What can bacterial genome research teach us about bacteria–plant interactions?

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Biological research is changing dramatically. Genomic and post-genomic research is responsible for the accumulation of enormous datasets, which allow the formation of holistic views of the organisms under investigation. In the field of microbiology, bacteria represent ideal candidates for this new development. It is relatively easy to sequence the genomes of bacteria, to analyse their transcriptomes and to collect information at the proteomic level. Genome research on symbiotic, pathogenic and associative bacteria is providing important information on bacteria–plant interactions, especially on type-III secretion systems (TTSS) and their role in the interaction of bacteria with plants.

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Abbreviations

avr	avirulence gene
GI	genomic island
ORF	open reading frame
PGPR	plant-growth-promoting rhizobacteria
Pst	*Pseudomonas syringae* pv. tomato
Rs	*Ralstonia solanacearum*
TTSS	type-III secretion system
Xac	*Xanthomonas axonopodis* pv. citri
Xcc	*Xanthomonas campestris* pv. campestris
Xf	*Xylella fastidiosa*
***Xf*-ALS**	*X. fastidiosa* strain Dixon
***Xf*-CVC**	*X. fastidiosa* 9a5c
***Xf*-OLS**	*X. fastidiosa* strain Ann1
***Xf*-PD**	*X. fastidiosa* strain Temecula

Introduction

Bacteria–plant interactions can be subdivided into three classes: symbiotic, pathogenic and associative. The symbiotic interaction is characterized by the formation of root nodules that are colonised by a microsymbiont. As a result, the microsymbiont is able to fix atmospheric nitrogen and to deliver fixed nitrogen to the macrosymbiont. Phytopathogenic interactions are more diverse. Phytopathogenic bacterial species have developed specific methods to attack plant cells and to use plant substances for their own growth. In the associative interaction, both the bacteria and the plant profit from each other. The bacteria live on plant exudates and either protect the plant by suppressing plant pathogens or stimulate plant growth by providing specific bacterial substances.

Because bacteria–plant interactions play an important role in agriculture, a lot of effort has been put into analysing these interactions in detail. The first sequenced genome of a symbiotic bacterium was that of *Mesorhizobium loti* [1], followed by that of *Sinorhizobium meliloti* [2]. Amongst phytopathogenic bacteria, the genome of *Xylella fastidiosa* (*Xf*) [3] was the first to be sequenced. Sequencing projects are underway for associative bacteria but none has been completed.

Although the genomic sequences of plant-colonising bacteria have only been established recently, review articles that summarise and compare the findings already exist. The review by Van Sluys *et al.* [4] is an interesting article that compares the genome sequences of seven plant-colonising bacteria. The genomes included are those of *Agrobacterium tumefaciens* [5,6], *M. loti* [1], *S. meliloti* [2], *Xanthomonas campestris* pv. campestris (*Xcc*) [7], *Xanthomonas axonopodis* pv. citri (*Xac*) [7], *Xf* [3] and *Ralstonia solanacearum* (*Rs*) [8]. The article concentrates on genome structure and metabolic pathways. It highlights type-III secretion systems (TTSS) for the control of host compatibility and reports on cell-wall-degrading enzymes and genes that are involved in overcoming the oxidative burst that is induced in the plant host.

Another recent review, by Weidner *et al.* [9], deals with genomic insights into symbiotic nitrogen fixation. This article covers genomic aspects of both the micro- and the macrosymbiont, and concentrates on the interactions between *M. loti* and *Lotus* species and between *S. meliloti* and *Medicago*. Of special interest, this review article also reports on the use of transcriptomics and proteomics techniques to analyse the interaction between *Rhizobium* sp. NGR234 and *S. meliloti*.

Further advances have been made since the publication of these reviews. Importantly, the genomes of the symbiotic bacterium *Bradyrhizobium japonicum* [10••] and the phytopathogen *Pseudomonas syringae* pv. tomato (*Pst*) [11••] have been sequenced and published. Furthermore, recent articles have also highlighted findings from transcriptomic and proteomic approaches. In this review, therefore, we discuss these new findings and concentrate in particular on TTSS in symbiotic and phytopathogenic interactions. In addition, associative bacteria–plant interactions are considered for the first time in the light of bacterial genome research.

Rhizobium–legume symbiosis viewed by bacterial genome research

The *Rhizobium*–legume symbiosis, which is characterized by the formation of root nodules, is the most important bacteria–plant interaction. For this reason, the genomes of three microsymbionts, namely *B. japonicum* USDA110 [10••], *M. loti* MAFF303099 [1] and *S. meliloti* 1021 [2], have already been sequenced. The structure of their genomes differs significantly (Table 1). The *S. meliloti* genome consists of a chromosome and two megaplasmids, the *M. loti* genome of a chromosome and two large plasmids, whereas *B. japonicum* only harbours one chromosome.

The *B. japonicum* genome, which was sequenced only recently, consists of a single circular chromosome and is outstanding because of its relatively large size of 9 105 828 bp [10••]. GC skew analysis and the presence of genes that are involved in replication indicate that the *B. japonicum* chromosome contains an *oriC* region around the coordinate of 700 kb. Altogether, 8317 putative protein-coding open reading frames (ORFs), 50 tRNA genes, one *rrn* operon, a split transfer-messenger RNA molecule (tmRNA) and the RNA component of RNase P have been identified within the *B. japonicum* chromosome. The presence of genes that encode proteins that are typically required for conjugative plasmid transfer suggests that one or more parts of the chromosome originated from lateral gene transfer. This assumption is supported by the observation that the G+C content of some regions of the chromosome is considerably lower than the average value of 64.1%. The largest region of low G+C content (59.4%) is 680 kb in size and contains all of the known nodulation and nitrogen fixation genes. In addition, several other genes that seem to be related to symbiosis are present in this region. One example is a *dctA*-like gene that is thought to encode a dicarboxylate transporter. Genes that are potentially required for molybdate uptake and hydrogen utilisation (e.g. the *hup* gene cluster) also reside in the symbiotic gene region. Multiple changes in the

Table 1

The structure of published bacterial genomes relevant for bacteria–plant interactions.

Organism	Abbreviation	Genome size (kb)	Genome structure	Size (bp)	Reference
A. tumefaciens C58 DuPont (UWash)	*At*	5674	Circular chromosome	2 841 490	[5]
			Linear chromosome	2 075 560	
			AT plasmid	542 780	
			Ti plasmid	214 234	
A. tumefaciens C58 Cereon	*At*	5673	Circular chromosome	2 841 481	[6]
			Linear chromosome	2 074 782	
			AT plasmid	542 869	
			Ti plasmid	214 233	
B. japonicum USDA110	*Bj*	9105	Chromosome	9 105 828	[10••]
M. loti MAFF303099	*Ml*	7596	Chromosome	7 036 074	[1]
			Plasmid pMLa	351 911	
			Plasmid pMLb	208 315	
P. syringae pv. tomato DC3000	*Pst*	6538	Chromosome	6 397 126	[11••]
			Plasmid pDC3000A	73 661	
			Plasmid pDC3000B	67 473	
R. solanacearum GMI1000	*Rs*	5810	Chromosome	3 716 413	[8]
			Megaplasmid	2 094 509	
S. meliloti 1021	*Sm*	6690	Chromosome	3 654 135	[2]
			Megaplasmid pSymA	1 354 226	
			Megaplasmid pSymB	1 683 333	
X. axonopodis pv. citri 306	*Xac*	5273	Chromosome	5 175 554	[7]
			Plasmid pXAC33	33 700	
			Plasmid pXAC64	64 920	
X. campestris pv. Campestris ATCC33913	*Xcc*	5076	Chromosome	5 076 188	[7]
X. fastidiosa 9a5c	*Xf*-CVC	2679	Chromosome	2 679 306	[3]
			Plasmid pXF51	51 158	
			Plasmid pXF1	8072	
X. fastidiosa Temecula1	*Xf*-PD	2519	Chromosome	2 519 802	[52]
			Plasmid pXFPD1.3	1345	

nucleotide sequence of the *hup* cluster most likely resulted in the formation of non-functional pseudogenes in the symbiotic region. The presence of 100 transposase-encoding/insertion-sequence-related elements within the symbiotic region indicates plasticity and ongoing restructuring. Work on *S. meliloti* has shown that the structure of rhizobial genomes can be highly dynamic (e.g. [12]).

Little is known about the communication between *B. japonicum* cells. However, it is known that quorum sensing and the production of acyl-homoserine lactone occurs in many plant-colonising bacteria, including some rhizobial strains [13–15]. The genome sequence of *B. japonicum* reveals the presence of the *blr1063* gene, which putatively encodes an autoinducer synthetase. Upstream of it, a transcriptional regulator belonging to the LuxR family is encoded by *blr1062*. In addition, *B. japonicum* can produce an autoinducer that differs from the usual acyl-homoserine lactone-type. This molecule, called bradyoxetin, is involved in the cell-density-dependent regulation of nodulation gene (*nod*) expression [16].

Recently, a *B. japonicum* TTSS that influences the interaction with host plants has been described [17,18]. TTSSs are highly conserved multi-protein complexes that span the entire bacterial cell envelope [19]. They are conserved among animal and plant pathogenic bacteria [20,21]. In response to environmental and host-derived signals, TTSSs transport a wide variety of effector proteins either into the extracellular medium or into the cytoplasm of eukaryotic host cells. TTSSs that were previously thought to be unique to pathogenic bacteria have now been shown to exist in several rhizobia [22].

The TTSSs of rhizobia are induced by plant-derived flavonoids [22–24]. To date, *nopA*, *nopX* and *nopL* from *Rhizobium* sp. strain NGR234 [24,25•] and *nopX* of *Sinorhizobium fredii* [26] are the only genes that are known to encode proteins secreted by the TTSS. However, the functions of these proteins are still unknown. Mutations within the type-III gene clusters of *B. japonicum*, *Rhizobium* sp. strain NGR234 and *S. fredii* affect nodule development. Depending on the host, the symbiotic capacity of mutant strains is impaired, improved or unaltered [18,24,27,28].

Interestingly, *S. meliloti* [2] and the symbiotic island of *M. loti* strain R7A [29] do not contain such a system. But these two strains encode proteins that are similar to the VirB proteins of *A. tumefaciens*, which comprise a type-IV secretion system. A type-IV secretion system was also found by sequencing the symbiotic plasmid of *Rhizobium etli* CFN42 [30].

In the near future, much more genomic information concerning the microsymbionts of the *Rhizobium*–legume symbiosis will become available because two further *Rhizobium* genome projects have been started. Sequencing of the genomes of *Rhizobium leguminosarum* bv. Viciae 3841, the microsymbiont of *Vicia*, *Pisum*, *Lathyrus* and *Lens*, and of *R. etli* CFN42, the microsymbiont of *Phaseolus* is ongoing (Table 2).

The genome of an endophytic bacterium known to infect grasses and to fix atmospheric nitrogen, namely *Azoarcus* sp. BH72, has also been sequenced and is of special interest (Table 2). The *Azoarcus* genome sequence should help to determine whether endophytic and endosymbiotic bacteria infect and colonise plants in a comparable manner.

Transcriptomics and proteomics contribute to the functional genomics of rhizobia

The basis upon which we are able to study rhizobia has changed because of the availability of complete genome sequences. Knowing the complete sequence of a genome is, however, only the first step towards understanding how all of the components of a bacterial cell work together. Information about a predicted gene can be deduced from similarities between its sequence and those of genes or motifs of known function, from the location of a gene in an operon or a gene cluster, from mutant phenotypes, and from expression patterns. Gene-expression analyses comprise monitoring changes in the levels of RNA (transcriptome) and protein (proteome). In the post-genomic era, experimental approaches have moved from the targeted investigation of individual genes to the investigation of thousands of genes in parallel experiments.

Before complete genome sequences and array techniques for gene-expression profiling became available, approaches such as transposon mutagenesis, RNA fingerprinting and promoter probe assays were used to identify loci that are associated with symbiosis in *S. meliloti* [31,32]. The gene fusion technique was expanded to the genome-wide scale using a Tn*5* derivative that contained the promoterless reporter gene *luxAB*. This approach was used by Milcamps *et al.* [33] to identify loci that are induced by carbon and nitrogen deprivation. Perret *et al.* [34] pioneered transcriptome analysis in rhizobia by producing the first transcriptome map of the *Rhizobium* sp. NGR234 symbiotic plasmid. This map was generated by the hybridisation of complex labelled RNA samples to Southern blots of polymerase chain reaction (PCR) fragments.

In recent years, DNA macro- and microarrays have developed into powerful tools for large-scale expression analysis at the level of transcription. The availability of the complete genome sequence of *S. meliloti* 1021 [2] has led to the development of pilot macroarrays, one containing 214 and another 34 *S. meliloti* genes, which have been analysed under a variety of symbiotic and non-symbiotic conditions [35,36]. Recently, comprehensive macroarrays

Table 2

Completed or ongoing bacterial genome projects relevant for bacteria–plant interactions.

Organism	Abbreviation	Host	Interaction	Status	Website/institution
A. tumefaciens C58-DuPont	*At*	Dicotyledons	Pathogenic	Completed	www.tigr.org
A. tumefaciens C58 (Uwash)	*At*	Dicotyledons	Pathogenic	Completed	www.agrobacterium.org
Azoarcus sp. BH72	*Az*	Grasses	Endophytic	Ongoing	www.Genetik.Uni-Bielefeld.DE/GenoMik/partner/bremen.html
B. cenocepacia J2315 (formerly *cepacia*)	*Bc*	Various plants	Pathogenic	Ongoing	www.sanger.ac.uk/Projects/B_cenocepacia/
B. cepacia R1810	*Bc*	Various plants	Pathogenic	Ongoing	www.jgi.doe.gov/JGI_microbial/html
B. japonicum USDA110	*Bj*	Soybean	Symbiotic	Completed	www.kazusa.or.jp/rhizobase/Bradyrhizobium/
C. michiganensis subsp. michiganensis	*Cmm*	Tomato	Pathogenic	Ongoing	www.Genetik.Uni-Bielefeld.DE/GenoMik/partner/bi_eichen.html
C. (*Corynebacterium*) *michiganensis* subsp. sepedonicus ATCC 33113	*Cms*	Potato	Pathogenic	Ongoing	www.sanger.ac.uk/Projects/C_michiganensis/
E. carotovora subsp. Atroseptica	*Eca*	Potato	Pathogenic	Ongoing	www.sanger.ac.uk/Projects/E_carotovora/
Leifsonia xyli subsp. xyli (*Clavibacter xyli* sp. xyli)	*Lxx*	Sugarcane	Pathogenic	Ongoing	aeg.lbi.ic.unicamp.br
M. loti MAFF30309 (Ml)	*Ml*	*Lotus*	Symbiotic	Completed	www.kazusa.or.jp/rhizobase/Mesorhizobium/
P. fluorescens Pf0-1	*Pf*	Root colonising	Associative, PGPR	Ongoing	www.jgi.doe.gov/JGI_microbial/html/pseudomonas/pseudo_homepage.html
P. fluorescens Pf-5	*Pf*	Root colonising	Associative, PGPR	Ongoing	www.ars-grin.gov/ars/PacWest/Corvallis/hcrl/Pf5genome/status.htm
P. fluorescens SBW-25	*Pf*	Root colonising	Associative, PGPR	Ongoing	www.sanger.ac.uk/Projects/P_fluorescens/
P. syringae pv. syringae B728a	*Pss*	Bean	Pathogenic	Ongoing	www.jgi.doe.gov/JGI_microbial/html/
P. syringae pv. tomato DC3000	*Pst*	Tomato, *Arabidopsis*	Pathogenic	Completed	www.tigr.org
P. syringae pv. phaseolicola1448A	*Psp*	Bean	Pathogenic	Ongoing	pseudomonas-syringae.org/psp_home.html
R. solanacearum GMI1000	*Rs*	Wide host range	Pathogenic	Completed	sequence.toulouse.inra.fr/R.solanacearum
R. etli CFN42	*Re*	*Phaseolus*	Symbiotic	Ongoing	www.cifn.unam.mx/
R. leguminosarum bv. viciae 3841	*Rlv*	*Vicia, Pisum, Lathyrus, Lens*	Symbiotic	Ongoing	www.sanger.ac.uk/Projects/R_leguminosarum/
S. meliloti 1021	*Sm*	*Medicago*	Symbiotic	Completed	sequence.toulouse.inra.fr/S.meliloti.html
X. axonopodis pv. aurantifolii B	*Xaa*	*Citrus*	Pathogenic	Ongoing	genoma4.iq.usp.br/projects/aurantifolii/
X. axonopodis pv. aurantifolii C	*Xaa*	*Citrus*	Pathogenic	Ongoing	genoma2.fcav.unesp.br/aurantifolii
X. axonopodis pv. citri 306	*Xac*	*Citrus*	Pathogenic	Completed	cancer.lbi.ic.unicamp.br/xanthomonas/
X. campestris pv. campestris 8004	*Xcc*	Crucifers	Pathogenic	Completed	cancer.lbi.ic.unicamp.br/xanthomonas/
X. campestris pv. campestris ATCC33913	*Xcc*	Crucifers	Pathogenic	Ongoing	cancer.lbi.ic.unicamp.br/xanthomonas/
X. campestris pv. campestris B100	*Xcc*	Crucifers	Pathogenic	Ongoing	www.Genetik.Uni-Bielefeld.DE/GenoMik/partner/bi_niehaus.html
X. campestris pv. vesicatoria	*Xcv*	Tomato, pepper	Pathogenic	Ongoing	www.Genetik.Uni-Bielefeld.DE/GenoMik/partner/halle.html
X. fastidiosa 9a5c	*Xf*-CVC	*Citrus*	Pathogenic	Completed	aeg.lbi.ic.unicamp.br/xf/
X. fastidiosa Temecula1	*Xf*-PD	Grapevine	Pathogenic	Completed	aeg.lbi.ic.unicamp.br/world/xfpd/
X. fastidiosa Dixon (pv. almond)	*Xf*-ALS	Almond	Pathogenic	Ongoing/draft	www.integratedgenomics.com/genomereleases.html www.jgi.doe.gov/
X. fastidiosa Ann1 (pv. oleander)	*Xf*-OLS	Oleander	Pathogenic	Ongoing/draft	www.integratedgenomics.com/genomereleases.htm www.jgi.doe.gov/

and microarrays comprising all of the 6207 currently predicted protein-coding genes of *S. meliloti* 1021 have been generated [37•,38]. As a step towards understanding the physiology of *S. meliloti* in its free-living and symbiotic forms, these arrays were employed for expression profiling under oxic and microoxic conditions and in both cultured cells and nodule bacteria. The transcriptome profiles highlighted a profound modification of gene expression during bacteroid differentiation, with 16% of genes showing altered expression. Transcriptome profiling generated under conditions of limited oxygen indicated that up to 5% of *S. meliloti* genes are regulated by oxygen. The low-oxygen and symbiotic transcriptomes included numerous genes that had not previously been identified as being induced under these conditions. Genome-wide studies of gene expression will advance our understanding of *S. meliloti*'s versatility in adapting to life both in the soil and as an organelle-like endosymbiont inside a plant cell, and will lay the foundation for future studies.

Proteomics approaches have been used to study protein patterns in several rhizobial species including *R. leguminosarum*, *R. etli* and *S. meliloti* [39–44]. The proteomics approach is most advanced for *S. meliloti*. Reference maps for the early and the late exponential growth phase, as well as protein patterns for bacteroids, were established before the genome sequence of *S. meliloti* became available [40,45]. The *S. meliloti* genome sequence paved the way for more sophisticated profiling of protein patterns. Djordjevic *et al.* [46•] used a combination of two-dimensional (2D) gel electrophoresis and peptide mass fingerprinting to investigate the protein patterns of nodule bacteria and of cultured bacteria in response to various stress conditions. They identified 1180 protein products derived from 810 genes (13.1% of the predicted genes), demonstrating that proteomic analysis is a powerful approach for global analysis of protein profiles and to screen for the processing and modification of proteins.

A large number of studies that include proteomic and transcriptomic approaches have followed this stage-setting work or have been initiated recently, confirming the widespread acceptance of these methods in the investigation of rhizobia–legume symbioses.

Phytopathogenic bacteria and the genomic boost

The published sequenced genomes of phytopathogenic bacteria are listed in Table 1. Altogether, the genome sequences of eight different phytopathogens belonging to the genera *Agrobacterium*, *Pseudomonas*, *Ralstonia*, *Xanthomonas* and *Xylella* are available. Once again, their genome structures differ significantly. Most of these phytopathogens contain a circular chromosome and, with the exception of *Xcc* ATCC 33913, plasmids of varying sizes. *A. tumefaciens* C58 harbours an novel linear chromosome in addition to a circular one.

The analysis of genomic data from phytopathogenic bacteria has remarkably expanded the repertoire of putative genes thought to control pathogenic interactions with plants. These include genes that are required for adhesion, phytotoxin production, resistance to oxidative stress, the degradation of plant cell walls, the production of plant hormones, the production and injection of effectors into host cells and interference with host defences. Altogether, these genes represent about 6% of the genes identified in the genomes of bacterial plant pathogens. A recent review that compared the genomes of plant-colonising bacteria described these different functions in detail [4]. Here, we relate only how genomics has enhanced research on *Xylella* and TTSS.

Xf strains infect the xylem vessels of higher plants. They are exclusively transmitted by specific leafhoppers and do not seem to have an alternative habitat [47]. They are difficult to grow on laboratory media, and this obstacle has impaired their study by classical approaches. *Xylella* is most closely related to the genus *Xanthomonas*, but its genome is much smaller than that of *Xanthomonas* (Table 1). This genome reduction may be related to its restricted habitat. *Xf* may therefore represent a minimal model pathogen in which to study host adaptation [48,49].

Several pathogenicity determinants that have been identified in *Xanthomonas* seem to be conserved in the *Xylella* genome [3,48–52]. In particular, the *rpf* gene cluster of *Xcc* (Table 1), which regulates the expression of virulence factors, is highly conserved in *Xf*. In *Xcc*, this cluster controls the production and perception of a diffusible signal factor (DSF) [53]. Recent bioassays indicate that *Xf* does indeed produce a molecule that is similar to DSF, suggesting that the control of some pathogenicity programs is conserved in both pathogens [54].

The recent sequencing of three other *Xf* strains (strain Temecula, which causes Pierce disease of grapevine [*Xf*-PD] [52]; strain Dixon [*Xf*-ALS], which causes almond lead scorch; and strain Ann1 [*Xf*-OLS]), which causes oleander leaf scorch [50,51]) has allowed comparative studies (Table 2). Three large chromosomal rearrangements and other smaller rearrangements were detected in comparisons of the *Xf*-9a5c (*Xf*-CVC) and *Xf*-PD genomes. All of these rearrangements were found in strain-specific genes and were phage-associated. These comparisons also defined two genomic islands (GIs), one specific to each of the *Xf*-CVC and *Xf*-PD genomes [52]. Interestingly, part of the *Xf*-CVC GI (GI-CVC1) was partially conserved in *Xac*, which is also pathogenic to citrus [52]. Comparison of *Xf*-CVC with gapped-genomic sequences from *Xf*-ALS and *Xf*-OLS revealed genes that are unique to each strain, pointing out potential differences in their adhesion mechanisms [50,51]. Furthermore, it appears that a series of conjugational-related ORFs found in *Xf* strains could have been acquired from soil-inhabiting bacteria [50].

Microarrays containing 2200 ORFs from *Xf*-CVC were used to compare the genomes of 11 *Xf* isolates obtained from different hosts. This work confirmed the existence of flexible pools of genes that represent up to 18% of the genome and are related to prophages, plasmids and GIs [55]. Interestingly, transcriptome analyses revealed that the expression of genes in these putative horizontally acquired elements is co-ordinated and influenced by environmental conditions [55]. Microarray analyses also showed that genes that are probably involved in adhesion or in adaptation to the host environment seem to be specifically induced in *Xf*-CVC bacteria that are freshly isolated from host plants, rather than in bacteria that have been submitted to several passages in axenic cultures [56]. Finally, analysis of the *Xf*-CVC proteome, combined with a codon-usage study of the most-expressed proteins, revealed a low biased distribution that might be related to

the slow growth capacity of this pathogen [57]. An interesting question is whether this codon bias represents a specific adaptation to nutrient-limited environments and/or whether it constitutes a way to limit bacterial growth rate, thus allowing the pathogen to escape plant defences.

Until now, the genomes of the phytopathogenic genera *Xylella* and *Xanthomonas* have been reviewed most often. Our knowledge on these genera will be expanded in the near future because further genome projects are in progress (Table 2). We should mention that the genomes of two further *P. syringae* strains, *P. syringae* pv. syringae B728a and *P. syringae* pv. phaseolicola 1448A, are being sequenced (Table 2). The genomes of *Erwinia carotovora* subsp. atroseptica and *Burkholderia cepacia* R1810 are also being sequenced.

The genome projects for Gram$^+$ *Clavibacter* strains are of special interest. The genomic sequencing of *Clavibacter michiganensis* subsp. Michiganensis (a tomato pathogen) and of *C. michiganensis* subsp. Sepedonicus (a potato pathogen) is under way (Table 2). In addition, the genome of *Leifsonia xyli* subsp. xyli (formerly *Clavibacter xyli* subsp. xyli) is being sequenced. Little information is available on the interaction of Gram$^+$ bacteria with their host plants, and so it is expected that these genome projects will unravel how these pathogenic bacteria infect and colonise susceptible plants.

Genomics of phytopathogenic bacteria reveals a myriad of novel TTSS substrates

In plant pathogens, TTSS are encoded by *hrp* and *hrc* genes that are essential for pathogenicity and the elicitation of the hypersensitive response (HR). Two types of proteins appear to transit via the Hrp machinery: effectors that interfere with the functions of plant cells and helper proteins that facilitate the translocation of effectors into host cells [58]. The effector proteins include avirulence gene (*avr*) products that induce host resistance in plants that carry matching resistance genes (*R*).

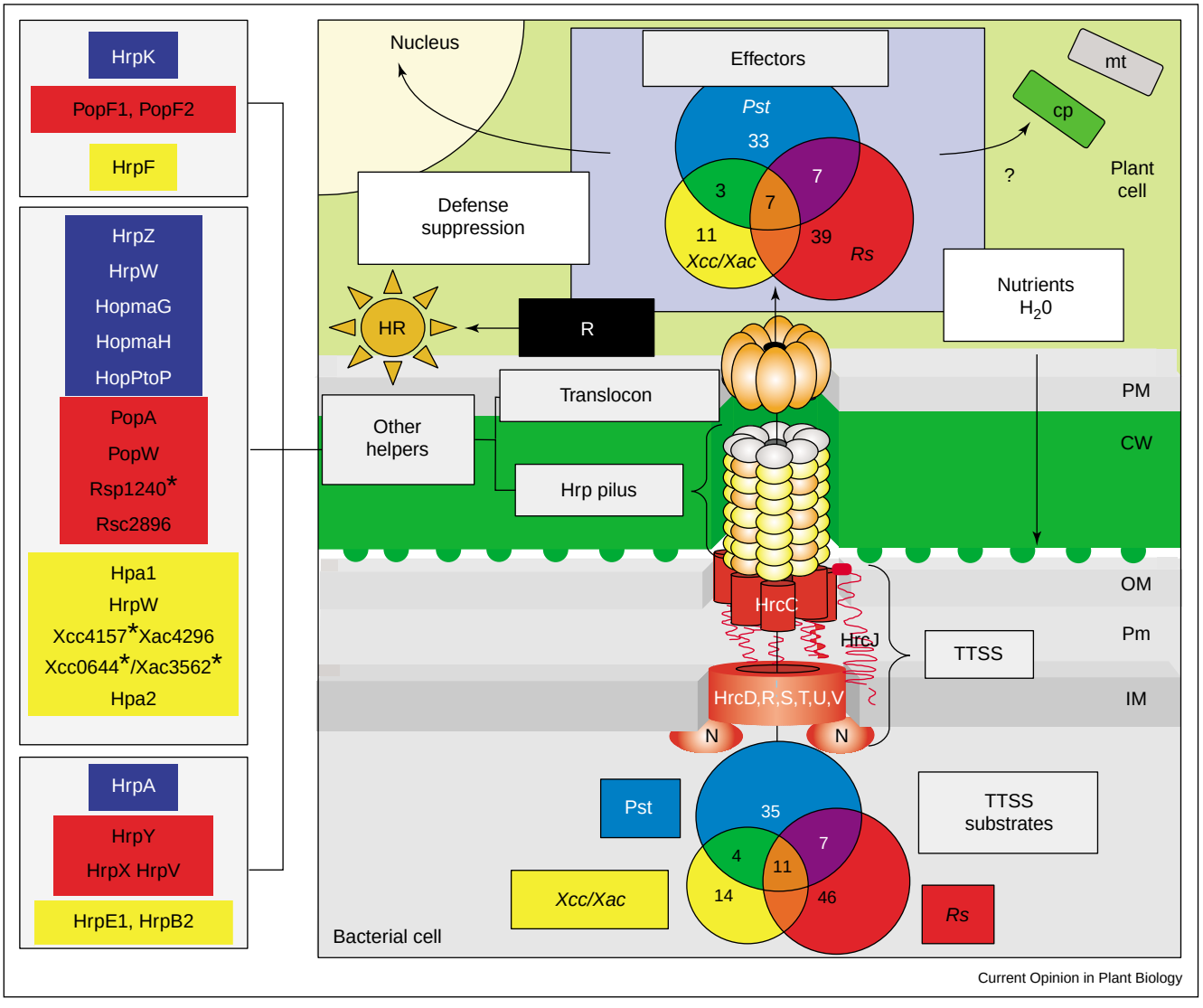
hrp clusters were found some time ago in *Rs*, *Pst* and *Xanthomonas* spp, but the number of identified effectors remained low for many years ([20]; Tables 1 and 2). Genome sequencing has revealed a considerable repertoire of novel TTSS effector candidates in these pathogens (Figure 1). The strategies used to detect such novel proteins have already been reviewed in detail [58,59]. In *Rs*, 57 novel TTSS-substrate candidates have been identified, mainly on the bases of homology or the presence of eukaryotic features [8]. A major surprise from this analysis was the discovery of several *avr*-like genes in *Rs*. Two of these genes were subsequently shown to be true *avr* genes in interactions with *Petunia* and *Arabidopsis* [60,61•]. The genomic sequences of *Xcc* and *Xac* were used to detect genes that have a PIP box in their promoter regions [7]. The PIP box is a consensus nucleotide sequence that is present in several genes that are regulated by HrpX, a regulatory protein that positively regulates *Xanthomonas hrp* genes [62]. Consequently, 17 and 20 HrpX-regulated gene candidates were identified in *Xcc* and *Xac*, respectively. Interestingly, several genes that seem to belong to this regulon are not direct TTSS substrates. Furthermore, this work revealed approximately 20 new TTSS-effector candidates in both *Xcc* and *Xac*.

In *Pst*, novel TTSS substrates were recently revealed by regulation, secretion and translocation functional assays [63••,64,65••,66••,67]. These studies involved genome mining for the presence of a hrp box in promoter regions [64,66••,67]. The hrp box is a *cis*-element found in the promoter regions of *hrp*, *hrc* and effector genes that is presumably recognised by HrpL sigma factor [68]. Also in *P. syringae*, comparison of Avr and Hrp outer proteins (Hops) revealed similar amino-acid biases in the amino-terminal secretion regions of these proteins. Genome-wide surveys for these properties were then used to characterise new TTSS substrates [65••,66••]. Altogether, 57 TTSS effectors or helpers were identified in the *Pst* genome [11••,63••,64,65••,66••,67]. Interestingly, more than half of these TTSS effectors are clustered in one of several regions that contain multiple effector genes [11••]. The putative function of some of these effectors has been discussed in previous reviews [58,59]. Interestingly, 17 of the putative *Pst* effector/helper proteins are conserved in the *Rs* genome [11••], five of which were not detected during the previous analysis of *Rs* genome [8]. Similarly, ten of the *Pst* effectors are conserved in the *Xcc* and *Xac* genomes, thus defining five novel candidates in both species. Finally, four effector and four helper proteins seem to be conserved in *Pst*, *Xcc*, *Xac* and *Rs*. These results suggest that some of the mechanisms that facilitate the translocation of effectors through the plant cell wall and membranes are conserved. Furthermore, the conserved effectors perform core functions that are indispensable for bacterial pathogenesis on plants. Thus, some of the conserved effectors display similarities with ADP ribosyl transferases and might therefore be crucial for altering plant signal transduction pathways [11••]. The characterisation of host plant targets is now a major challenge in determining the mode of action of all of these effectors. The model plant *Arabidopsis*, a host plant for *Pst*, *Xcc* and *Rs*, will certainly accelerate this quest.

Genomic approaches for associative bacteria

Plant-growth-promoting rhizobacteria (PGPR) are characterised by their abilities to colonise plant roots without causing disease and to confer benefit to the plant. This benefit generally comes from the production of growth stimulators or through the suppression of pathogens [69,70]. Most genomic effort for PGPR has been devoted to species of *Pseudomonas fluorescens*, a bacterium that is an

Figure 1



Model for Hrp-TTSS in entirely sequenced phytopathogenic bacteria: *P. syringae* pv. tomato (*Pst*), *R. solanacearum* (*Rs*), *X. campestris* pv. campestris (*Xcc*) and *X. axonopodis* pv. citri (*Xac*). Hrp-TTSS allow the injection of bacterial effector proteins into plant cells. The TTSS basal body (drawn in red) spans both bacterial membranes. (Hrc proteins for which a localisation has been shown or predicted are represented.) The basal body is associated with a pilus (Hrp pilus), which presumably traverses the plant cell wall (not drawn to scale). Helper proteins may facilitate this process. A translocon embedded in the plasma membrane (PM) of the plant cell allows the delivery of effector proteins into the plant cell cytoplasm. Colour-coded circles represent pools of TTSS substrates for each pathogen (blue: *Pst*; red: *Rs*; yellow: *Xcc* and *Xac*). Numbers in circles show the number of TTSS substrates (in bacterial cells) or effectors (in plant cells) that have been identified by genomic and functional analyses of each pathogen. (For *Xcc* and *Xac*, the numbers correspond to substrates shared by the two bacteria and substrates that are specific to each pathovar.) Numbers in intersecting areas correspond to TTSS substrates that are shared by pathogens. TTSS substrates comprise helper proteins, Hrp pilus components, translocon proteins and effectors. Proteins belonging to the translocon are shown in the top left box of the diagram with the same colour code (*Pst*: blue; *Rs*: red; *Xcc* and *Xac*: yellow). Helper proteins and Hrp pili proteins are shown in the central left box and in the bottom left box, respectively. Effector proteins are injected into plant cell cytoplasm and can be targeted to different locations (i.e. nucleus [21] and presumably chloroplasts [59]). In susceptible hosts, they presumably suppress plant defences and/or promote nutrient and water leakage. In resistant plants, effectors (named Avr proteins) can be recognised by resistance sentinel proteins (R) and trigger the activation of specific defence responses that are associated with the hypersensitive response (HR). Proteins that possess a putative signal peptide and might not represent TTSS substrates are marked with an asterisk. cp, chloroplast; CW, cell wall; IM, inner membrane; mt, mitochondria; OM, outer membrane; Pm, periplasm.

efficient coloniser of plant roots and possesses species-specific PGPR properties. Three species of *P. fluorescens*, Pf0-1, Pf5, and SBW25, are being sequenced (Table 2). The latter two have been best studied. Pf5 produces the anti-fungal metabolites pyoluteorin and 2,4-diacetyl phloroglucinol, whereas SBW25 has an unknown mechanism

for conferring protection against pathogens. Draft sequences are available for all three strains; the SBW25 genome is likely to be the first to be finished, the complete annotated sequence was due in January 2004.

Understanding which genes or biological processes define and distinguish associative and infective interactions is a major problem. For this reason, it will be of great benefit to have the full genome sequence of three associative bacteria to compare with three pathovars of *P. syringae* (Table 2). It had been considered that possession of a TTSS was a defining trait of a pathogen, but SBW25 possesses an active TTSS that is expressed in the rhizosphere [71,72]. Furthermore, Southern blotting suggests that TTSS may be common in non-pathogenic species of *P. fluorescens* [72]. Hence, a rethink of what constitutes a pathogenicity determinant might be required. It may be that there is a continuum from pathogenic to associative interactions.

Rhizosphere colonisation is the key event that underpins an associative interaction with a plant. Perception of the plant, chemotaxis towards and along the root, and adhesion and colonisation at the root surface (rhizoplane) are the stages in this association. Plant exudates are the drivers of the plant–microbe interaction, and *P. fluorescens* exhibits a chemotactic response to these exudates [73•]. This response is needed for efficient colonisation of the entire root zone and can be separated from the requirement for motility [73•]. Investigation of the responses of gene expression to root exudates (and of gene expression *in situ*) will help to determine how a bacterium mediates a functional response to the presence of a plant. It may also explain why, even in associative interactions, host specificity plays such a significant role.

Functional genomics will also be important in clarifying the importance of phase variation, which is defined as the ability to switch phenotype reversibly, in PGPR function. Two recent studies have highlighted potential roles for phase variation. Sanchez-Contreras *et al.* [74] identified three phenotypic variants that were selected during the colonisation of alfalfa roots by *P. fluorescens* F113. Specific variants preferentially colonised different root zones. Significantly, there was also variation in several traits that are important for rhizosphere colonisation and biocontrol activity. Van den Broek *et al.* [75•] also identified phenotypic variation in *Pseudomonas*, again with linked effects on biocontrol traits such as the production of anti-fungal metabolites. Both of these studies established a link between phase variation and the GacS/GacA system, which is a global regulatory system that controls many traits that are associated with production of secondary metabolites. Van den Broek *et al.* [75•] suggest that mutation and reversion in the *gacS* locus may be the driver of phase variation, although the involvement of the site-specific recombinase (*sss*) may suggest another mechanism [74]. The major question is whether phase variation is simply selection for mutations or whether it constitutes a genuine regulatory system for facilitating association with plants. Although not conducted in a plant system, one recent study shows how a population of *P. fluorescens* SBW25 evolved during transition from individual to cooperating cells [76•]. This has important implications for the colonisation of roots as there is a major transition from free-living in the rhizosphere to living in an attached colony on the root surface, which may develop as a biofilm. Once the full genome sequences of the *P. fluorescens* strains are available, it will be possible to use transcriptome analysis to understand changes that occur in response to encountering a conditioned rhizosphere, to discover what happens during the transition from rhizosphere to rhizoplane, and to determine which traits distinguish associative and infective bacteria.

Conclusions

Most of the sequenced genomes of bacteria that are involved in bacteria–plant interactions belong to phytopathogenic bacteria. To date, seven genomes of phytopathogens have been sequenced and published, whereas the sequencing of only three genomes of symbiotic bacteria have been reported (Table 1). No genome project is yet finished for an associative plant-growth-promoting rhizobacterium. This situation will change in the near future as a further 21 genomes of bacterial species/strains that interact with plants are currently being sequenced (Table 2). These 21 genomes include three genomes of associative bacteria that have plant-growth-promoting properties.

One lesson that has been learnt from bacterial genome research concerns the wide distribution of bacterial TTSS, which evidently play a role in interactions with plant cells. It is especially interesting that genes specifying TTSS were found in the genomes of symbiotic, phytopathogenic and associative bacteria.

Post-genomic research is still a developing field. To date, only a limited number of studies concerning transcriptome or proteome analyses have been published. These studies, which were concentrated predominantly on symbiotic bacteria, were aimed at learning more about the functions of genes that are involved in bacteria–plant interactions. In the near future, the number of post-genomic studies will increase tremendously as the wealth of sequence data obtained facilitates the analysis of the different types of interactions between bacteria and plants.

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61. • Deslandes L, Olivier J, Peeters N, Feng DX, Khounlotham M, Boucher C, Somssich I, Genin S, Marco Y: **Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus**. *Proc Natl Acad Sci USA* 2003, **100**:8024-8029.

The authors show that PopP2, a type-III effector of *Rs* that belongs to the YopJ/AvrRxv family, is an avirulence protein that is recognised by RRS1-R. RRS1-R is the first member of the TIR-NBS-LRR subclass of R proteins and contains a WRKY motif that is characteristic of some plants transcriptional factors. Using a Split-Ubiquitin two-hybrid assay, the authors show that RRS1-R and PopP2 interact in yeast. Furthermore, using PopP2 or RRS1-R proteins fused to green fluorescent protein (GFP), they show that both proteins colocalise to the nucleus of *Arabidopsis* protoplasts after transient expression.

62. Wengelnik K, Bonas U: **HrpXv, an AraC-type regulator, activates expression of five of the six loci in the *hrp* cluster of *Xanthomonas campestris* pv. vesicatoria**. *J Bacteriol* 1996, **178**:3462-3469.

63. •• Boch J, Joardar V, Gao L, Robertson TL, Lim M, Kunkel BN: **Identification of *Pseudomonas syringae* pv. tomato genes induced during infection of *Arabidopsis thaliana***. *Mol Microbiol* 2002, **44**:73-88.

The authors used a promoterless *hrcC* gene to design a modified *in-vivo* expression technology (IVET) assay to trap *P. syringae* pv. tomato promoters that are induced during *Arabidopsis* infection. More than 500 *in-planta*-expressed (*ipx*) loci were identified. Many *ipx* fusions are dependent on HrpL, a regulator of the *hrp* regulon, and several of the identified genes either corresponded to known effectors or have since been confirmed to be effectors. However, most of the loci found in this work have yet to be characterised.

64. Fouts DE, Abramovitch RB, Alfano JR, Baldo AM, Buell CR, Cartinhour S, Chatterjee AK, D'Ascenzo M, Gwinn ML, Lazarowitz SG *et al.*: **Genomewide identification of *Pseudomonas syringae* pv. tomato DC3000 promoters controlled by the HrpL alternative sigma factor**. *Proc Natl Acad Sci USA* 2002, **99**:2275-2280.

65. •• Guttman DS, Vinatzer BA, Sarkar SF, Ranall MV, Kettler G, Greenberg JT: **A functional screen for the type III (Hrp) secretome of the plant pathogen *Pseudomonas syringae***. *Science* 2002, **295**:1722-1726.

The authors used a truncated AvrRpt2 that was deprived of its secretion signal to construct a transposon that generates transitional fusions. This transposon was used to identify 13 type-III effectors in *P. syringae* pv. maculicola. The authors noticed that the amino-terminal regions of these effectors have a biased amino-acid composition. They used this information and the presence of hrp boxes to perform a bioinformatic analysis on the *P. syringae* pv. tomato draft genome sequence. This led to the identification of 38 additional effectors, some of which have since been shown to be secreted or translocated (see [11••,58,59]).

66. •• Petnicki-Ocwieja T, Schneider DJ, Tam VC, Chancey ST, Shan L, Jamir Y, Schechter LM, Janes MD, Buell CR, Tang X *et al.*: **Genomewide identification of proteins secreted by the Hrp type III protein secretion system of *Pseudomonas syringae* pv. tomato DC3000**. *Proc Natl Acad Sci USA* 2002, **99**:7652-7657.

The analysis of a large number of known type-III effectors of *Pst* revealed a typical secretion pattern in the first 50 amino acids. This pattern and the presence of hrp boxes in promoter regions were used to analyse the draft genome sequence of *Pst*. Sixty putative effectors were detected, seven of which were shown to be Hrp-secreted. Several putative effectors have since been confirmed (see [11••,58,59]).

67. Zwiesler-Vollick J, Plovanich-Jones AE, Nomura K, Bandyopadhyay S, Joardar V, Kunkel BN, He SY: **Identification of novel *hrp*-regulated genes through functional genomic analysis of the *Pseudomonas syringae* pv. tomato DC3000 genome**. *Mol Microbiol* 2002, **45**:1207-1218.

68. Xiao Y, Hutcheson SW: **A single promoter sequence recognized by a newly identified alternate sigma factor directs expression of pathogenicity and host range determinants in *Pseudomonas syringae***. *J Bacteriol* 1994, **176**:3089-3091.

69. Bloemberg GV, Lugtenberg BJ: **Molecular basis of plant growth promotion and biocontrol by rhizobacteria**. *Curr Opin Plant Biol* 2001, **4**:343-350.

70. Walsh UF, Morrissey JP, O'Gara F: ***Pseudomonas* for biocontrol of phytopathogens: from functional genomics to commercial exploitation**. *Curr Opin Biotechnol* 2001, **12**:289-295.

71. Rainey PB: **Adaptation of *Pseudomonas fluorescens* to the plant rhizosphere**. *Environ Microbiol* 1999, **1**:243-257.

72. Preston GM, Bertrand N, Rainey PB: **Type III secretion in plant growth-promoting *Pseudomonas fluorescens* SBW25**. *Mol Microbiol* 2001, **41**:999-1014.

73. • De Weert S, Vermeiren H, Mulders IH, Kuiper I, Hendrickx N, Bloemberg GV, Vanderleyden J, De Mot R, Lugtenberg BJ: **Flagella-driven chemotaxis towards exudate components is an important trait for tomato root colonization by *Pseudomonas fluorescens***. *Mol Plant Microbe Interact* 2002, **15**:1173-1180.

The authors demonstrate that *Pseudomonas* exhibits chemotaxis towards tomato root exudates. Furthermore, by constructing *cheA* mutants, they establish that chemotaxis is required for efficient colonisation along the plant root. This is an important separation of chemotaxis from motility as traits that are important for rhizoplane colonisation.

74. Sanchez-Contreras M, Martin M, Villacieros M, O'Gara F, Bonilla I, Rivilla R: **Phenotypic selection and phase variation occur during alfalfa root colonization by *Pseudomonas fluorescens* F113**. *J Bacteriol* 2002, **184**:1587-1596.

75. • Van den Broek D, Chin-A-Woeng TF, Eijkemans K, Mulders IH, Bloemberg GV, Lugtenberg BJ: **Biocontrol traits of *Pseudomonas* spp. are regulated by phase variation**. *Mol Plant Microbe Interact* 2003, **16**:1003-1012.

This study and that of Sanchez-Contreras and colleagues [74] establish the consequences of phase (phenotypic) variation for rhizosphere function of *Pseudomonas* spp. The authors of these two papers propose a model that suggests that the modification of *gacA* and *gacS* is the trigger for phase variation. Given the relatively high frequency with which *gacS* and *gacA* mutants occur, this has implications for plant–*Pseudomonas* interactions. There is some debate regarding the significance of phase variation, and these studies pave the way for functional genomics to explore its biological relevance.

76. • Rainey PB, Rainey K: **Evolution of cooperation and conflict in experimental bacterial populations**. *Nature* 2003, **425**:72-74.

This study examines the evolutionary aspects of the selection of mutants during growth in an (artificial) ecosystem. This has correlations with the selection of mutants in the plant rhizosphere, and many implications for studies on *gacS* mutants and phase variation.