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Role of the PhoP-PhoQ System in the Virulence of *Erwinia chrysanthemi* Strain 3937: Involvement in Sensitivity to Plant Antimicrobial Peptides, Survival at Acid pH, and Regulation of Pectolytic Enzymes

Arancha Llama-Palacios, Emilia López-Solanilla, and Pablo Rodríguez-Palenzuela*

Departamento de Biotecnología—UPM, E.T.S. Ingenieros Agrónomos, Madrid, Spain

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***Erwinia chrysanthemi* is a phytopathogenic bacterium that causes soft-rot diseases in a broad number of crops. The PhoP-PhoQ system is a key factor in pathogenicity of several bacteria and is involved in the bacterial resistance to different factors, including acid stress. Since *E. chrysanthemi* is confronted by acid pH during pathogenesis, we have studied the role of this system in the virulence of this bacterium. In this work, we have isolated and characterized the *phoP* and *phoQ* mutants of *E. chrysanthemi* strain 3937. It was found that: (i) they were not altered in their growth at acid pH; (ii) the *phoQ* mutant showed diminished ability to survive at acid pH; (iii) susceptibility to the antimicrobial peptide thionin was increased; (iv) the virulence of the *phoQ* mutant was diminished at low and high magnesium concentrations, whereas the virulence of the *phoP* was diminished only at low magnesium concentrations; (v) in planta Pel activity of both mutant strains was drastically reduced; and (vi) both mutants lagged behind the wild type in their capacity to change the apoplastic pH. These results suggest that the PhoP-PhoQ system plays a role in the virulence of this bacterium in plant tissues, although it does not contribute to bacterial growth at acid pH.**

Bacteria of the family *Enterobacteriaceae* are able to colonize a wide range of natural habitats, and many species cause diseases in both animals and plants. Bacterial cells experience several types of stresses in natural situations, such as starvation, lack of iron, oxidative damage, heat, or acid pH (18). Most enteric bacteria, including *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, are neutrophiles, they grow better at approximately neutral pH, but they are often exposed to acid stress in natural and pathogenic conditions (7, 42). This type of stress has been described as the combined biological effect of the low pH (inorganic component) and the weak organic acids (organic component) present in the milieu (7).

Plant pathogenic enterobacteria have to grow in the apoplast, the pH of which usually ranges between 5.0 and 6.5 but can be as low as 4.0. This acid pH is due to the abundance of organic acids, as citric or malic acid, and proton pumping from nearby cells (22). This is in contrast with animal pathogenic bacteria, which may be confronted with a more acidic pH in the animal's stomach.

Not surprisingly, some bacterial species have evolved mechanisms to withstand acidic stress. For example, for *S. enterica* serovar Typhimurium, an acid tolerance response has been described (17, 46), which enables the bacterium to survive to extremely low pH (3.0 to 4.0) if it became previously adapted to a mild acid pH (5.5 to 6.0). Moreover, acid resistance of *E. coli* has been described as the capacity of the bacterium to withstand an acidic pH of 2.5 or below if the cell is in stationary phase (10).

* Corresponding author. Mailing address: ETS Ingenieros Agrónomos, Avda. Complutense s/n, E-28040 Madrid, Spain. Phone: 34 91 3365705. Fax: 34 91 3365757. E-mail: pablo@bit.etsia.upm.es.

Erwinia chrysanthemi is a plant-pathogenic bacteria belonging to the *Enterobacteriaceae*. It causes soft-rot disease in a wide range of plant hosts, being able to infect essentially any nonlignified tissue (39). The typical maceration symptoms are mostly the result of secreted pectolytic enzymes that degrade the cell walls, ultimately leading to cell lysis and necrosis of the whole tissue (3, 4). In the initial stages of infection, the bacteria have to establish a population in the acidic plant apoplast. However, as the infection progresses, a concomitant alkalinization of the milieu occurs (37).

Most studies of *E. chrysanthemi* have been performed with two model strains: EC16, isolated from chrysanthemum plants (11), and 3937, isolated from saintpaulia plants (30). Although they produce similar symptoms and they attack a wide range of plants, the two strains have different virulences in different plant hosts. In addition, EC16 secretes four major pectic lyase isoenzymes in culture (PelABCE) (28), whereas 3937 secretes five isoenzymes (PelABCDE) (9). Little is known about the genetic relatedness of the strains.

Our previous work with strain EC16 has revealed that mutants in the *phoP-phoQ* operon are altered in their ability to grow at acid pH and also showed (i) decreased survival at acid pH in plant tissues, (ii) increased susceptibility to antimicrobial peptides, (iii) decreased virulence in several hosts, (iv) reduced production of pectolytic enzymes, and (vi) reduced ability to alkalinize plant tissues (31). The *phoP-phoQ* operon has been described as a key factor controlling virulence in *S. enterica* serovar Typhimurium (23, 35). PhoQ is a sensor histidine kinase that autophosphorylates in response to environmental conditions, and PhoP is a transcriptional regulator which controls the expression of genes that are essential for virulence, particularly involved in survival within macrophages, survival at acid pH, and resistance to antimicrobial peptides (16). The

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source or reference
E. coli DH5α	*supE44 Δlac U169* (ϕ80 *lacZ*M15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	24
E. chrysanthemi		
3937	Wild-type strain	30
BT123	Δ(*phoP*)::Tn*7* Cam^r, derivative of 3937	This work
BT124	Δ(*phoO*)::Tn*7* Cam^r, derivative of 3937	This work
Plasmids		
pGEM T-easy	Amp^r	Promega
pBluescript II SK(−)	Amp^r	Stratagene
pB113	pGEM carrying 3937 *phoP* and *phoQ* genes	This work
pB114	pBluescript II carrying 3937 *phoP* mutant	This work
pB115	pBluescript II carrying 3937 *phoQ* mutant	This work

PhoP-PhoQ system also controls the expression of a set of around 40 proteins including proteases, phosphatases, and cation transporters (15).

It has been reported that the magnesium concentration in the bacterial environment is one of the signals that controls the PhoP-PhoQ system in *S. enterica* serovar Typhimurium (19). Also, we have previously reported that low magnesium concentrations induce expression of the *phoQ* gene in *E. chrysanthemi* EC16 (31). It is likely that this condition is commonly found by these pathogens in their respective hosts.

In this work, we have constructed *phoP* and *phoQ* mutants in strain 3937 and studied the phenotypes of these mutants with respect to (i) virulence, (ii) survival at acid pH, (iii) sensitivity to antimicrobial peptides, and (iv) pectic enzyme production. This regulatory system appears to have an important role in the pathogenicity of this strain, as in strain EC16. However, the phenotypes of the *phoP* and *phoQ* mutants differed in both strains with respect to the above-mentioned features.

MATERIALS AND METHODS

Microbiological methods. The bacterial strains and plasmids used in this study are described in Table 1. Strains of *E. coli* were cultivated at 37°C in Luria-Bertani medium. Strains of *E. chrysanthemi* were cultivated at 28°C in nutrient broth (NB; Difco, Detroit, Mich.), King's B medium (29), or modified basal medium A (MBMA) (44) (citric acid monohydrate was added instead of sodium citrate to buffer in the lower pH ranges) (17, 45) supplemented with 0.2% glycerol and 250 μM potassium phosphate (pH 7.0) (40). Antibiotics were added to the media at the following concentrations (micrograms per milliliter): ampicillin, 100; and kanamycin, 20.

Short-chain organic acid inhibition and enzymatic assays. Short-chain organic acid inhibition assays were performed by using the method described by López-Solanilla et al. (33). Organic acids were added to the basal medium A cultures without citric acid monohydrate at pH 5.5 and 7.0, as follows: acetic acid, benzoic acid, butyric acid, citric acid, formic acid, lactic acid, and propionic acid at 50, 100, and 200 μg/ml.

Culture filtrates containing a mixture of extracellular pectic enzymes were obtained as previously described (34). For the determination in planta of the enzymatic activities, chicory disks of 1 cm in diameter were inoculated with a suspension containing 10^7 bacterial cells and incubated at 28°C. After 4 h, the tissues were treated as indicated for the culture filtrates. The extracellular pectic lyase (Pel) activity was determined by monitoring the increase of absorbance at 232 nm as a result of the 4,5-unsaturated reaction products. This assay was performed as described by Collmer et al. (13).

DNA manipulation and sequencing. The *E. chrysanthemi* 3937 *phoP-phoQ* operon was amplified by PCR with the oligonucleotides 5′-GAGAAACTGAAAG AACTGACCCGCG-3′ and 5′-CTTAATTCACGGACGAGGCGG-3′ (based on the *phoP-phoQ* operon sequence of *E. chrysanthemi* EC16) (31), cloned in pGEMT-easy (Promega, Madison, Wis.), and designated pB113 (Table 1). Plasmid pBluescript SK(−) (Stratagene, La Jolla, Calif.) was used for subcloning. Tn*7* in vitro mutagenesis was performed with the genome priming system kit (GPS-1; New England Biolabs, Beverly, Mass.). Two mutagenized constructions (pB114 and pB115) (Table 1) bearing the Tn*7* transposon within the *phoP* and *phoQ* genes, respectively, were selected and marker exchanged into the chromosome as previously described (26). The marker exchange was verified by DNA blot hybridization (data not shown). The corresponding mutant strains (BT123 and BT124) (Table 1) were selected for further analysis. Standard molecular cloning techniques employed in this study were performed as described previously (41). DNA sequencing of both strands was done by the chain termination method on double-stranded DNA templates with an ABI Prism dye terminator cycle sequencing kit (Perkin-Elmer, Norwalk, Conn.) in a 3100 DNA sequencer (Perkin-Elmer). Sequence alignments were performed at the National Center for Biotechnology Information (online) with the BLAST network service (1) and with The Institute for Genomic Research BLAST (http://www.tigr.org).

Susceptibility, lethality, and virulence assays. Susceptibility to antimicrobial peptides was assayed as previously described (32). To perform lethality assays, 10 ml of MBMA at pH 3.0, 3.5, 4.0, 4.5, 5.5, and 7.0 was inoculated with 100 μl of a suspension containing 10^6 bacterial cells of *E. chrysanthemi* 3937 wild-type or *phoP* or *phoQ* mutant strains. Cells were incubated for 4 h at 28°C with shaking, and then a portion of each sample was diluted and plated on nutrient broth agar plates for assessment of bacterial viability. Three replicates were performed in each case.

Witloof chicory leaves were purchased from a local supermarket. The bacterial cells from an overnight NB liquid medium culture were washed with 10 mM or 10 μM $MgCl_2$ and then resuspended in an appropriate volume of buffer to obtain the desired inoculum concentration. Virulence assays on chicory leaves were performed as previously described (5). Briefly, each chicory leaf was inoculated at two locations with *E. chrysanthemi* 3937 wild-type and *phoP* or *phoQ* mutant strains. Forty leaves were pair-inoculated with 10 μl of a suspension containing 10^3 bacterial cells in 10 mM $MgCl_2$ or 10 μl of a suspension containing 10^4 bacterial cells in 10 μM $MgCl_2$. The reason for using a different level of inoculum was to achieve a 100% frequency of infection for the wild-type strain in both experiments. Chicory leaves were incubated for 32 h in a moist chamber at 28°C. The virulence in this assay is measured as the macerated area. The differences between wild-type and mutant strains were statistically assessed with a paired Student's *t* test. To estimate the bacterial populations attained in plant tissues, 10 μl of a suspension containing 10^4 bacterial cells in 10 μM $MgCl_2$ was inoculated in chicory leaves. The tissue was incubated in a moist chamber at 28°C for 24 h and ground, and the bacterial populations were estimated by dilution plating. To monitor pH variations in chicory leaves upon infection, the following pH indicator solutions (0.1%) were prepared: (i) bromocresol purple (Merck, KGaA, Darmstadt, Germany), which is yellow at a pH value below 5.2 and purple at a pH value above 6.8, and (ii) phenol red (Merck), which is yellow at a pH value below 6.4 and red-violet at a pH value above 8.2. The chicory leaves were inoculated with 10 μl of a suspension containing 10^4 bacterial cells of *E. chrysanthemi* 3937 and *phoP* and *phoQ* mutant strains. After different times of incubation at 28°C, the changes in the pH were monitored by adding the pH indicator solutions at the inoculation point.

RESULTS

Sensitivity to short-chain fatty acids in *E. chrysanthemi* 3937. It has been known for a long time that short-chain organic acids present in the intestine can exert antimicrobial

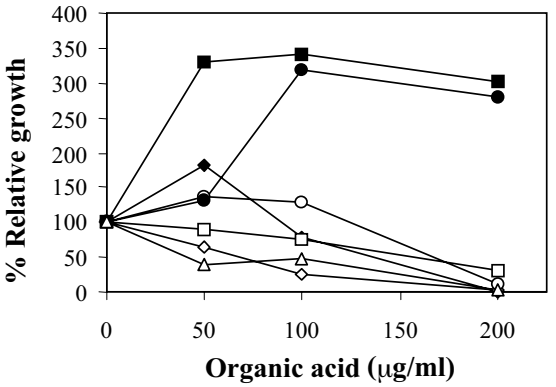

FIG. 1. Susceptibility of *E. chrysanthemi* strain 3937 to several short-chain organic acids at pH 7.0: ♦, acetic acid; ◇, benzoic acid; □, butyric acid; ■, citric acid; ○, formic acid; ●, lactic acid; △, propionic acid. *E. chrysanthemi* 3937 at pH 5.5 did not grow at this incubation time. Relative growth is expressed as the percentage of optical density attained by the cultures after 24 h in the presence of the indicated organic acid concentration with respect to the optical density attained in the absence of the organic acid. Results show the means of three replicates. Standard error bars are too small to be represented.

activity (2, 12). Furthermore, the addition of a mixture of these acids has been used to prevent *Salmonella* infections in chicken farms (25). Therefore, we decided to analyze the sensitivity of *E. chrysanthemi* 3937 to several weak organic acids, such as acetic, benzoic, butyric, citric, formic, lactic, and propionic acids. The sensitivity was measured as described in Material and Methods. At pH 7.0, it was found that the bacteria were inhibited by all of the acids assayed except citric and lactic acids, as shown in Fig. 1.

Growth and survival of strain 3937 and *phoP* and *phoQ* mutants in acid medium. To study the behavior of strain 3937 in acid medium, growth curves at different pHs in MBMA were done. We observed that 3937 did not grow at pH 5.0 and 5.5 and grew poorly at pH 6.0 (Fig. 2). The growth curves at different pHs of the *phoP* and *phoQ* mutants were compared with that of the wild type (data not shown). Interestingly, these mutants did not show diminished ability to grow at acid pH, in contrast with behavior reported for the same mutations in strain EC16 (31).

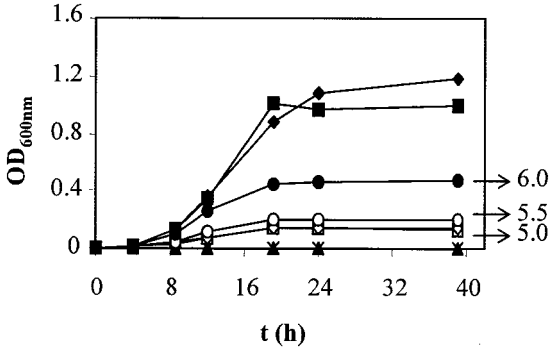

FIG. 2. Growth curves of *E. chrysanthemi* strain 3937 in MBMA at different pHs: ♦, pH 7.0; ■, pH 6.5; ●, pH 6.0; ○, pH 5.5; □, pH 5.0; ◇, pH 4.5; ▲, pH 4.0; △, pH 3.5; ∗, pH 3.0. Results show the means of three replicates. Standard error bars are too small to be represented.

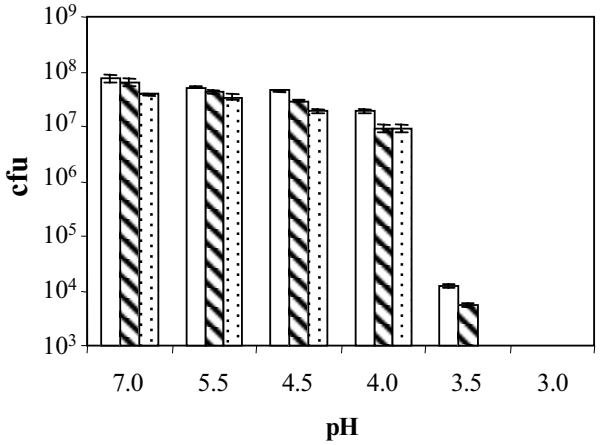

FIG. 3. Survival at different pHs of *E. chrysanthemi* 3937 wild-type (empty bars), *phoP* (diagonally striped bars), and *phoQ* (dotted bars) strains. Each strain (10^4 CFU) was incubated for 4 h at the indicated pH at 28°C, and the appropriate dilutions were plated onto NB agar plates. Results show the means and standard errors of three replicates.

Also, we investigated the survival of wild-type and *phoP* and *phoQ* mutant strains after acid treatment, performing lethality assays as indicated in Material and Methods. Differences between the wild type and *phoP* and *phoQ* mutants were found at several pHs (Fig. 3). At pH 3.5, the difference between the wild type and *phoQ* mutant was 4 orders of magnitude.

Virulence and enzymatic assays. To investigate the possible effect on virulence of the *phoP* and *phoQ* mutations, chicory leaves were inoculated with *E. chrysanthemi* 3937 and *phoP* and *phoQ* strains. Since it is known that magnesium levels affect the PhoP-PhoQ system, we decided to perform the inoculations at high and low magnesium concentrations. Necrotic areas of the developed lesions were measured after 32 h. At high magnesium concentrations, statistically significant differences were found only between the wild-type and *phoQ* mutant strains (Table 2). In contrast, the virulence tests performed at low magnesium concentrations showed that both mutants were significantly less virulent than the wild-type strain. The necrotic areas produced by the *phoP* and *phoQ* mutants were 33 and 41%, respectively, of that of the wild type.

TABLE 2. Effects of Δ(*phoP*)::Tn*7* and Δ(*phoO*)::Tn*7* mutations on the virulence of *E. chrysanthemi* 3937 on witloof chicory leaves at high and low Mg^{2+} concentrations

Strain	$MgCl_2$ concn	Size of lesion (cm^2) (mean ± SE)[a]
3937 (wild type)	10 mM	1.75 ± 0.03
BT123 (*phoP*)	10 mM	1.20 ± 0.11[b]
3937 (wild type)	10 mM	2.64 ± 0.17
BT124 (*phoQ*)	10 mM	1.71 ± 0.10[c]
3937 (wild type)	10 μM	1.16 ± 0.17
BT123 (*phoP*)	10 μM	0.39 ± 0.03[c]
3937 (wild type)	10 μM	1.23 ± 0.16
BT124 (*phoQ*)	10 μM	0.50 ± 0.04[c]

[a] Values are the products of the length and width of the necrotic area.
[b] Differences between wild-type and mutant strains are not significant according to the Student *t* test ($P < 0.05$).
[c] Differences between wild-type and mutant strains are significant according to the Student *t* test ($P < 0.05$).

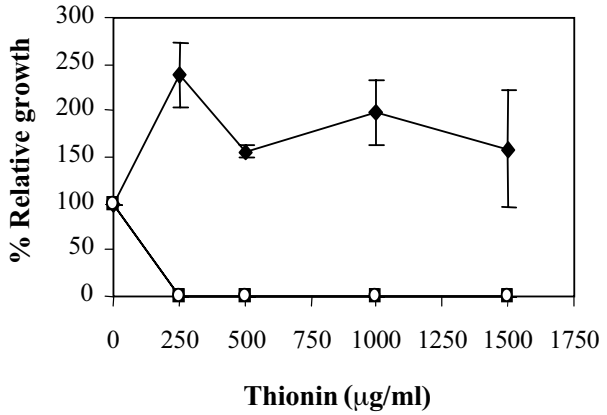

FIG. 4. Susceptibility of the *E. chrysanthemi* 3937 wild type (♦) and the *phoP* (■) and *phoQ* (○) mutants to thionin. Relative growth is expressed as the percentage of optical density attained by the cultures after 6 h in the presence of the indicated peptide concentration with respect to the optical density attained in the absence of the peptide. Results show the means and standard errors of three replicates.

The bacterial populations attained in plant tissues by the wild-type and mutant strains were estimated. In a high magnesium (10 mM) concentration, the bacterial population of *phoP* was 78.5% of that of the wild type and that of *phoQ* was 86.6%. In a low magnesium (10 μM) concentration, the populations of *phoP* and *phoQ* were 35.3 and 56.3%, respectively. In general, there is a good agreement between the level of growth in planta and the virulence of the different strains (Table 2).

To study the possible role of the *phoP-phoQ* operon in the production of extracellular pectate lyase, this activity was analyzed in planta. The pectate lyase activity was reduced in the *phoP* and *phoQ* mutants. It was found that relative enzymatic activity of the mutants, expressed as the percentage of activity attained in the spectrophotometric assay with respect to that of the wild type, was 4.65 and 8.95% for *phoP* and *phoQ*, respectively. These results were the means of three replicates, and the standard error was less than 10% of the mean value.

Sensitivity to antimicrobial peptides. The sensitivity of *phoP* and *phoQ* mutant strains to antimicrobial peptides, derived from plants and animals, was compared with that of the wild type. Thionins are cysteine-rich antimicrobial peptides, found in many plant species, which show antimicrobial activity towards several fungal and bacterial pathogens at concentrations in the 50- to 500-μg/ml range (21). Protamine is a lineal antimicrobial peptide that it is frequently used as an antibiotic. Wheat thionin and salmon protamine were selected for in vitro inhibition tests. As shown in Fig. 4, the mutants were significantly more susceptible to thionin, although no differences were found with respect to protamine. Curiously, the wild-type strain appears to grow more in the presence of the antimicrobial peptide thionin. This effect has been previously reported (32), and it is probably due to peptide degradation and utilization by the bacteria. In contrast, other phytopathogenic bacteria are inhibited at concentrations in the 50- to 500-μg/ml range (21).

Modification of apoplastic pH during infection. Since it has been reported that the pH value of the intercellular environment changes as a consequence of *E. chrysanthemi* colonization (37), we decided to carry out a comparative study during the course of the infection by using the wild-type and mutant strains with 10 μM magnesium chloride. Chicory leaves were inoculated as described in Materials and Methods with bacterial cells of the wild-type and *phoP* and *phoQ* mutant strains. pH indicator solutions were added at the inoculation site in a time-course experiment, and both mutants lagged behind the wild type in their ability to modify the apoplastic pH around the infection site (Fig. 5). In longer experiments, we have consistently observed that both wild-type and mutant strains eventually change the apoplastic pH to a pH value above 8.2 (data not shown).

FIG. 5. pH modification of the chicory apoplast shown by color changes of pH indicator solutions after inoculation with a suspension of *E. chrysanthemi* 3937 wild-type (WT) and *phoP* and *phoQ* mutant strains in 10 μM $MgCl_2$ at 6 h. BP, bromocresol purple (pH 5.2 to 6.8); PR, phenol red (pH 6.4 to 8.2).

DISCUSSION

The two-component regulatory system PhoP-PhoQ is a key feature for the pathogenicity of several gram-negative bacteria (20). Our previous work with *E. chrysanthemi* strain EC16 indicates that this system controls a set of characteristics that act coordinately to help the establishment and survival of the bacterial population in plant tissue, namely, the abilities to grow at acid pH, alkalinize the external pH, and withstand the

action of antimicrobial peptides. These features seem to be far more important at the low inocula prevailing in natural infections and may play an important role in adapting the bacterium to the changing conditions found along its life cycle.

In this work, we have focused on the role of the PhoP-PhoQ system in *E. chrysanthemi* strain 3937. Surprisingly, these mutants were not affected in their ability to grow at acid pH, in contrast with the results reported for EC16 (31) and other bacteria in the *Enterobacteriaceae*, such as *S. enterica* serovar Typhimurium and *Yersinia pestis* (6, 38). Apparently, the PhoP-PhoQ system of strain 3937 is not involved in the control of growth at acid pH and probably is not a key feature of the pathogenicity of this strain. Actually, the data obtained in the survival in acid pH experiments suggest that this system is involved in the control of this feature. On the other hand, the PhoP-PhoQ system of strain 3937 had a higher effect in the total pectate lyase activity in planta than that of strain EC16. In 3937, the reduction in Pel activity in the mutants was 90 to 95%, whereas in EC16, the reduction was only about 60% (31). This is congruent with the idea that the basic strategy of strain 3937 relies on a rapid release of plant cell compounds.

Another difference found between the *phoQ* mutants of both strains is related to the sensitivity to antimicrobial peptides. In EC16, the mutant showed an increased sensitivity to antimicrobial peptides from both animal and plant origin (protamine and thionin) (31), whereas in the 3937 mutant, only the sensitivity to thionin was altered (Fig. 4). A possible explanation of the differential effect between antimicrobial peptides could be based in the particular set of genes activated by PhoP-PhoQ; this set of genes is not known at present (neither in strain 3937 nor in strain EC16). For example, it cannot be ruled out that the PhoP-PhoQ system of strain EC16 activates a mechanism effective against protamine, which is not regulated by this system in the 3937 strain.

Several works with other *Enterobacteriaceae* have shown that magnesium is an important factor in the regulation of the PhoP-PhoQ system (19, 27, 36). It has been reported that PhoQ acts as a magnesium sensor of bacteria in animal tissues (19, 43). Our results clearly indicate that the magnesium concentration plays an important role in the altered pathogenic features of the *phoP* and *phoQ* mutants of strain 3937. As shown in Table 2, the mutants had a noticeably diminished virulence at low magnesium concentrations. There are very few data regarding the availability of magnesium for bacteria during infection in the plant apoplast. It is generally true that magnesium is abundant in plants (8, 22), but it could be tightly linked to other components to the plant cell wall. The model resulting from these data is that *E. chrysanthemi* cells encountered low magnesium levels in the apoplast before the infection; this magnesium level induces the PhoP-PhoQ system, which helps the cell to tolerate several types of stresses. A prediction of this model is that *phoP* and *phoQ* mutants survive poorly in the apoplast, and the reduced number of bacterial cells could explain the reduced virulence and reduced pectate lyase activity. Our finding of a good agreement between the virulence of the strains and the bacterial populations attained in plant tissues is congruent with this model.

The fact that the mutation of *phoQ*, coding a sensor kinase, had a larger effect on virulence at high magnesium concentrations than the mutation of *phoP*, encoding a transcriptional regulator, could look puzzling at first sight. Although we do not have a satisfactory explanation, this result suggests that alternative sensory proteins could phosphorylate PhoP or/and alternative regulator proteins could be activated by PhoQ.

Considering the published data (31) and those presented in this work, it is possible to compare the behavior of the two strains with respect to growth curves in minimal medium at different pHs, sensitivity to organic acids, survival at acid pH, and the modification of apoplastic pH along the infection process. It was shown that EC16 grew better at low pH (31) than 3937 (Fig. 2). In contrast, the survival at low pH of EC16 (31) was lower than that of 3937 (Fig. 3). Moreover, the ability to change the apoplastic pH of EC16 was lower than that of the strain 3937 (data not shown). Considering these data altogether, we can hypothesize that both strains are using different strategies to cope with acid stress. Strain 3937 relies on survival at low pH, together with a higher ability to modify the pH of the milieu, whereas the EC16 strategy is likely based on the capacity to grow under the initial conditions. The fact that strain 3937 possesses an additional major isozyme of pectate lyase is congruent with this hypothesis because it enables strain 3937 to more rapidly change the apoplastic pH due to a faster release of plant cell contents.

Plant tissues are extraordinarily rich in weak organic acids, for example, the titratable acidity of lemon fruit is as high as 6 M; in most vegetative tissues, the content of free titratable acids is 0.2 to 0.4 g per 100 g of fresh tissue (8). We have found that strain 3937 was inhibited at a 200-μg/ml concentration of acetic, benzoic, butyric, formic, and propionic acids (Fig. 1). These inhibitory concentrations are well bellow the total titratable acidity found in plant tissues, which suggests that these compounds could constitute a barrier for the proliferation of bacterial cells. The strains 3937 and EC16 showed differences with respect to their ability to grow in the presence of these organic acids: EC16 showed a higher ability for growing in the presence of all of the organic acids assayed, except citric and lactic acids (data not shown). These results are in line with the fact that EC16 grows better in acidic conditions.

Although *E. chrysanthemi* is considered a broad-host-range pathogen, it is known that different strains have different virulences according to the host plant (39). Surprisingly, the natural variability of strains in soft-rot erwinias has received little attention, in spite of the pioneering work of Dickey (14), which showed that strains of *E. chrysanthemi* isolated in the same host usually show similar characteristics, independent of geographic origin. Since plant tissues differ with respect to apoplastic pH and the type and amount of organic acids and antimicrobial peptides, among over features, we can expect that different strains have evolved finely tuned regulatory mechanisms to improve the colonization of particular host plants. Clearly, the genetic basis of strain specificity in this bacterium poses an interesting question, which merits further investigation.

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