suggesting a horizontal transfer of the LPS cluster in one or both strains that may have contributed to host adaptation. However, because only these two isolates were used, the significance of the LPS variation for virulence and host specificity remained unclear. When six additional *X. vasicola* strains, isolated from sugarcane or maize, were analyzed, two different LPS types were found and the difference did not correlate with the plant from which the pathogen was isolated (142). Hence, there may be no simple one-to-one relationship between LPS structures and host plant species and/or tissues. However, it is possible that adaptation to sugarcane by *X. vasicola* strains may have been strain-specific but ultimately led them to cause the same disease.

Changes in the LPS structure have been associated with host range in some *Xanthomonas* spp. For instance, *Xanthomonas alfalfae* subsp. *citrumelonis* (*Xac*) causes leaf spot on rutaceous and leguminous plants. An *opsX* mutant (for outer-membrane polysaccharide) of *Xac*, affected in a glucosyltransferase involved in LPS core assembly and altered in its LPS structure, had lost its virulence on citrus while it could still colonize bean plants (77). The *opsX* mutation caused pleiotropic effects, such as alterations in growth, colony morphology, and exopolysaccharide (EPS) production. Therefore, it is unclear whether changes in LPSs alone were responsible for this change in host range. Indeed, LPS mutants have been shown to be affected in their sensitivity to antimicrobials but also in their type III secretion system (T3SS), which could offer an alternative explanation for the observed change in host range (140). Certainly, more work, including comparative genomics paired with synthetic biology (strain engineering), is required to decipher to what extent LPS contributes to host range. Variation in LPS structure may affect not only the specific attachment to plant cell surfaces but also the escape from distinct plant recognition mechanisms. We also lack important information on how LPSs shape interactions of *Xanthomonas* spp. outside the plant (e.g., with bacteriophages) or with insect vectors.

Evasion of Microbe-Associated Molecular Pattern Recognition and Impact on Host Specificity

ETI: effector-triggered immunity

The flagellum is certainly the best studied MAMP. Flagella are complex, multicomponent nanomotors that propel the bacterium in its microenvironment. The conserved and abundant FliC flagellin protein betrays the bacteria to the plant defense surveillance system and was one of the first MAMPs discovered in plant-pathogenic bacteria (44). The FLS2 (flagellin sensing) pattern recognition receptor from *Arabidopsis* recognizes a conserved peptide of the flagellin, flg22, at the plant plasma membrane (28). Another motif of FliC, called flgII-28, is recognized independently of FLS2 by tomato and other solanaceous plants (but not by *Arabidopsis*), most likely via another pattern recognition receptor (24). Since these discoveries, it has been speculated that bacteria may escape recognition by variation or modification (decoration, e.g., glycosylation) of key flagellin residues, downregulation of flagellin expression, loss of the flagellum, or bacteria-driven enzymatic degradation of flagellin (111). Variations in Flg22 residues required for FLS2-mediated recognition are observed in xanthomonads, allowing evasion of FLS2-mediated immunity (128). Noneliciting variants of flg22 are present not only in *Xcc* (55, 128) but also in xanthomonads pathogenic to, for example, cannabis (70).

The elongation factor Tu (EF-Tu) is another potent MAMP conserved in bacteria that is perceived by the Brassicaceae-specific EF-Tu receptor (EFR) (154). Interestingly, some *Xanthomonas* pathogenic to Brassicaceae express EF-Tu variants that evade EFR recognition (112), thus potentially contributing to their greater fitness on Brassicaceae plants. However, perception of specific MAMPs was never strictly correlated to host-range restriction in *Xanthomonas*. Therefore, avoidance of MAMP perception seems to result from plant-bacterial coevolution to improve bacterial fitness rather than strictly determine host range. Although it does not strictly affect host specificity, AvrXa21 is an interesting example of a MAMP that restricts the range of rice plants that can be infected by *Xoo*. AvrXa21 is strongly recognized by the Xa21 plasma membrane receptor-like kinase and confers immunity to Xa21-expressing rice plants (9). Future studies may decipher specific interactions between MAMPs and MAMP receptors as driving forces for pathovar evolution, similar to the gene-for-gene interaction in race-specific resistance (48).

Type III Effector Proteins as Major Host-Specificity Determinants

With the known exception of *X. albilineans*, *Xanthomonas* pathogenicity on plants is dependent on the presence of a T3SS that injects bacterial T3Es directly into the host cell. T3Es play a central role in the outcome of the interaction between bacteria and their host plants. Indeed, once inside the host cell, numerous T3Es were shown to suppress PTI (56). The specific recognition by the plant of a T3E triggers HR, resulting in effector-triggered immunity (ETI). Finally, some T3Es were shown to suppress ETI (130). Overall, T3Es seem to act synergistically or antagonistically on different pathways of the host cell, creating a physiological host status that would be optimal for the proliferation of the pathogen (**Figure 2**).

Host specificity of strains may be shaped by the presence of various T3Es acting as host-limiting T3Es by inducing ETI. As an example of a host-limiting factor, the T3E XopAG (a.k.a. AvrGf1) explains the inability of *Xcci*-A^{w} to colonize grapefruit. Indeed, delivery of XopAG in grapefruit cells triggers HR, and its deletion in *Xcci*-A^{w} restores the ability of the bacterium to grow on grapefruit to levels close to that of *Xcci* A strains (i.e., causing it to be pathogenic to most commercial citrus) (71). The deletion of *xopAG* does not restore the ability of *Xcci*-A^{w} to grow on sweet orange nor does it increase the host range on which the strain is able to produce canker-like lesions, showing that the *Xcci*-A^{w} host range is shaped by multiple host-limiting factors (71).

Generalist: organism with an extended host range; i.e., it interacts with multiple hosts with little selectivity

TAL: transcription activator-like

However, when expressed by *Xcci*-A strains in grapefruit, *xopAG* or its homolog *avrGf2* induces an HR reaction (50). Conversely, T3Es may act as host-range broadening factors by suppressing ETI induced by another T3E. For example, the *Xanthomonas euvesicatoria* (*Xe*) T3E AvrBsT suppresses the AvrBs1-induced ETI in ECW-10R pepper plants (129). Recent studies highlighted that some T3Es may play a role in suppressing both PTI and ETI. For example, in *Xe*, XopQ suppresses cell death controlled by the ETI-associated MAP (mitogen-activated protein) kinase MAPKKKa in tomato and pepper (130), whereas in *Xoo*, XopQ was described to suppress DAMP-induced PTI in rice (120). Another example is that of XopB, which suppresses flg22-induced PTI as well as ETI induced by XopG, AvrBsT, and XopJ in *Nicotiana benthamiana* (116).

Because T3Es participate in host defense suppression and promote pathogen multiplication and dispersion, it has been suggested that the effectome, i.e., the complete repertoire of T3Es, may reflect the adaptation of strains to various hosts. To challenge this hypothesis, several studies investigated the distribution of known T3E genes in strain collections chosen to represent the known phylogenetic diversity of *Xanthomonas* pathovars. The strains used reflected a wide range of collection dates, locations, and host plants to maximize the natural genetic variation in T3E repertoires. These studies revealed that T3E repertoires differ between *Xanthomonas* species and also between strains within species (41, 55, 57–59, 112). Among pathogenic strains of *Xanthomonas*, *Xcr*, *X. arboricola* pathovars *celebensis*, *poinsettiicola*, and *fragariae*, and some strains of *Xanthomonas* spp. isolated in Rwanda have reduced T3E repertoires (5, 17, 59). *Xanthomonas sacchari* and strains of *X. arboricola* and "*Xanthomonas cannabis*" do not present known T3Es (67, 70, 126). Interestingly, in several *Xanthomonas* species, a correlation was observed between pathovars and the composition of T3E repertoires (57–59), further suggesting that T3E repertoires play a major role in determining the host specificity of strains. A role of two T3Es (AvrBsT and XopQ) in shaping the host range of *Xanthomonas perforans* among solanaceous species has recently been revealed (117). However, within pathovars, the picture is much more complicated: Whereas in some cases T3E variations observed within a pathovar were linked to the host of isolation of the strains (57, 58), in other cases no correlation could be observed between T3E variations and pathotypes or races (12, 51, 55, 112). These results highlight the complexity of the genetic basis of host specificity in plant-pathogenic bacteria. The presence of some T3E genes appears to be variable, depending on the pathovar or the strain. For instance, *xopAC* is unique to *X. campestris* strains (55). Interestingly, many of these variable T3E genes are associated with mobile genetic elements in *Xanthomonas* genomes. HGT of T3Es was suggested to be among the driving forces of the specialization of *Xanthomonas* strains on diverse crops (97).

Nonpathogenic strains of *Xanthomonas* devoid of T3SSs and T3Es (or featuring a very restricted number of T3E genes) were isolated from several ecosystems (42, 70, 131). An emerging picture of the acquisition of pathogenicity in strains of *Xanthomonas* involves as a first step the acquisition of master regulators, such as HrpG and HrpX, and key CWDEs (70). The acquisition of a T3SS and core T3Es as a second step could lead to the emergence of generalist pathogenic strains (70, 97). The extensive acquisition of novel T3Es and subsequent shaping of T3E repertoires through HGT events was hypothesized to account for specialization of strains on specific hosts (70, 95, 97). On top of HGT, selection of advantageous alleles seems to contribute to host adaptation (153).

Transcription activator-like (TAL) effectors are T3Es (almost) specific to *Xanthomonas* with unique properties. A survey for TAL effector genes among available genome sequences and in PCR- or RFLP-based analyses chosen from the literature revealed that 12 *Xanthomonas* species out of 29 carry at least one TAL effector gene (**Figure 1**). As expected, species without TAL effectors included the pathogen *X. albilineans*, which lacks the Hrp T3SS, and *X. vasicola*, *Xanthomonas vesicatoria*, *Xanthomonas fragariae*, and *X. oryzae* (US strains), which have possibly lost or never acquired TAL effector genes. Yet, absence of TAL effector genes in a few strains can be

misleading because the chosen strain might not be representative of its kind, as illustrated in *Xcc* (18). Hence, the analysis of additional strains might change our perspective on the conservation of TAL effector genes among *Xanthomonas* species. TAL effector genes tend to be over-represented in some lineages, such as pathovars of *X. oryzae*, with up to 27 family members in some *Xoc* strains. Depending on the pathovar, TAL effector genes may be either chromosomal or carried by plasmids, potentially reflecting a more recent acquisition in the latter case. TAL effectors act as plant transcription factors that are injected into the host cell by the T3SS to reprogram the host plant transcriptome. Although the mode of action of TAL effectors has become clearer in the past decade, notably through the discovery of the TAL effector–DNA binding code (15, 98), our understanding of their virulence function is still restricted to a few genes of a limited number of pathosystems. Yet, some patterns of TAL activity are emerging, such as the induction of membrane transporters to provide nutrients in the apoplast, or of transcription factors leading to developmental changes within host tissues. Citrus bacterial canker was shown to rely on the PthA series of TAL effectors from *X. citri*, which are responsible for the formation of pustules upon the induction of the transcription factor CsLOB1 (63). *Xoo* induces members of the *SWEET* family of sugar (e.g., sucrose) efflux transporters to potentially feed the bacteria (reviewed in 66). In *Xcc*, one CUT system, required for full pathogenicity on *Arabidopsis*, is involved in sucrose utilization (14). Although TBDT-mediated transport is an active, energy-consuming process for the bacterium, these genes are overrepresented in *Xanthomonas* species (14), suggesting that the benefits must be higher than the cost of their use. Determining whether *Xoo* uses a CUT system dedicated to the absorption of TAL effector–mediated sucrose delivery would be of interest (**Figure 2**). Such a finding would highlight a key feature of *Xanthomonas* adaptation based on an intimate interplay between T3SSs and the TonB-ExbBD system allowing nutrient capture in hosts, which is also illustrated by the coregulation of several TBDT and CWDE genes with T3SS genes (14, 129). *Xoc* activates the sulfate transporter *OsSULTR3;6*, which facilitates bacterial egress by as yet unknown mechanisms (25). This is reminiscent of the putative function of *Xcm* Avrb6 (148) and *Xe* AvrBs3, which leads to cell hypertrophy upon induction of the transcription factor–encoding gene *UPA20* (76). Interestingly, strains of *Xoc* transformed with *Xoo* TAL effectors targeting *OsSWEET14* cause higher leaf streak lesions but are unable to colonize the vascular tissues (135). Importantly, TAL effectors target susceptibility genes whose promoter sequences may vary among plant species. Because the specific induction of several of these susceptibility genes is needed for disease and bacterial multiplication, TAL effectors from a given *Xanthomonas* are adapted to the promoter sequence of the corresponding host. Thus, TAL effectors are archetypal host-specificity factors reflecting the coadaptation of *Xanthomonas* species and pathovars and their corresponding host plants.

Host-specificity factors: factors that allow an organism to exploit a particular feature of a given host

CAN GENOMICS HELP TO IDENTIFY NOVEL CANDIDATE FUNCTIONS INVOLVED IN HOST SPECIFICITY?

Currently, GenBank lists approximately 400 *Xanthomonas* genome sequences. Since the release of the first two *Xanthomonas* genome sequences in 2002 (32), approximately 50 complete genomes have been sequenced. The vast majority of the other genomes produced by next-generation sequencing yielded fragmented genome assemblies of uneven quality and propagation of severe misannotations while preventing the analysis of global genomic rearrangements. Transcriptomic data were not routinely used to improve structural gene annotation until recently (112, 115). All in all, we are now left with rich genomic resources of varying quality with respect to assembly and annotation, and with varying representativeness of the different *Xanthomonas* species and pathovars. This heterogeneity may impact the potential of these resources and demands standardized

reannotation of the genomes before using them for robust comparative analyses. However, third-generation sequencing technology will allow low-cost (re)sequencing of complete genomes (145), resolving most of the assembly problem. This breakthrough is expected to strongly boost our understanding of the dynamics of genomes and host specificity.

Whole-Genome Comparisons

As discussed above, host specificity in *Xanthomonas* is a complex process involving different functions ranging from chemotaxis with host manipulation. Initial analyses focused mainly on particular gene families. Whole-genome analyses can unveil new molecular determinants of host specificity. Genic and/or nongenic pathovar-specific signatures contain potential traces of host adaptation. Such signatures comprise (*a*) single nucleotide polymorphisms (SNPs) resulting from convergent (or divergent) evolution, (*b*) genomic islands or recombination resulting from HGT, and/or (*c*) positive selection resulting from host-driven selection pressure. A role of HGT in host adaptation was suggested by comparative genomics of 26 bean-pathogenic bacteria, with more than 100 genes appearing to have been acquired from *X. citri* pv. *phaseoli* var. *fuscans* by an ancestor of *Xap* (4). Likewise, comparison of 21 (153) and 43 (51) citrus-pathogenic strains suggested that acquisition and loss of genes may be associated with the broadening in host range of a single *Xcci* pathotype. Also, an over-representation of recombinants within positively selected genes was found in a comparative study of *X. campestris* and *X. citri* genomes, suggesting that HGT may be the cause for the observed adaptive diversification (65). Therefore, construction of robust phylogenies using genome-wide SNPs is essential to distinguish the neutral evolutionary history of pathovars and species from that of HGT (51).

Exploring Genome Plasticity and Effector Evolution

Several pathogenicity determinants such as TAL effector genes are often located in regions corresponding to mobile insertion cassettes (46), and knowledge about the structure, contents, and evolutionary history of those regions will greatly help in understanding the importance of recombination and HGT in host and tissue specialization. The recent sequencing and resequencing of several *X. oryzae* strains indeed revealed a dynamic genome structure and an important plasticity in TAL effector genes through HGT, duplication, and recombination (19). More generally, broad sequence analyses of genes coding for pathogenicity determinants together with their genomic environment will help to determine not only their functions but also the mechanisms by which they appear and evolve in a given population (88, 124).

The Larger, the Better

Although it is a cliché to say that conclusions will improve when more samples are analyzed, increasing the genomic coverage of the genus *Xanthomonas* is instrumental in unraveling the processes responsible for host specificity. This concerns three different scales: First, the growing number of genomic data will allow investigation of the extent these processes can be generalized to a broader panel of cases. Second, this will also provide a larger coverage of the diversity within species/pathovars, which should improve the robustness of comparative genomic investigations by reducing the amount of false-positive candidates. Finally, this should benefit from recent advances in the field of population genomics, which have the potential to improve our understanding of the evolutionary and demographic history of populations. The application of phylogenomics or approximate Bayesian computation inference approaches such as BEAST (38) or DIYABC

(30) can help in reconstructing the splits between populations and estimating past variation in population sizes such as bottlenecks and clonal expansion that can be directly linked with host specialization events. It is particularly important to sequence polyphyletic pathovars because each of them regroups strains with functional convergence for their host (57). Traces of HGT between phylogenetically distant strains can be found by direct genome comparisons in such pathovars; thus, they can be used as pioneer models for whole-genome studies of host specificity.

Complementarily, comparison of the transcriptomes of strains with differential host or tissue adaptation will allow assessment of the importance of gene expression regulation in adaptive processes and narrowing genomic analyses to genes that are specifically expressed (or repressed) during pathogenesis and in specific conditions. Most transcriptome studies on *Xanthomonas* were done in vitro (54, 71, 112), but experiments in other pathosystems show that the challenges of in planta bacterial transcriptome analyses can be overcome to identify factors related to niche adaptation and pathogenicity (93). Finally, complementing genomic data with genomes from nonpathogenic strains or strains from nonplant habitats will not only provide new comparative data to analyze host adaptation but may also unveil the importance of such reservoirs in emergent processes.

CONCLUSIONS

Here, we propose that host specificity of plant-pathogenic bacteria is the end result of multiple adaptive traits that first appear with the perception of the host and continue to multiplication inside the plant tissues. However, functional evidences are still lacking for many functions hypothetically involved in host specificity. T3Es are to date probably the gene class for which there is the most evidence for host range definition in pathogenic *Xanthomonas*, and we expect formal proofs to be presented in the coming years.

Controlling emerging diseases requires a deep understanding of the basis of plant bacteria coevolution associated with host shifts/jumps and proper management practices so that deployment of resistance genes or novel chemicals can be successful and long-lasting. However, adaptation-driven emergences are complex to evidence. This is an interesting challenge to our community, and will require a multidisciplinary approach (comparative genomics, functional genomics, population genomics, metagenomics, transcriptomics, and proteomics as well as microbial ecology, epidemiology, and phytopathology). Such an integrative approach combining -omics data and experimental studies has already been initiated on animal (including human) bacterial diseases. Comparable studies will undoubtedly emerge for plant-associated bacteria to improve our understanding of the adaptive and demographic processes that drive genetic changes in pathogen populations when submitted to selective pressures from their hosts. For instance, to our knowledge, there are no reports addressing host specificity in xanthomonads using experimental evolution, although this approach has proven to be extremely powerful in other phytopathogenic bacteria such as *Ralstonia solanacearum* when combined with genomics (53). In this bacterium, a single transcriptional regulator was shown to be responsible for host jump, highlighting that comparative genomics alone might not be sufficient to address host-specificity issues. In the near future, we believe that such approaches, in combination with full genome analyses and in planta transcriptomics, may give us the keys to decipher host specificity in *Xanthomonas*.

DISCLOSURE STATEMENT

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