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Review Article

Molecular aspects of phaseolotoxin biosynthesis produced by *Pseudomonas syringae* pv. *phaseolicola*

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ABSTRACT

Background/Objective. Phaseolotoxin is produced by one of the most important and studied phytopathogens in the agricultural area: *Pseudomonas syringae* pv. *phaseolicola*. This bacterium causes halo blight, a disease that devastates the bean crop. The success of *P. syringae* pv. *phaseolicola* is related to its genetic information, which allows it to synthesize deleterious metabolites for its host, such as phaseolotoxin. This research aimed to analyze the molecular basis of the mechanism of action, immunity, genetics involved in the biosynthesis of phaseolotoxin, molecular diagnostic strategies, and molecular techniques developed in Mexico to manage bean halo blight.

Materials and Methods. The search and analysis of the most relevant scientific information regarding the biosynthesis of phaseolotoxin and the molecular studies of the pathogenicity and virulence factors of *P. syringae* pv. *phaseolicola* has contributed to the development of molecular strategies focused on the diagnosis and management of halo blight in beans.

Results. *P. syringae* pv. *phaseolicola* produces phaseolotoxin, responsible for forming the chlorotic halo characteristic of halo blight, this toxin is an inhibitor of OCTase, an enzyme that participates in the arginine synthesis pathway in beans. The Pht and Pbo chromosomal regions contain genes involved in the synthesis and immunity of phaseolotoxin, and the expression of these genes is regulated by the GacS/GacA system and temperature. The identification of genes involved in the synthesis of pathogenicity and virulence factors, such as phaseolotoxin, has allowed the development of strategies for diagnosis and management of the disease

based on DNA amplification and the use of molecular markers that facilitate the identification of bean cultivars resistant to the pathogen.

Conclusion. Molecular studies have contributed to understanding how the *phaseolicola* pathovar produces phaseolotoxin. This information has been essential to understanding how bacteria have evolved from non-pathogenic to pathogenic variants. In addition, they provide information that allows the development of new strategies for timely diagnosis and contributes to strategies for managing halo blight.

Keywords: Pathogenicity, Virulence, Phytotoxins, Horizontal Transference, Genetic Regulation.

Introduction

The common bean (*Phaseolus vulgaris*) is a highly important food and commercial crop worldwide. This crop is widely adapted to environments with moderate growth temperatures and its popularity is due to it being easy to produce (Foyer *et al.*, 2016). The quality, yield and production of this crop is currently affected by numerous phytosanitary problems, some of which are created by diverse microorganisms that limit the quality of agricultural products and are responsible for enormous economic losses, in addition to being a significant threat for the world's food security (Sundin *et al.*, 2016). Among these microorganisms is *Pseudomonas syringae*, one of the most important pathogens for this crop and others such as tomato, kiwifruit, tobacco, lime, oat, wheat, soybean, cucumber, rice and others (Chen *et al.*, 2022). The *P. syringae* species includes 62 pathovars, depending on the host they infect, including most of the phytopathogens of the *Pseudomonas* genus (Bull *et al.*, 2010). The success of a phytopathogen to infect, colonize and induce a series of symptoms in its host is directly related to its genetic information. This information, represented by the genes, helps it synthesize and secrete metabolites that are deleterious to the plant (Lamichhane *et al.*, 2015). The diverse *P. syringae* pathovars are known for producing a wide spectrum of metabolites that act as pathogenicity and virulence factors; among these are phytotoxins, which directly damage the plant cells and influence the development of the disease. *P. syringae* pv. *phaseolicola* is the causal agent of the halo blight disease in bean, which can be devastating, particularly when adverse weather conditions persist. The presence of a chlorotic halo that characterizes this disease is precisely caused by phaseolotoxin, a phytotoxin produced by *P. syringae* pv. *phaseolicola* (Arrebola *et al.*, 2011). The genetics related to the synthesis of phytotoxins has become remarkably important,

since the genes coding for the enzymes involved in the assembly, maturation and even those required for secretion are clustered in chromosomal fragments known as genomic islands. These islands contain all the elements needed to be transferred horizontally between the different pathovars. These genetic transfer mechanisms are highly relevant because the bacteria acquire new pathogenic abilities (Melnyk *et al.*, 2019). Advances in the genetic study of pathogenicity and virulence factors, such as phytotoxins, favor the understanding of how pathogens acquire and produce the molecular arsenal needed for the development of diseases in plant systems (Ichinose *et al.*, 2013).

Considering that every *P. syringae* pathovar produces a diversity of phytotoxins, this revision will focus on the analysis of one of those cases: the genetic involved in the biosynthesis of the phaseolotoxin produced by the *phaseolicola* pathovar and its involvement in the development of the halo blight in bean. This information essentially contributes to the understanding of how this phytopathogenic bacterium has acquired genes needed to synthesize phaseolotoxin and how it has coordinated its endogenous regulation system for this purpose. In addition, the potential of this type of genetic studies on the development of strategies for the diagnosis and timely management of diseases is analyzed.

P. syringae pv. *phaseolicola*

Halo blight is one of the main diseases that affects the bean crop and it is caused by the bacterium *P. syringae* pv. *phaseolicola*. This bacterium persists in the seed and activates after the seed germination; it can survive as a parasite in the plant tissue and as an epiphyte on the surface of leaves. The bacterium has been observed in most bean-producing regions of the world and it has become a highly destructive phytopathogen for this crop, causing losses that range between 50 and 100% of the production (Arnold *et al.*, 2011; Lépiz-Ildefonso *et al.*, 2015). The bacterium can be found in the different phenological stages affecting leaves, stem, pods and seeds. In leaves, small, aqueous lesions appear which later become necrotic; approximately one week after infection, one or more lesions become surrounded by a necrotic halo (Figure 1 A and B). Pods also develop reddish-brown, aqueous and sunken lesions (Figure 1 C and D). Infections in pods can be transferred to seeds, causing them to shrink, discolor or become smaller than normal. The bacterium develops in a range of 28-30 °C with a relative humidity higher of over 80%, although more severe symptoms appear at temperatures ranging between 18 and 20 ºC. A reduction in temperature induces the production of phaseolotoxin, which is responsible for the formation of the chlorotic halo and the characteristic yellowing observed during the development of the disease (Arnold *et al.*, 2011; Xin *et al.*, 2018).

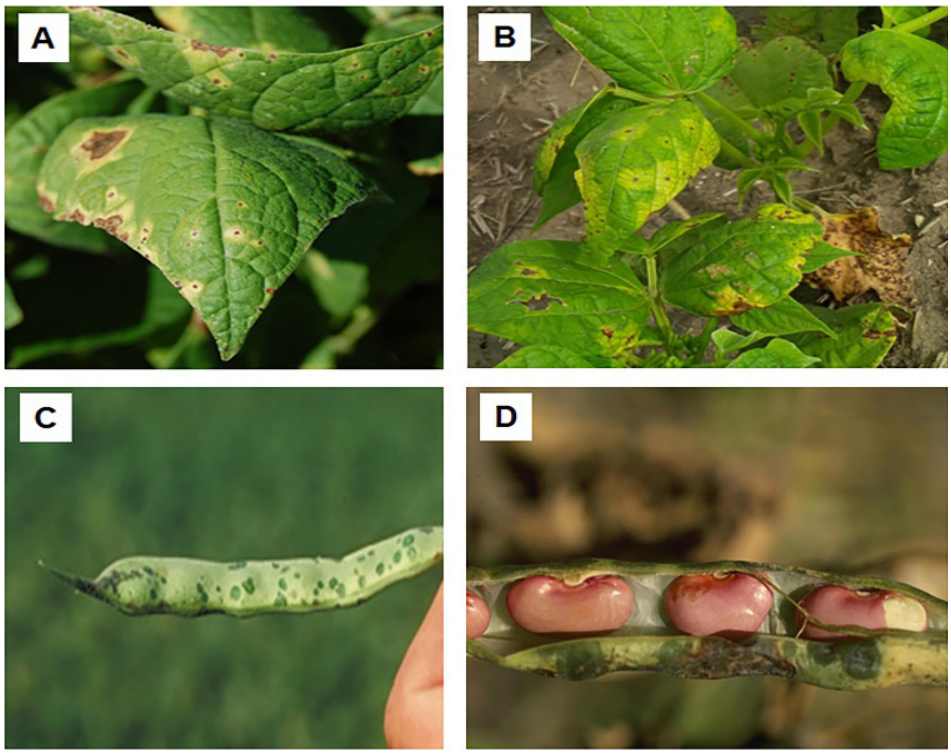

Figure 1. Symptoms produced by *P. syringae* pv. *phaseolicola* on bean. A y B, Symptoms on leaves. C y D, Symptoms on pods. Source: Adapted from Schwartz, (2008); Harveson, (2009).

Phaseolotoxin and its action mechanism

From a chemical point of view, the structure of phaseolotoxin is unusual and has two different components: an inorganic residue, N^{δ}- (N'-Sulphodiaminophosphinyl) and a tripeptide, L-ornithyl-alanyl-homoarginine (Figure 2) (Arrebola *et al.*, 2011). This toxin acts as a reversible inhibitor of the enzyme ornithine carbamoyltransferase (OCTase EC 2.1.3.3.), codified by the gene *argF*. OCTase catalyzes the conversion of ornithine and carbamoylphosphate into citrulline in the arginine synthesis pathway. In the plant cell, phaseolotoxin is hydrolyzed due to the action of peptides to produce N^{δ}-(N´-sulphodiaminophosphynil)-L-ornithine (called octicidin or PSOrn), which is an irreversible inhibitor of OCTase and the predominant form in which the toxin is found in infected bean plants (Sawada *et al.*, 2002). This inhibition causes an accumulation of ornithine and an arginine deficiency. As the toxin spreads through the foliar tissue, it alters the synthesis of the chlorophyll, causing chlorosis and growth inhibition in plant tissues, finally leading to the death of host cells. In addition, the systemic invasion of the plant is facilitated by the

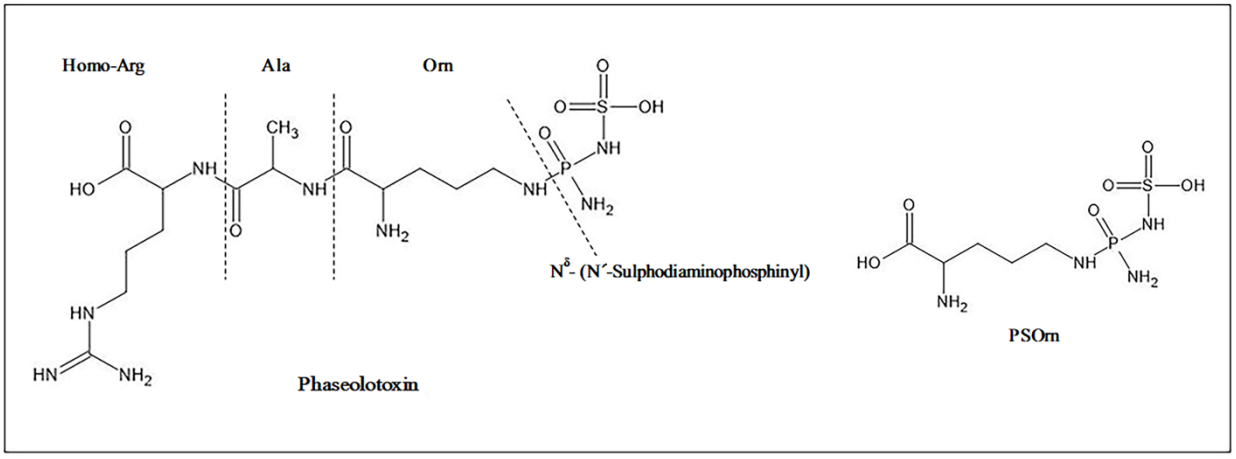

Figure 2. Phaseolotoxin structure and PSOrn, product of phaseolotoxin degradation by plant peptidases.

effect of the phaseolotoxin, thus contributing significantly to the virulence of the pathogen (Arnold *et al.*, 2011).

Originally, phaseolotoxin was considered to be restricted to *P. syringae* pv. *phaseolicola*, although it is also produced by the pathovar *actinidiae*, which infects kiwifruit (*Actinidia chinensis*) (Fujikawa and Sawada, 2019), and by a strain of the pathovar *syringae*, isolated from *Vicia sativa* (Tourte and Manceau, 1995). Several isolates belonging to the pathovar *phaseolicola* synthesize phaseolotoxin when grown at 18-20 °C, although its degree of production may vary between isolates. On the other hand, strains have been identified that belong to the pathovar *phaseolicola*, which are unable to produce it, since they lack all or part of the genes required for their synthesis (Gonzalez *et al.*, 2003).

Immunity to phaseolotoxin

Initially, determining how *P. syringae* pv. *phaseolicola* was immune to its own toxin was a mystery. The toxic effect of phaseolotoxin was evident, since it does not only inhibit OCTase from the bean, but also a large variety of plant and bacterial OCTases, and even from mammals (Bender *et al.*, 1999). In these organisms, it causes an auxotrophy phenotype to arginine, which is alleviated by adding arginine or citrulline to the growth medium. By contrast, *P. syringae* pv. *phaseolicola* strains that produce phaseolotoxin are insensitive to its toxin and do not require arginine or citrulline supplements. Everything was clarified when the gene *argK,* which codifies for a phaseolotoxin-resistant OCTase, was identified and characterized (Mosqueda *et al.*, 1990). Thus, *P. syringae* pv. *phaseolicola* was observed to present two OCTase activities; one activity that is sensitive to the toxin produced at 28 °C and the activity of the resistant OCTase, which guarantees the optimum

supply of arginine required for bacterial growth, under conditions of phaseolotoxin synthesis (Lopez-Lopez *et al.*, 2004). The knowledge on the bacterial resistance to the effect of its toxins has been used in several strategies to develop resistant plants. In the case of phaseolotoxin, *argK* has been used as transgene in tobacco plants (*Nicotiana tabacum*) and has been successful in developing plants that are resistant to the effect of phaseolotoxin (De la Fuente-Martinez *et al.*, 1992). Studies such as these can be extrapolated to other crops of agronomic interest, allowing for the generation of cultivars that are resistant to the attack of pathogens.

The genetics behind the phaseolotoxin biosynthesis

Diverse studies have focused on the chemistry of phaseolotoxin, its form of action and its contribution to pathogenicity, although little is known about its biosynthesis. Through genetic analyses, relevant information has been provided to elucidate how this toxin is synthesized. The first insight into the genes involved in its synthesis was the identification and characterization of *amtA* and *desI*, which codify for an aminotransferase and one desaturase of fatty acids, respectively (Hernandez-Guzman and Alvarez-Morales, 2001; Zhang and Patil, 1997). We currently have the description and analysis of a 30,245 pb region of the chromosome of *P. syringae* pv. *phaseolicola* NPS3121, called the Pht Region (Figure 3A), which contains 23 genes required for the synthesis of phaseolotoxin, including the genes *argK*, *amtA* and *desI* (Aguilera *et al.*, 2007). The Pht Region is delimited by insertion sequences and transposases, suggesting that this fragment constitutes a genomic island that could have been acquired through horizontal transfer events (Murillo *et al.*, 2011). The precise function of the codified genes in the Pht Region remains uncertain and some of these genes have been studied further (Aguilera *et al.*, 2012; Arai y Kino, 2008; Chen *et al.*, 2015; Gonzalez-Villanueva *et al.*, 2014). Thus, it has been possible to assign a function to them in the phaseolotoxin synthesis and regulation process (Table 1).

Phaseolotoxin is composed of a tripeptide and its synthesis has been proposed to be via a mechanism called a non-ribosomal peptide synthesis. Enzyme complexes such as Non-Ribosomal Peptide Synthases (NRPS) and/or Polyketide Synthases (PKS) participate in this process. The gene PSPPH_4550, identified as an NRPS, participates in the phaseolotoxin synthesis (De la Torre-Zavala *et al.*, 2011). Interestingly, the gene PSPPH_4550 is codified outside of the Pht region and is part of another genomic island called the Pbo Region. This fragment contains 16 genes (Figure 3B) some of which codify for NRPS or PKS (Table 2), suggesting its participation in the synthesis of the tripeptide (Guardado-Valdivia *et al.*, 2021).

To date, multiple genes involved in the phaseolotoxin synthesis have been identified (Table 1 and 2), and functions have been assigned to some of them.

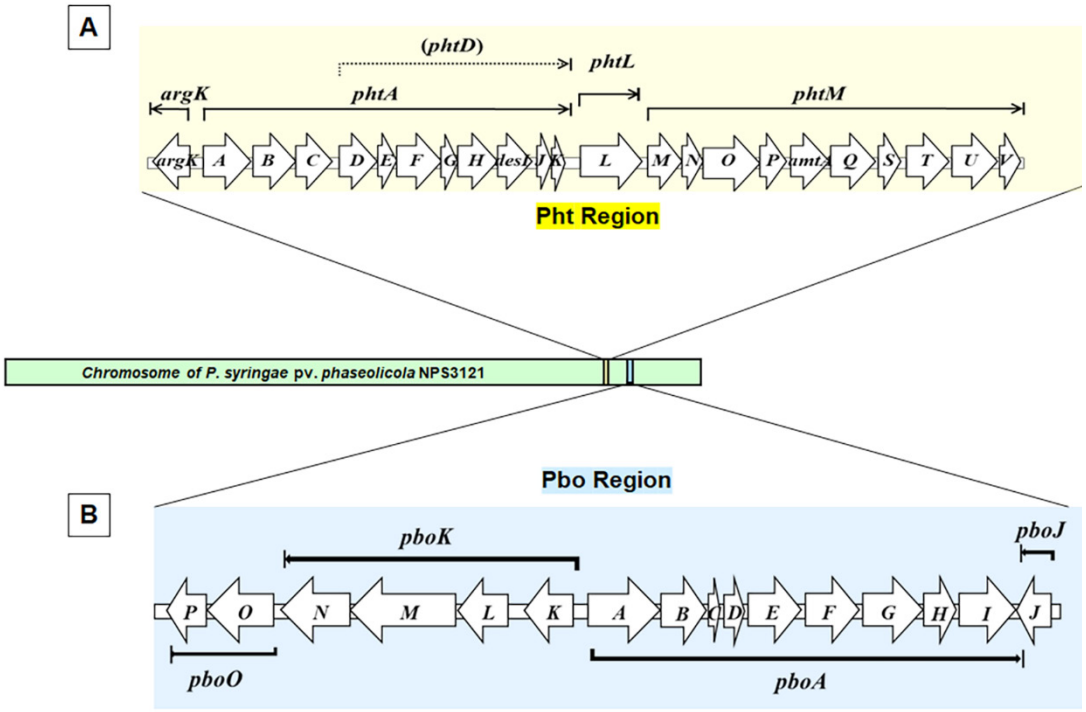

Figure 3. Graphic representation of the Pht and Pbo Regions on *P. syringae* pv. *phaseolicola* chromosome. A, Pht Region operons. B, Pbo Region operons. Each arrow represents one gene, the direction of the arrow indicates the direction of transcription.

Table 1. Function of *pht* genes involved in phaseolotoxin synthesis.

Gene	Protein coded	Function	Reference
argK	Ornithine carbamoyltransferase	Catalyze the reaction between carbamoyl phosphate and ornithine to form citrulline under phaseolotoxin synthesis conditions.	Lopez-Lopez *et al*., 2004
amtA	Amidinotransferase	Catalyze the reversible transfer reaction of amidino groups to synthesize L-homoarginine.	Hernandez-Guzman y Alvarez-Morales, 2001
desI	Fatty acid desaturase	Dehydrogenate fatty acids, creating double bonds between carbon-carbon; temperature perception.	Zhang y Patil, 1997
phtU	L-aminoacid ligase	Catalyze the condensation of amino acids.	Arai y Kino, 2008
phtQ	Peptide ligase	Catalyze the formation of peptide bonds.	Chen *et al*., 2015
phtL	Pyruvate phosphate dikinase	Transfer phosphate groups from pyrophosphate and phosphoenolpyruvate to an AMP molecule to synthesize ATP, phosphate, and pyruvate.	Gonzalez-Villanueva *et al*., 2014
phtA	Nucleoside triphosphate hydrolase	Hydrolyze the beta-gamma phosphate bond of a nucleoside triphosphate.	Aguilera *et al*., 2012

Table 2. Prediction of the function of *pbo* genes involved in the phaseolotoxin synthesis.

Gene	Protein coded	Function	Reference
pboO	Synthetase c	Form amide bonds in the synthesis process of non-ribosomal peptides.	Guardado-Valdivia *et al*., 2021
pboM	Ketoacyl transferase	Catalyze the elongations of fatty acids in the synthesis of polyketides.	Guardado-Valdivia *et al*., 2021
pboA	AMP-binding enzym	Condense and adenylate in the process of synthesis of non-ribosomal peptides.	De la Torre-Zavala *et al*., 2011
pboB	Ketoacyl synthase	Catalyze the decarboxylating Claisen condensation of fatty acids in the synthesis of polyketides.	Guardado-Valdivia *et al*., 2021
pboC	Phosphopantheine binding domain	Bind amino acids and activated fatty acids in the synthesis of non-ribosomal peptides.	Guardado-Valdivia *et al*., 2021

However, there is still much work to be done to elucidate the functions of the rest of the genes, and particularly, those genes involved in the synthesis of the inorganic compound of the toxin.

Regulation of the phaseolotoxin synthesis

It is evident that the phytopathogens require genetic mechanisms that give them the ability to adapt in response to their conditions. In phytopathogenic bacteria, the regulation systems of two components allow for the adaptation to different conditions in response to the environmental signals, transferring the signal to transcription factors, which activate the gene expression (Sultan *et al.*, 2021). *P. syringae* pv. *phaseolicola* has several two-component systems, although the GacS/GacA system is of great importance due to its role in the control of virulence and pathogenicity determinants, ecological adaptation, *Quorum sensing* (*Qs*) systems, and the synthesis of diverse secondary metabolites (Latour, 2020). The GacS/GacA system controls all genes involved in the synthesis and regulation of the phaseolotoxin in *P. syringae* pv. *phaseolicola* NPS3121 known to date (De la Torre-Zavala *et al.*, 2011; Ramirez-Zapata *et al.*, 2020). Other global regulators, such as the IHF transcriptional factor, also participates in this process. The function of IHF as a regulator of the expression of virulence factors has been widely reported (Arvizu-Gomez *et al.*, 2011; Reverchon *et al.*, 2021). These findings reveal that some genes acquired through horizontal transfer events can be integrated into the preexisting global regulation systems (Redondo-Salvo *et al.*, 2020). In this sense, the genes involved in the biosynthesis of phaseolotoxin were not the exception.

For *P. syringae* pv. *phaseolicola* NPS3121, the descent in temperature is also a determining factor in the production of phaseolotoxin. This change controls the

transcription of the *pht* and *pbo* genes (Aguilera *et al.*, 2007; Guardado-Valdivia *et al.*, 2021). The information gathered so far allows to propose a signaling and regulatory method for the biosynthesis of phaseolotoxin in *P. syringae* pv. *phaseolicola* NPS3121 (Figure 4). This model schematically represents the stimulus and signaling cascade responsible for triggering the synthesis of phaseolotoxin.

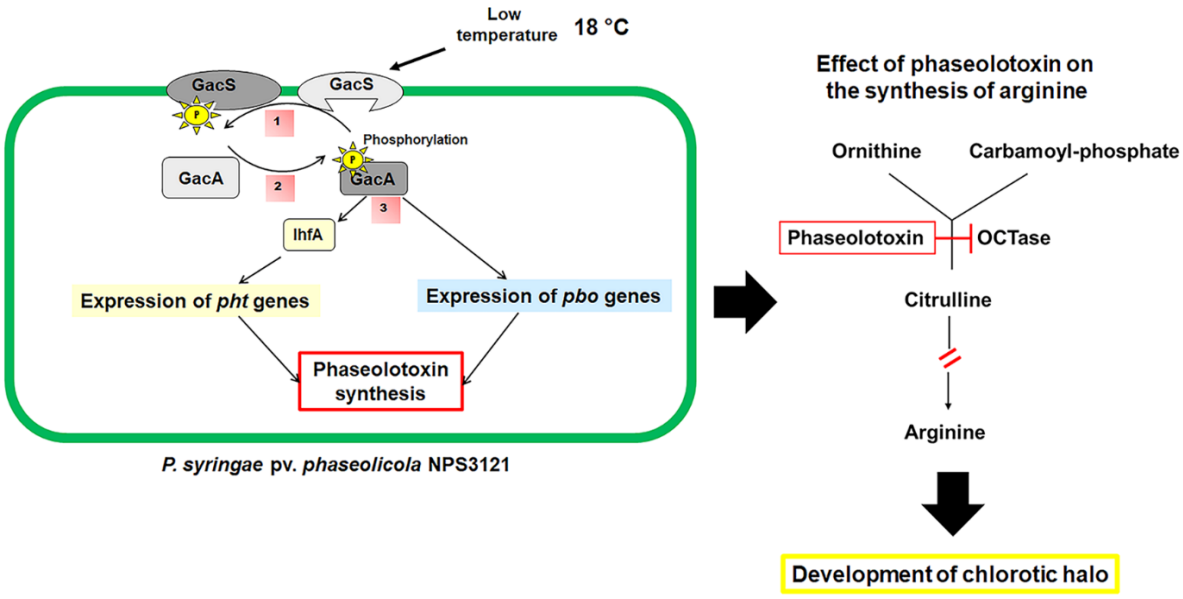

Figure 4. Model of signaling and regulation of phaseolotoxin biosynthesis in *P. syringae* pv. *phaseolicola* NPS3121. Temperature is sensed by the GacS membrane sensor, which autophosphorylates therefore (1). Phosphorylated GacS transfers phosphate to the response regulator GacA (2). GacA controls the expression of *pht* genes, mediated by the IHF regulator. GacA also controls the transcription of the *pbo* genes (3). Finally, phaseolotoxin is synthesized, which inhibits bean OCTase and prevents the synthesis of arginine. Consequently, the chlorotic halo develops.

Transfer of the Pht and Pbo Regions between pathovars

In bacteria, the horizontal transfer of genes through accessory genetic elements such as plasmids, transposons, prophages or genomic islands helps them acquire and express genes from a wide range of species (Carraro *et al.*, 2017). The Pht region has been suggested to constitute a genomic island that *P. syringae* acquired using horizontal transfer mechanisms. This has been supported by the similarity of the organization and the sequence of the different versions of the Pht region identified in the pathovars *phaseolicola* and *actinidae*, which clearly denote a common origin and a preserved functionality (Murillo *et al.*, 2011). Additionally, finding

P. syringae pv. *phaseolicola* strains lacking the Pht region has also supported this proposal (Gonzalez *et al.*, 2003). The Pbo Region also comprises another genomic island. Despite both islands participating in the synthesis of phaseolotoxin, there is no correlation between the possession of the Pbo Region and the Pht Region, suggesting that its acquisition was carried out independently, and once acquired, they were incorporated into the chromosome of *P. syringae* pv. *phaseolicola*, remaining under the control of the preexisting global regulation systems (De la Torre-Zavala *et al.*, 2011; Guardado-Valdivia *et al.*, 2021). Pathogenic bacteria have evolved from related non-pathogenic organisms, due to the acquisition of genetic material that codifies for multiple pathogenicity and virulence factors. In regards to the *P. syringae* pathovars, it is important to understand how the genetic mobility mechanisms have contributed to their pathogenicity and virulence, because, in the end, this is what has turned this species into one of the 10 most important phytopathogenic bacteria in the world (Mansfield *et al.*, 2012). In terms of the pathovar *phaseolicola*, studies on the molecular genetics of its interaction with bean and the evolution of its pathogenicity and virulence have contributed with important discoveries to the field of the plant-microorganism interactions, leading *P. syringae* pv. *phaseolicola* to be considered a model for the study of plant-pathogen molecular interaction (Arnold *et al.*, 2011).

Pseudomonas syringae pv. *phaseolicola* molecular detection strategies

One of the most important requirements to manage plant diseases is the timely identification of the causal agent. There are currently biotechnological techniques that accelerate the achievement of results and have enabled the timely identification of phytopathogenic bacteria. Additionally, they have complemented traditional diagnostic processes such as visual examination, pathogen culturing, microscopy and pathogenicity tests.

In terms of the detection and identification of *P. syringae* pv. *phaseolicola*, diverse molecular strategies have been developed in order to carry out an accurate and timely detection. Within the first strategies reported is the Enzyme-Linked Immunosorbent Assay (ELISA). The antisera obtained specifically recognized the *phaseolicola* pathovar, without showing a cross-reaction with other *P. syringae* pathovars (Wyatt *et al.*, 1989). Around the same time, a strategy was developed based on the hybridization of DNA probes carrying genes involved in the synthesis of phaseolotoxin. This technique helped detect and identify the pathovar *phaseolicola* from seeds and macerated samples of tissue with lesions (Schaad *et al.*, 1989).

With the development of the Polymerase Chain Reaction (PCR), strategies were implemented to detect and identify multiple phytopathogens and *P. syringae* pv. *phaseolicola* was not an exception. Specific oligonucleotides were designed for

this purpose from the available genes involved in the biosynthesis of phaseolotoxin (Mosqueda-Cano and Herrera-Estrella, 1997; Schaad *et al.*, 1995; Schaad *et al.*, 2007). Table 3 describes some of the oligonucleotides used for the identification and the regions they amplify.

Table 3. Oligonucleotides used to identify strains of *P. syringae* pv. *phaseolicola* producing phaseolotoxin.

Name	Sequence	Target	Reference
BRL519 BRL520	TTCATTCAAACCTCGCCCGTGTG TGAAAGGAGCCGCCGAAACTATTG	*amtA*	Aguilera *et al*., 2007
PA5.1 PA3.1	AGCTTCTCCTCAAAACACCTGC TGTTCGCCAGAGGCAGTCATG	*desI*	Schaad *et al*., 1995
62a 63a	CAATGAAGATTACAAGCCTG GCTAGCTATCAGGGGACGAC	*argK*	Mosqueda-Cano y Herrera-Estrella, 1997
P3004L P3004R	CTGTCTGGCAGCCACTACAAAG GGCTGCAAATTGTGGGATTT	Tox-Region	Rico *et al*., 2006
AVR1-F AVR1-R	CCGCCGTAGCAGGGCTTCAC GGACCAATCTCTTTTCTCAA	*phtL*	Gonzalez *et al*., 2003
PHTE-F PHTE-R	AATATAGGCTTCAACTTCCTC CCAGGTCAACTCACTTCCG	*phtE*	Gonzalez *et al*., 2003
P25156 Ptx1I5c	GCAAAAACGAAAACACCAGGCT ATCGCGCTGATCCGGAAAGG	*phtA*	Aguilera *et al*., 2017
P12556 P11311	TCCGGTTATCGCTTCAGGTCG GCAGTTTCTGATCTTGGGCCC	*phtM-N*	Aguilera *et al*., 2017

It is important to consider the existence of *P. syringae* pv. *phaseolicola* isolates that do not produce phaseolotoxin and therefore cannot be detected using these oligonucleotides (Rico *et al.*, 2006). Additionally, the production of phaseolotoxin is not only a characteristic of the pathovar *phaseolicola*, so these assays could also detect other pathovars (Tamura *et al.*, 2002). According to this, new methodologies have been designed, such as the TaqMan real-time PCR, in which specific oligonucleotides based on the site-specific recombinase gene were used. With this strategy, we were able to differentiate between phaseolotoxin producing and non-producing *P. syringae* pv. *phaseolicola* strains (Cho *et al.*, 2010). Another DNA amplification technique, known as a Loop-Mediated Isothermal Amplification (LAMP), has been adapted to facilitate the characterization and quick and specific identification of the pathovar *phaseolicola* (Li *et al.*, 2009). The molecular diagnosis of *P. syringae* pv. *phaseolicola* is crucial for the efficient management of halo

blight in bean crops. Although the molecular diagnosis can be more expensive and require specialized infrastructure, its high precision, speed and capacity to detect infections in an early and quantitative way make it a crucial tool in the fight against this pathogen. In time, molecular diagnosis techniques are expected to continue improving and become more accessible, which will allow for a more efficient management of bacterial diseases in agriculture.

Molecular strategies to manage halo blight in bean. An alternative for agriculture in Mexico

In Mexico, the management of halo blight in bean has become a challenge for farmers, due to the aggressive nature of the disease and its ability to spread quickly (Félix-Gastélum *et al.*, 2016). *P. syringae* pv. *phaseolicola* is widely distributed in the country (Figure 5), and is therefore a risk for the country's bean production (Félix-Gastélum *et al.*, 2016; Jiménez-Hernández *et al.*, 2023; Navarrete and Acosta-Gallegos, 2000; Quiñones-Aguilar *et al.*, 2018). For this reason, for the management of halo blight, strategies have been developed such as the application of agricultural antibiotics, the elimination of harvest residues, crop rotation or the use of resistant bean varieties, and others (Schwartz *et al.*, 2005). By combining these measures, it has been possible to control the development of the disease, although it has not been enough.

Figure 5. Representative map of the distribution of *P. syringae* pv. *phaseolicola* in Mexico. The states marked with yellow color indicate the presence of this bacteria.

Based on advances in biotechnology, molecular strategies have emerged as a powerful tool that can complement conventional strategies and achieve a more efficient and sustainable management of pathogens. Genetic resistance is one of the most effective strategies to control plant diseases. In Mexico, the use of molecular markers has favored the identification of bean varieties, such as Flor de Mayo M38, San Rafael, Pinto Laguna 80 and Pinto Saltillo, which are resistant to *P. syringae* pv. *phaseolicola* (Jiménez-Hernández *et al.*, 2023). Despite this, resistance in the commercial varieties available in Mexico continues to be limited, therefore the development of new resistant varieties with novel molecular techniques such as CRISPR/Cas9 genetic editing (Thomas *et al.*, 2024) may be an alternative to specifically introduce genetic resistance in the bean varieties. In addition, technologies based on the so-called "omic sciences" offer valuable information to understand the interaction of *P. syringae* pv. *phaseolicola* with the bean. Proteomic analyses of the halo blight-resistant bean varieties provide information to understand the nature of this resistance (Cooper *et al.*, 2020; Oblessuc *et al.*, 2022), as well as generating information that could be used to develop control strategies based on the analysis of genetic and protein markers of these varieties. The use of bacteriophages in biological control is also a promising and eco-friendly alternative for the management of pathogenic bacteria. In 2018, Quiñones-Aguilar *et al*. isolated native bacteriophages native to the state of Zacatecas and evaluated their lytic activity against virulent strains of *P. syringae* pv. *phaseolicola*. In this way, they identified a bacteriophage capable of reducing the damage caused by the *phaseolicola* pathovar in green beans by up to 60% (Quiñones-Aguilar *et al.*, 2018). In Mexico, molecular techniques focused on managing halo blight and other crop diseases are limited, making it necessary to promote the development of new strategies to complement the management of halo blight in bean crops in Mexican agriculture.

Conclusions

Pseudomonas syringae pv. *phaseolicola* is a bacterial species with a great pathogenic ability, and has therefore become a study model for the understanding of the plant-pathogen molecular interaction. The regions Pht and Pbo, which contain genes involved in the biosynthesis of phaseolotoxin, play a key role in the development of halo blight. Evidence shows that the acquisition of the Pht and Pbo regions was carried out via horizontal transfer events, a common activity among phytopathogens. Thus, they acquire foreign genetic material and control it through its endogenous regulation system, which, in the case of the genes *pht* and *pbo*, were incorporated into the global regulation systems of the components, GacS/

GacA and of the regulator IHF. For *P. syringae* pv. *phaseolicola*, acquiring the Pht and Pbo regions has been a favorable event, since it helped it increase its degree of virulence in such a way that the result of this new ability is to devastate the bean crop with greater efficiency. Molecular studies of the pathogenicity and virulence factors of *P. syringae* pv. *phaseolicola*, such as in the case of phaseolotoxin, offers valuable information for the design of diagnostic strategies and the management of the halo blight. The development of new molecular strategies will favor the early diagnosis, the identification of new pathogenic strains and the development of resistant crops, which will, in turn, reduce the economic impact of diseases in agronomically important crops such as the bean.

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