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## Plant-microbe interactions and the new biotechnological methods of plant disease control

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**Abstract** Plants constitute an excellent ecosystem for microorganisms. The environmental conditions offered differ considerably between the highly variable aerial plant part and the more stable root system. Microbes interact with plant tissues and cells with different degrees of dependence. The most interesting from the microbial ecology point of view, however, are specific interactions developed by plant-beneficial (either non-symbiotic or symbiotic) and pathogenic microorganisms. Plants, like humans and other animals, also become sick, but they have evolved a sophisticated defense response against microbes, based on a combination of constitutive and inducible responses which can be localized or spread throughout plant organs and tissues. The response is mediated by several messenger molecules that activate pathogen-responsive genes coding for enzymes or antimicrobial compounds, and produces less sophisticated and specific compounds than immunoglobulins in animals. However, the response specifically detects intracellularly a type of protein of the pathogen based on a gene-for-gene interaction recognition system, triggering a biochemical attack and programmed cell death. Several implications for the management of plant diseases are derived from knowledge of the basis of the specificity of plant-bacteria interactions. New biotechnological products are currently being developed based on stimulation of the plant defense response, and on the use of plant-beneficial bacteria for biological control of plant diseases (biopesticides) and for plant growth promotion (biofertilizers).

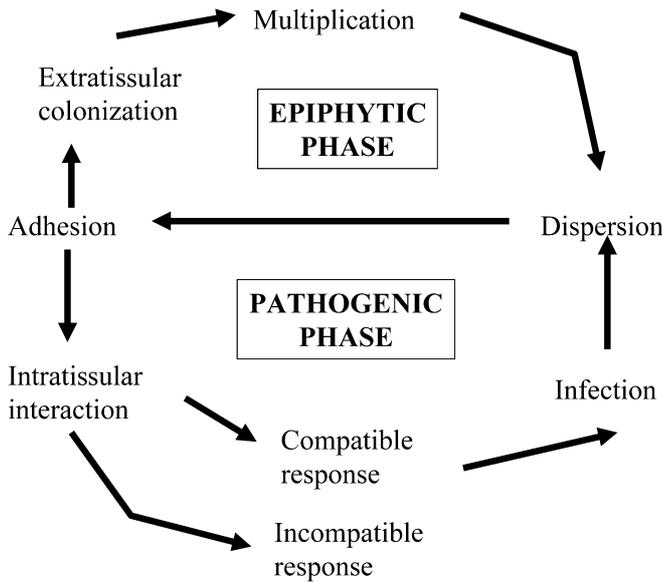
**Keywords** Plant-microbe interaction · Plant disease · Hypersensitivity reaction

### Introduction

Microorganisms have developed several strategies to adapt themselves to the plant environment, including beneficial or detrimental interactions. Beneficial interactions are caused by symbiotic or non-symbiotic bacteria and by a highly specialized type of fungi, the mycorrhizae. The pathogenic or detrimental interactions of microbes with plants involve viroids, viruses, bacteria and fungi, and lead to infectious diseases affecting only the plant kingdom. Losses in crop production due to plant disease average 13% in the world and severely limit production of food [2]. The 11,000 diseases that have been described in plants are caused by 120 genera of fungi, 30 types of viruses, and eight genera of bacteria (including two genera of mollicutes) [2]. Thus, many plant diseases are caused by fungi, in contrast to the situation in animals, in which fungal diseases are less frequent. Since the normal state of plants is to be healthy, development of disease requires the coincidence of a susceptible host, a virulent pathogen, and a favorable environment. Also, suitable conditions for disease development are less frequent than one would expect because the pathogen population and the host plant change during their life cycle according to the stage of development (Fig. 1). These stages affect the virulence in the pathogen and the susceptibility in the host plant.

Disease is also strongly dependent on environmental conditions, especially in pathogens that have an epiphytic phase which is strongly dependent on water availability on the plant surface [2]. Temperature and surface wetness are environmental parameters that strongly affect microbial epiphytic life, as they determine growth rate, germination, taxis and other essential processes for colonization of the plant surface. However, temperature and wetness levels often exhibit strong changes, even

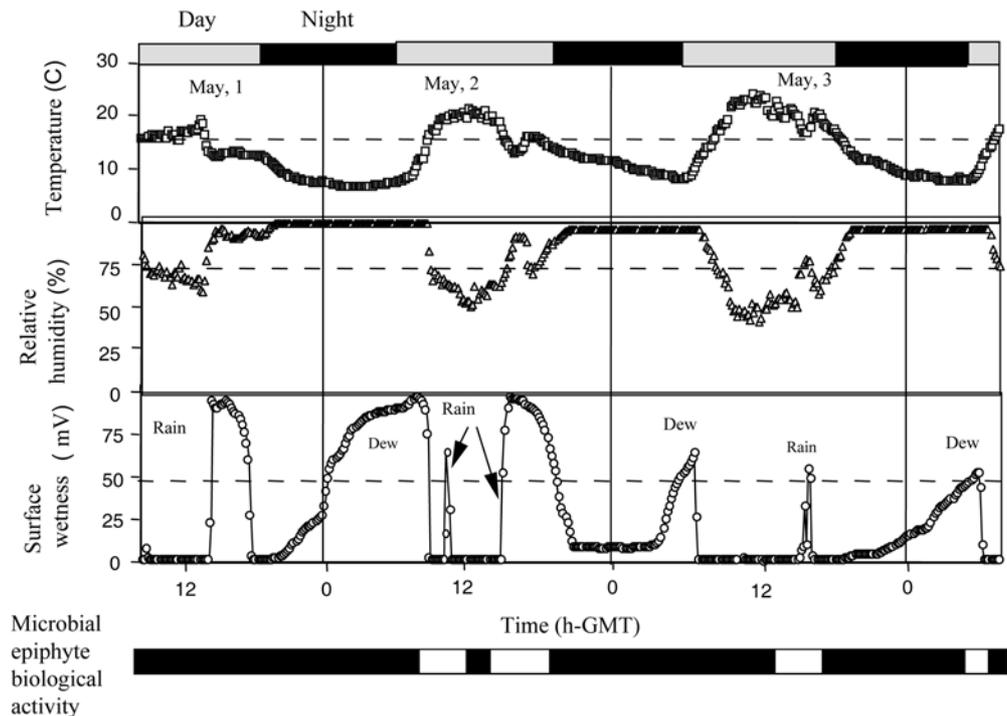
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**Fig. 1** Biological cycle of a typical plant pathogen that has an epiphytic phase and a pathogenic phase

within short day-night cycles (Fig. 2), thus limiting the frequency of conditions suitable for the infection of plant tissues. Disease spread among plants is also limited by the fact that most plant-pathogen interactions are highly specific and because plants defend themselves from microbial attack. Due to the interest in basic knowledge of plant disease and the economic importance of plant disease control with respect to crop production, a vast amount of research has been dedicated to understanding the basis of the specificity of plant-pathogen interactions, how plants defend themselves

**Fig. 2** Dynamics of plant temperature, relative humidity, and surface wetness in a pear tree within a commercial orchard in Girona, Spain, measured every 10 min over a period of 3 days. A relative index of epiphytic activity based on the coincidence of warm temperatures (>15 °C) and wetness (>50 mV) is represented as white horizontal bars in the lower part of the figure



from infection by pathogens, and the role of plant-beneficial bacteria in the control of plant pathogens.

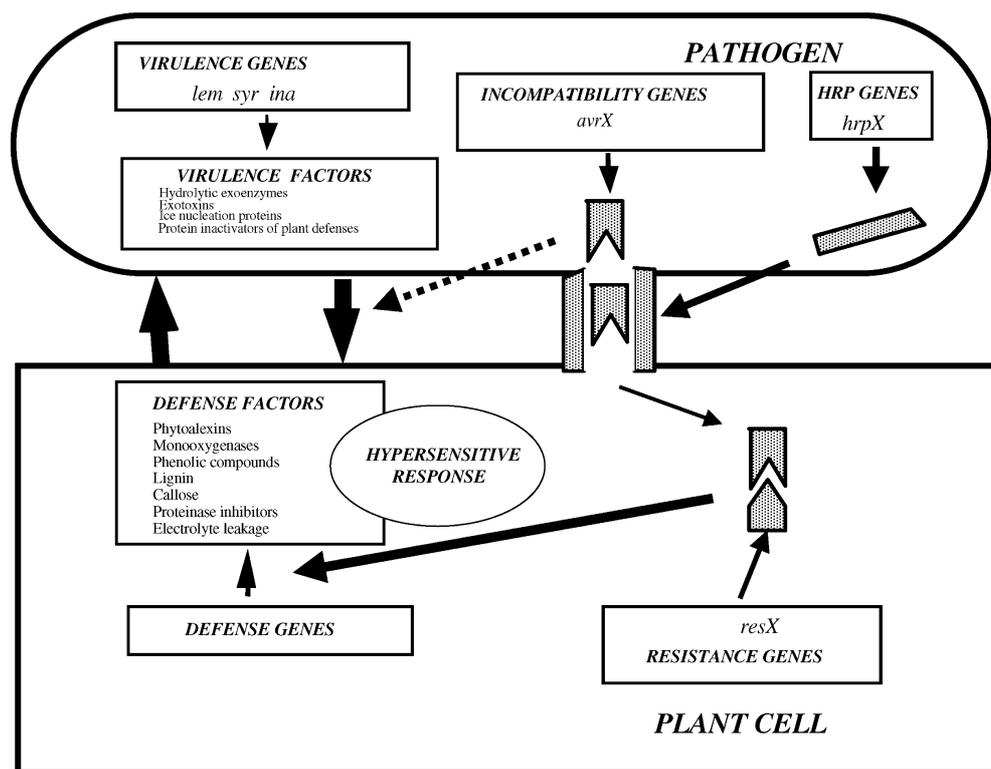
**Basis of the specificity of plant-bacteria interaction**

The specificity of interaction between pathogens and plant hosts is a general phenomenon. For many plant pathogens, races and pathovars, which are groups of host-range pathogens at the subspecies level, can be recognized. Also, many races and pathovars of pathogens show specific host-range within cultivars of host plants, an agronomic differentiation of plants of the same species but differing in certain agricultural properties.

The genetic basis of this strong specificity is explained by the gene-for-gene elicitor-receptor model [4,7]. This model takes into account avirulence (*avr*) genes in the pathogen which are homologous to the resistance (*R*) genes in the host plant. A complementary combination of *avr* and *R* genes results in an incompatible plant-pathogen interaction (rejection) and triggers defense mechanisms in the host cells. By contrast, a non-complementary combination of *avr* and *R* genes (compatible) results in infection (Fig. 3).

Many studies on the basis of specificity of plant-microbe interaction have made use of plant pathogenic bacteria of the genera *Pseudomonas*, *Erwinia* and *Xanthomonas*. A group of genes has been implicated consisting of the hypersensitivity reaction (HR) and pathogenicity (*hrp*) genes which control the capacity of bacteria to develop HR in non-host plants. The transcription of *hrp* genes is controlled by a contact-dependent signal transduction cascade, comprising a type III

**Fig. 3** Simplified schematic representation of the elicitor-receptor model in plant-bacteria interactions. The virulence genes and factors in the pathogen are confronted with the defense genes and factors in the plant host cell (left). Incompatibility genes in the bacterial pathogen encode avirulence factors (*avrX*) (center) which are secreted by a type III secretion system controlled by the *hrp* genes (*hrpX*) (right). The avirulence factors are detected by the resistance genes (*resX*) or factors in the plant cell which trigger the hypersensitive reaction and defense



secretion system which is homologous to and has common features with animal pathogenic bacteria such as *Yersinia*, *Shigella*, *Salmonella* and *Escherichia* [16]. The first confirmation of the role of *hrp* genes was provided by the discovery of harpins, which are proteinaceous elicitors of the HR, in *Pseudomonas syringae* and *Erwinia amylovora* [23].

A second group of genes, the avirulence (*avr*) genes, code for most of the virulence-associated proteins introduced into the host cell by the type III secretion system controlled by the HRP system, and trigger programmed plant defense responses such as HR [6].

Studies have also been carried out with *Agrobacterium tumefaciens*, a member of the family Rhizobiaceae, that causes tumors in several plants. *A. tumefaciens* has been taken as a model for inter-kingdom genetic exchange in plants because the ultimate cause of disease is the transfer of a T-DNA region of the Ti plasmid to the plant cell, its integration into the chromosomes, and the expression of its encoded plant regulator genes [20]. T-DNA is cleaved from the Ti plasmid, and the resultant single strands are coated with Vir proteins and secreted to the host-plant cell by means of a type IV secretory pathway. In fact, the plant parasite is a DNA-based genetic element which multiplies synchronously with the plant cell genome.

The genetic basis of the interaction has recently been confirmed by the fact that the complete sequences of the plant pathogenic bacteria *Ralstonia solanacearum* GMI1000, *Agrobacterium tumefaciens* C58, and *Xylella fastidiosa* are available, and sequencing is ongoing on *Burkholderia cepacia* J2315, *Clavibacter michiganensis*

subsp. *sepedonicum*, *Pseudomonas syringae* pv. *tomato* DC3000, *Spiroplasma citri*, and several pathovars of *Xanthomonas axonopodis* (Table 1).

### Defense response in plants

One of the most striking discoveries in plant pathology was the observation that plants can recognize pathogens [5, 6]. Each plant, either at the species or subspecies level, has a range of pathogens to which it is susceptible, and another to which it is resistant. Non-host plants of a given pathogen group develop a type of blocking necrosis against these pathogens when their tissues are invaded, which is called the hypersensitivity reaction (HR). An incompatible plant-pathogen reaction is typically detected on leaves of tobacco plant when it is inoculated with a bacteria that is pathogenic to non-tobacco plants. The resultant HR contrasts with the compatible reaction of a pathogen on its host plant, which consists of infection and invasion of plant tissues or organs, and often plant death (Fig. 4).

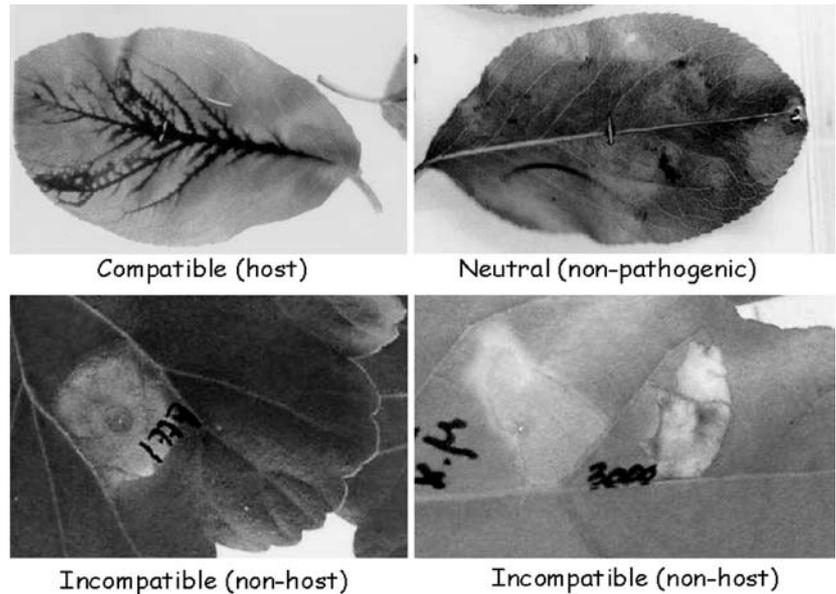
Plant defense mechanisms are characterized by a combination of constitutive and inducible responses. Constitutive responses consist of general barriers or preexisting biochemical defenses. Inducible responses can be localized or systemic and are more sophisticated because they involve the recognition of the pathogen by the host plant, signal transduction, and the expression of several genes. In a localized response, plant tissues react against pathogens by a type of programmed cell death consisting essentially of electrolyte leakage from the

**Table 1** Genomes of plant-associated bacteria currently sequenced and ongoing. Source: <http://wit.integratedgenomics.com/GOLD/>. CAU Cardiff University, CER Cereon Genomics, CNRS Centre National de la Recherche Scientifique, CSU Colorado State University, CWU Central Washington University, DUP Dupont, EDU Edinburgh University, EUC European Consortium, EUN European Union, FAD Fadesp, GHU Ghent University, INRA Institut National de la Recherche Agronomique, JAC Japanese Consor-

tium, JGI Joint Genome Institute, KDI Kasuza DNA Research Institute, MON Monsanto, OSU Ohio State University, SAC The Sanger Centre, STU Stanford University, TIGR The Institute for Genomic Research, UBI University of Birmingham, UCM University of Campinas, UOX University of Oxford, URI University of Richmond, USP University of Sao Paulo, UWA University of Washington

Bacteria	Genome size (kb)	Institution or company	Reference
Sequence complete			
<i>Ralstonia solanacearum</i> GMI1000	5,810	GEN, INRA, CNRS	[21]
<i>Agrobacterium tumefaciens</i> C58	4,915	CER, URI, MON, UWA, DUP, UCN	[9,24]
<i>Xylella fastidiosa</i> CUC	2,679	ECO, JCO	[22]
<i>Sinorhizobium meliloti</i> 1021	6,690	EUN, STU	[8]
<i>Mesorhizobium loti</i> MAFF303099	7,596	KDI	[12]
Sequence ongoing			
<i>Burkholderia cepacia</i> J2315	7,600	SAC, CAU, EDU, GHU	–
<i>Clavibacter michiganensis</i> subsp. <i>sepedonicum</i>	2,500	SAC, CSU, OSU	–
<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	6,100	TIGR	–
<i>Spiroplasma citri</i>	–	CWU	–
<i>Xanthomonas axonopodis</i> pvs	5,000–5,500	FAD, USP, UCM	–
<i>Pseudomonas fluorescens</i> Pf10–1	3,500	JGI	–
<i>Pseudomonas fluorescens</i> SBW25	6,600	SAC, UOX, UB	–
<i>Bacillus subtilis</i> 168	7,600	EUC, JAC	–

**Fig. 4** Reaction of plants to bacterial pathogens. *Upper panel* (left to right): infection and compatible reaction of *Pseudomonas syringae* on pear; neutral reaction caused by inoculation of *Pseudomonas fluorescens*, a non plant-pathogenic bacterium, on pear. *Lower panel* (left to right): incompatible or hypersensitive reaction of *Pseudomonas syringae* inoculated in geranium and tobacco



cytoplasm and an oxidative burst. In systemic defense, a signal spreads from the site of interaction as a response to chemicals, microorganisms, insects, mechanical damage or stress. The signal is mediated by several molecules which function as messengers in plants, for example, salicylic and jasmonic acid, or even volatiles such as nitric oxide and ethylene [4]. These messengers interact with specific binding proteins that are implicated in the transcriptional activation of pathogenesis-related (PR) genes in response to pathogen aggression. The products of several of these genes are enzymes, e.g. peroxidases, lipo-oxygenases, superoxide dismutases, and phenylalanine-ammonia-lyase (PAL), involved in the secondary metabolism of plants and specifically in

the synthesis of phenolic compounds, or are phytoalexins, glucanases and chitinases, with antimicrobial activity.

The inducible defense response in plants produces a limited variety of biochemical compounds with low specificity for the target pathogen. However, the specificity of the response in plants lies in the fact that they detect intracellularly specific types of proteins secreted by the pathogen (elicitors), using an interaction recognition system which triggers the defense response. There is now experimental evidence that *avr* genes are present in plant viruses, plant pathogenic bacteria, and plant pathogenic fungi [6]. Also, the plant genome of a model plant, *Arabidopsis thaliana*, has been sequenced and

sequencing of the genomes of an additional 30 plant species of agricultural interest is currently ongoing. About 30 plant genes are actually known to be involved in resistance to plant pathogens, most encoding proteins with leucine-rich repeats and which are highly conserved among angiosperms [1].

### New methods of plant protection based on knowledge of plant-microbe interactions

Knowledge of the molecular basis of plant-microbe interactions is now being applied to search for less aggressive crop protection methods based on the use of beneficial microorganisms and on stimulation of the plant defense response. Bacteria can establish intimate symbiotic associations with plants or live as epiphytes or endophytes in a more subtle relationship with the plant. The non-symbiotic, beneficial plant bacteria include plant-growth-promoting rhizobacteria (PGPR) and biological control agents (BCA). Some of the bacteria interacting with plants confer beneficial traits on the host but are unable to invade tissues and infect. The isolation of PGPR and BCA is a time consuming and inefficient process which is dependent on efficient screening methods. Very often it requires the analysis of thousands of isolates, yielding only a few useful ones, because not all isolates of a given species are active. For this reason a strong research effort is needed in order to find biochemical or molecular markers specifically associated with the best performance in beneficial bacteria.

PGPR are inhabitants of the rhizosphere, the volume of soil under the immediate influence of the plant root system. In the rhizosphere, secretion of organic compounds by the plant favors a large, active microbial population. Inoculation of plants with PGPR, mainly of the genera *Pseudomonas*, *Serratia*, *Azospirillum* and *Bacillus*, enhances growth of the root system and the whole plant, and often limits the growth of certain soil-borne plant pathogens [13]. The mechanisms of plant growth stimulation are strongly dependent on the bacteria and host plant. In some cases a relationship has been found with the synthesis of plant-growth regulators such as IAA, of siderophores which chelate iron, with biological control of soil-borne plant pathogens or plant deleterious microorganisms, or even with induction of the systemic defense response. BCA are found either in the aerial plant part or in the root system, which is colonized by an extremely abundant microbiota (Fig. 5). However, only a few of these microorganisms have been identified, because many are intimately associated with the plant and thus not easily cultivated in the laboratory.

BCAs are able to colonize or compete for nutrients and sites of pathogen interaction with plants, as well as to exert various types of antagonism against plant pathogens. In some cases of antagonism, biocontrol has been ascribed to the synthesis of antimicrobial compounds such as bacteriocins and novel antibiotic compounds [10] (Fig. 6). In other cases, BCA are able to

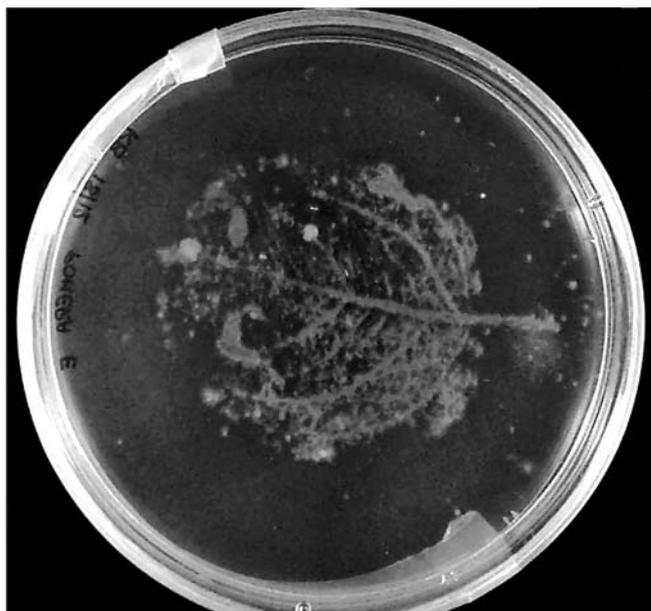


Fig. 5 Replica plating of a pear leaf onto the surface of a petri plate containing nutrient agar, and development of colonies of the culturable microbiota after incubation

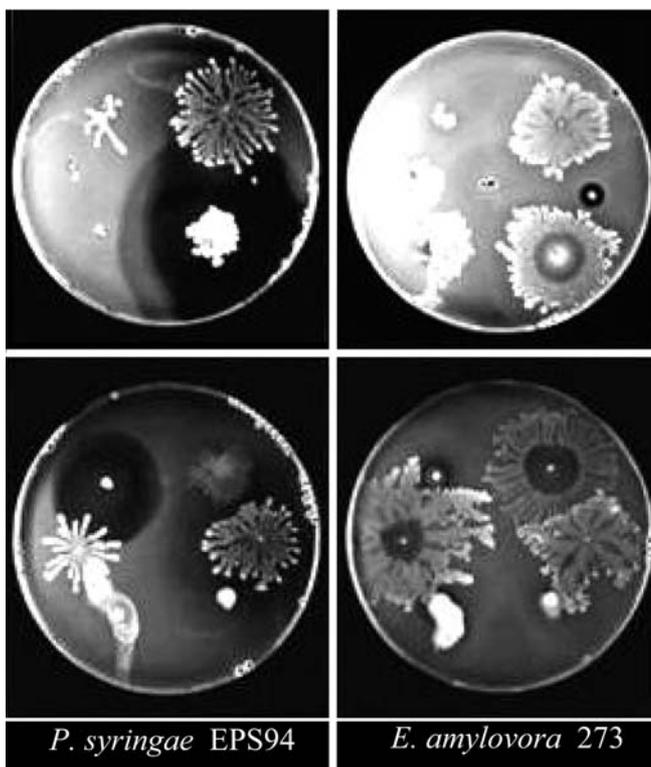


Fig. 6 In vitro antagonism of several bacterial colonies of putative beneficial bacteria against the plant pathogenic bacteria *Pseudomonas syringae* and *Erwinia amylovora*. Notice the inhibition halos and the invasive growth in several strains

well-colonize pathogen entry sites, hyperparasitism or to directly interfere with the pathogen. More rarely, BCA induce systemic acquired resistance (SAR) in the plant

by eliciting, to some degree, the HR response without infection, or by causing some kind of stress to the plant [19]. Most of the BCA which provide an efficient protection of plants against infection by plant pathogenic bacteria and fungi are non-pathogenic strains of the species *Pseudomonas*, *Erwinia* (synonymous of *Pantoea*), *Agrobacterium* and *Bacillus* [15, 18, <http://www.inra.fr/Internet/Produits/dpenv/sribad19.html>, U.S. Department of Agriculture]. Numerous strains of bacteria have been isolated which yield excellent results in the laboratory in vitro, ex vivo or even in planta under controlled environment conditions. However, few of these strains exert sufficient biological activity under the conditions encountered in the field or the food environment. The problem is often due to a low survival level of the BCA or PGPR in the field, or the too high concentration needed to exert activity. The practical use of PGPR or BCA as microbial fertilizers or pesticides, and their efficiency is strongly dose-dependent as with chemical pesticides [17]. This introduces a technical problem to be solved at a biotechnological level: the development of proper methods of industrial scale-up and fermentation, and the suitable formulation to be delivered as a commercial biofertilizer or biopesticide [3, 11].

An additional problem is the potential toxicological risk and the environmental impact associated with the introduction of BCA or PGPR into the food chain or environment. Appropriate studies have to be done even when BCA or PGPR have been isolated from the healthy plant environment. Due to all these restrictions, only a few products have arrived on the market. Inoculants of the plant-root system using PGPR consisting of formulations of *Bacillus subtilis* to increase plant growth and performance are currently under commercial development in several countries. Several BCAs that have successfully been used as competitors or antagonists of bacterial and fungal plant pathogens are also being commercially delivered [<http://www.inra.fr/Internet/Produits/dpenv/sribad19.html>], such as formulations of *Agrobacterium radiobacter* strain K84 to prevent crown gall tumors, *Pseudomonas fluorescens* against fire blight and frost damage, *Pseudomonas syringae* for control of post-harvest fruit rot, *Burkholderia cepacia*, against fungal root rot, and *Streptomyces griseoviridis* for control of many soil-borne fungal diseases.

A new generation of chemical compounds, produced either by chemical synthesis or by biotechnological processes, consists of molecules which do not exhibit in vitro antimicrobial activity against the pathogens but instead elicit plant defense responses. Several of these compounds are currently on the market in some countries and include derivatives of acetylsalicylic acid, e.g. benzotriadiazol (BTH) [1]. However, the most intriguing example is the registration and commercialization of the proteins encoded by the *hrp* genes from phytopathogenic bacteria for use in crop protection and as a plant enhancer. These proteins trigger several plant responses to

stress and their topical application has shown several beneficial effects [23]. Finally, transgenic plants have been produced with engineered genes encoding HR elicitors, such as harpins, or which overexpress *R* genes or PR proteins, such as chitinases. The transgenic plants have an increased resistance to many plant pathogenic bacteria and fungi.

In summary, the future of plant disease control, currently provided mainly by chemically synthesized fungicides and bactericides, will soon be complemented or substituted by new disease-control technologies emerging from the basic knowledge of plant-microbe interactions, and consisting of new biotechnological products

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