Calcium-Dependent Pectate Lyase Production in the Soft-Rotting Bacterium *Pseudomonas fluorescens*


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**ABSTRACT**


Pectate lyase (PL) is the principal or sole enzyme responsible for maceration of plant tissue caused by most strains of soft-rotting pseudomonads. Production of PL in four of 25 *Pseudomonas fluorescens* (or *P. marginalis*) strains examined was not induced by the enzyme substrate, polygalacturonic acid (PGA), but was induced by Ca<sup>2+</sup>. These four strains produced 10 times more PL in medium containing 1 mM CaCl<sub>2</sub> than in one containing no CaCl<sub>2</sub> supplement. Over 86% of total PL produced by these strains in CaCl<sub>2</sub>-supplemented medium was excreted into the culture fluid. Only a small portion (13%) of total PL produced by these strains in CaCl<sub>2</sub>-deficient medium was detected in the extracellular fraction. Ca<sup>2+</sup> was required not only the amount but also the final destillation of PL produced by these pseudomonads. Additionally, all four strains were unable to use PGA as a nutritional source when cultured in CaCl<sub>2</sub>-deficient medium, which indicates that the initial step of PGA degradation was mediated by Ca<sup>2+</sup>-dependent PL and not by Ca<sup>2+</sup>-independent polygalacturonase. The optimal Ca<sup>2+</sup> concentration required for PL production in one of these strains, CY091, was determined to be 0.2 mM. A linear correlation was observed between the amounts of PL produced and the concentrations of Ca<sup>2+</sup> included in the medium. Furthermore, the requirement of Ca<sup>2+</sup> for PL induction could be replaced by Sr<sup>2+</sup> but not by other divalent cations, such as Zn<sup>2+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup>, Mg<sup>2+</sup>, and Ba<sup>2+</sup>. Because of the indispensable role of Ca<sup>2+</sup> in PGA degradation and in PL production, the possibility of using the ion-chelating agent ethylenediaminetetraacetic acid (EDTA) for control of *Pseudomonas* rot was evaluated. EDTA exhibited bactericidal activity against *P. fluorescens* at a minimal inhibitory concentration of 4 mM. When assayed on potato tuber disks, EDTA at a concentration of 0.13 mM (40 ppm), which is 30-fold lower than the minimal inhibitory concentration, was effective in preventing *P. fluorescens* from growing and causing maceration in potato tuber tissue.

Additional keywords: enzyme export, potato rot control.

Pectolytic, fluorescent pseudomonads, mainly strains of *Pseudomonas fluorescens* (or *P. marginalis*) and *P. viridiflava*, account for over 40% of bacterial rot of fruits and vegetables in storage and during transit (15). These pseudomonads are unique among postharvest pathogens in that they are able to grow under refrigerated conditions and to use a wide variety of simple compounds as carbon (C) and energy sources (1, 15). Both *P. fluorescens* and *P. viridiflava* have been shown to cause diseases of plants in the field (1, 12). However, they appear to be more often associated with decay of plant products after harvest (12). Because of the psychrophilic nature of the bacteria, refrigeration currently employed to prolong the shelf life and to reduce microbial decays of fresh produce is ineffective in suppressing rot caused by *P. fluorescens* and *P. viridiflava*. No control measure specifically targeted against this group of soft-rotting pathogens is presently available, but such measures might be developed when more is learned about the biochemical and genetic mechanism by which these pathogens cause diseases in plants.

The ability of *P. fluorescens* and *P. viridiflava* to induce maceration of plant tissues is primarily due to their ability to produce pectolytic enzymes that are capable of degrading pectic components in plant cell walls. Although a few strains of *P. fluorescens* have been shown to produce polygalacturonase (21, 33), pectin methylesterase (21), and pectin lyase (28, 29), almost all strains of soft-rotting pseudomonads so far examined produce pectate lyase (PL) (5, 7, 10, 21, 35). Recently, we investigated the isoelectric focusing profiles of PL samples produced by 10 strains of *P. fluorescens* and eight strains of *P. viridiflava* (10) and found that the pectic enzyme system of these strains was much simpler than that of *Erwinia* (10). Unlike the multiple PL isozyme systems (pl 4.5-10.0) in *Erwinia* (2), all *P. fluorescens* and *P. viridiflava* strains examined produced a single alkaline PL with approximate isoelectric points of pl 9.7 and 10.0, respectively (10). Results from genetic studies with transposon mutagenesis (12) and gene cloning (11, 14) indicate that the alkaline PL produced by *P. fluorescens* and *P. viridiflava* is the principal or sole enzyme responsible for maceration of plant tissues.

At present, very little is known about the biochemical mechanism governing PL production in soft-rotting pseudomonads. Production of PL in the majority of *P. fluorescens* strains appears to be induced by pectic substrates (5, 7, 21, 33) or by plant tissue extracts (33, 35). However, in some strains, PL production is not affected by the type of C source included in the medium (21, 35). Recently, we investigated the mode of PL production in an unusual strain of *P. fluorescens* designated CY091 (11). We found that PL production in this strain, although not affected by the type of C source included in the medium, appeared to be induced by Ca<sup>2+</sup>. In this study, we surveyed the regulation of PL production in 24 other strains of *P. fluorescens* (15). We identified three additional strains that produced PL in a mode similar to that observed in strain CY091. Furthermore, we report here that the effect of Ca<sup>2+</sup> on PL production in strain CY091 is dose-dependent and that Ca<sup>2+</sup> is replaceable by Sr<sup>2+</sup>. Previously, we suggested that control of *Pseudomonas* rot might be achieved by manipulating free Ca<sup>2+</sup> available in plant tissue (11). Here, we report that the ion-chelating agent ethylenediaminetetraacetic acid (EDTA), at an extremely low concentration (0.13 mM), is sufficient to inhibit maceration of potato tuber tissue caused by *P. fluorescens*.

**MATERIALS AND METHODS**

Bacterial strains and culture media. Twenty-five soft-rotting strains of *P. fluorescens* previously isolated and characterized...
in our laboratory (15) were used. After initial screening, four strains (CY901, BC-05-1B, PJ-08-3, and LU-04-2B), which produced high levels of PL in media containing glucose, glycerol, or polyglacturonate (PGA), were identified and chosen for further studies. Strain CY901, which had been previously used to clone the PL gene and to demonstrate the Ca\(^{2+}\) dependency for PL production (11), was used to determine other parameters affecting enzyme production. A kanamycin-resistant derivative of strain CY901 (designated CY901B) was obtained by transposon mutagenesis as described elsewhere (16). Strain CY901B was prototrophic and showed no alteration in tissue-macerating ability. This strain was used to evaluate the efficacy of EDTA as a disease control agent in potato tuber assays (to be described later).

Media that were used during the study included 1) *Pseudomonas* agar (Difco Laboratories, Detroit, MI), 2) Luria broth (GIBCO/BRL Laboratories, Grand Island, NY), and 3) minimal salt (MS) standard medium (pH 7.1) containing 0.7% K\(_2\)HPO\(_4\), 0.2% KH\(_2\)PO\(_4\), 0.02% MgSO\(_4\)·7H\(_2\)O, 0.1% (NH\(_4\))\(_2\)SO\(_4\), 1 mM CaCl\(_2\), and 0.4% glucose, glycerol, or PGA (grade 1, no. P-3899, Sigma Chemical Co., St. Louis, MO). When required, CaCl\(_2\) in the standard MS medium was omitted or adjusted to various concentrations ranging from 0.01 to 1.00 mM. All cultures were incubated at 26 C and were shaken (120 rpm) when liquid media were used. The initial inoculum density in liquid media was in the range of 3·10\(^6\) cells per milliliter, and incubation lasted 60 h, unless otherwise indicated.

**Enzyme assays.** Polygalacturonase (3) and PL (12) activities were determined in accordance with methods previously described. One unit of PL activity was defined as the amount of enzyme that caused an increase of 1.0 absorbance unit per minute at 232 nm and 20 C. The reaction was carried out in a 0.5-ml volume containing 100 mM Tris HCl (pH 8.0), 1 mM CaCl\(_2\), 0.2% PGA, and enzyme sample. The specific enzyme activity was calculated and expressed as units of PL activity per 10\(^6\) cells. Cell numbers were estimated from optical density (OD600) readings; a sample with an OD600 of 1.5 was assumed to contain 10\(^6\) cells per milliliter. Activities of PL in cultures or in subcellular fractions were determined according to the methods reported previously (11).

**Effects of C sources, divalent salts, and EDTA on PL production.** Bacterial strains were grown in MS media containing various types and concentrations of C sources, divalent salts, and EDTA to determine 1) the effects of C sources and Ca\(^{2+}\) on PL production and 2) the optimal concentration and the dependency of Ca\(^{2+}\) for PL induction. Three C sources examined were glucose, glycerol, and PGA, and nine divalent salts tested were CaCl\(_2\), BaCl\(_2\), FeSO\(_4\), MnCl\(_2\), MgCl\(_2\), SrCl\(_2\), ZnCl\(_2\), NiCl\(_2\), and CuCl\(_2\). After incubation, total PL activities in cultures and partial enzyme activities in subcellular fractions were analyzed in accordance with methods previously described (11). Briefly, cells were separated from the culture medium by centrifugation (10,000 g for 10 min), and the clear supernatant was assayed for extracellular activity. The cell pellet was washed and resuspended in 50 mM Tris HCl (pH 7.2), and cells in the suspension were then disrupted by ultrasonication. After that, cell debris was removed by centrifugation (25,000 g for 20 min), and the clear supernatant was assayed for cell-bound activity. Occasionally, cells were disrupted by adding 1/200 volume of toluene to the culture or the cell suspension to release the cell-bound enzyme.

To determine whether the requirement for Ca\(^{2+}\) in PL production is dependent on the concentration of Ca\(^{2+}\), strain CY901 was grown in the MS-glycerol medium containing various concentrations (0.01-3.00 mM) of CaCl\(_2\). After incubation, total PL activities in the cultures were measured. To further demonstrate that PL production is inducible by Ca\(^{2+}\), two experiments were conducted. In the first experiment, strain CY901 was grown for 24 h in the MS-glycerol medium without the addition of CaCl\(_2\). After that, CaCl\(_2\) was added to the culture to a final concentration of 0.2 mM, and incubation continued for 36 h. In the second experiment, strain CY901 was grown for 24 h in the MS-glycerol medium with the addition of 0.2 mM of CaCl\(_2\). Then EDTA at a sublethal level (0.5 mM) was added to the culture, and incubation continued for another 36 h. Both PL activities and cell populations in the culture were monitored at 12-h intervals for 60 h.

**Inhibitory effect of EDTA on bacterial growth and tissue maceration.** Luria broth (20 ml) with or without 2 mM CaCl\(_2\) was inoculated with cells of strain CY901B grown overnight to an initial cell density of approximately 10\(^8\) cells per milliliter. The inoculated medium was then equally dispensed into a series of 10 tubes. Filter-sterilized EDTA stock solution (0.5 M) was added to tube 1 to a final concentration of 16 mM, and serial twofold dilutions of EDTA were made in tubes 2-9. Tube 10, which contained no EDTA, was used as a control. After incubation at 26 C with shaking (120 rpm) for 60 h, bacterial growth was recorded as indicated by an increase in turbidity in the tube. The minimal inhibitory concentration was defined as the minimal concentration of EDTA that was capable of preventing the increase of cell density in cultures. Similarly, 20 ml of sterile water with or without 2 mM CaCl\(_2\) was inoculated with cells of strain CY901B grown overnight to 10\(^8\) cells per milliliter. The suspension of bacteria in water was then equally dispensed in a series of 10 tubes (16 × 125 mm). EDTA was added again to the first tube to a final concentration of 16 mM and was serially diluted twofold in tubes 2-9. A surface-sterilized potato tuber disk, 8 × 6 × 3 mm, prepared as previously described (19), was placed in each tube. After incubation at 26 C with shaking for 60 h, each tube was vigorously vortexed for 1 min. The development of soft rot, as indicated by total disintegration of potato tuber tissue, was determined and recorded. To avoid the growth of bacterial contaminants, kanamycin was added to each tube at a final concentration of 10 \(\mu\)g/ml.

**RESULTS**

**Ca\(^{2+}\) requirement for pectin utilization.** Twenty-one of the 25 *P. fluorescens* strains examined in the study produced PL at levels four- to seven-fold higher in the medium containing PGA than in the medium containing glucose or glycerol. The presence of 1 mM CaCl\(_2\) in the MS medium was essential for these 21 strains to produce high levels of PL and to use PGA as the sole C source for growth. Four of the 25 strains examined produced nearly equal levels of PL in the medium containing either glucose, glycerol, or PGA (Table 1). Analysis of variance showed that production of PL by these four strains (CY901, BC-05-1B, PJ-08-30, and LU-04-2B) was not significantly \((P \geq 0.05)\) affected by the type of C source included in the medium. All four strains were unable to use PGA or pectin when cultured in the MS medium without the addition of 1 mM CaCl\(_2\). However, when glucose or glycerol was included as the C source, the growth of these four strains in the MS medium with or without 1 mM CaCl\(_2\) was not significantly different \((P \leq 0.01)\). The inability of these pseudomonads to grow in the MS-MS medium lacking 1 mM CaCl\(_2\) was therefore not due to the Ca\(^{2+}\) deficiency. In addition, polygalacturonase activity was not detected in culture filtrates prepared from any of these four strains. The action of Ca\(^{2+}\)-dependent PL, but not of Ca\(^{2+}\)-independent polygalacturonase.

**Table 1. Effect of carbon sources on pectate lyase production in four soft-rotting strains of Pseudomonas fluorescens**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Glucose</th>
<th>Glycerol</th>
<th>Polygalacturonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CY901</td>
<td>35.1 ± 4.2</td>
<td>41.8 ± 6.5</td>
<td>69.2 ± 3.1</td>
</tr>
<tr>
<td>BC-05-1B</td>
<td>28.5 ± 6.3</td>
<td>25.7 ± 3.9</td>
<td>47.2 ± 2.8</td>
</tr>
<tr>
<td>PJ-05-30</td>
<td>71.4 ± 5.3</td>
<td>80.6 ± 7.3</td>
<td>120.3 ± 6.7</td>
</tr>
<tr>
<td>LU-04-2B</td>
<td>90.5 ± 3.7</td>
<td>10.8 ± 6.3</td>
<td>98.5 ± 6.3</td>
</tr>
</tbody>
</table>

1. One unit of activity was defined as the amount of enzyme that caused an increase of 1.0 absorbance unit per minute at 232 nm and 20 C.

2. Grown in minimal salt media (see Materials and Methods) containing 1 mM CaCl\(_2\) and 0.4% glucose, glycerol, or polygalacturonate.

3. Values are the mean of three separate experiments plus minus the standard error. Analysis of variance indicated no significant \((P \geq 0.05)\) effect of carbon source on pectate lyase production.
Table 2. Effect of CaCl₂ on pectate lyase production in four strains of Pseudomonas fluorescens

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total activity (units/10⁹ cells)</th>
<th>Location (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without CaCl₂</td>
<td></td>
</tr>
<tr>
<td>CY091</td>
<td>4.8 ± 0.9</td>
<td>11 89</td>
</tr>
<tr>
<td>BC-05-1B</td>
<td>2.5 ± 0.7</td>
<td>8  92</td>
</tr>
<tr>
<td>PJ-08-30</td>
<td>19.1 ± 3.1</td>
<td>4  96</td>
</tr>
<tr>
<td>LU-04-2B</td>
<td>4.7 ± 1.2</td>
<td>13 87</td>
</tr>
<tr>
<td></td>
<td>With CaCl₂</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total activity (units/10⁹ cells)</td>
<td>Location (%)</td>
</tr>
<tr>
<td></td>
<td>Extracellular</td>
<td>Cell-bound</td>
</tr>
<tr>
<td>CY091</td>
<td>37.8 ± 5.8</td>
<td>93  7</td>
</tr>
<tr>
<td>BC-05-1B</td>
<td>31.2 ± 3.2</td>
<td>86 14</td>
</tr>
<tr>
<td>PJ-08-30</td>
<td>82.8 ± 6.1</td>
<td>91  9</td>
</tr>
<tr>
<td>LU-04-2B</td>
<td>93.4 ± 2.8</td>
<td>89 11</td>
</tr>
</tbody>
</table>

*Grown in minimal salt medium supplemented with glycerol (0.4%) (see Materials and Methods) and containing or lacking 1 mM CaCl₂.

One unit of activity was defined as the amount of enzyme that caused an increase of 1.0 absorbance unit per minute at 232 nm and 20 °C.

Values are the mean of three separate experiments plus or minus the standard error. Analysis of variance indicated significant (P < 0.01) effect of CaCl₂ on enzyme production. The enzyme activities that were cell-bound in cultures with or without CaCl₂ were not significantly different at P = 0.05.

Fig. 1. Effect of various concentrations of CaCl₂ on pectate lyase production in Pseudomonas fluorescens strain CY091. The bacterium was grown in minimal salt medium supplemented with glycerol (0.4%) and various concentrations of CaCl₂. Values are the mean of three independent experiments, and brackets indicate the standard error. One unit (U) of activity was defined as the amount of enzyme that caused an increase of 1.0 absorbance unit per minute at 232 nm and 20 °C.

Fig. 2. Ca²⁺ induction and ethylenediaminetetraacetic acid (EDTA) inhibition of pectate lyase production in Pseudomonas fluorescens strain CY091. The bacterium was first grown in minimal salt medium supplemented with glycerol (0.4%) and containing CaCl₂ (Ca) or not including CaCl₂ (●) for 24 h. Then, 0.5 mM EDTA or 0.2 mM CaCl₂ was added to the culture, and incubation continued for another 36 h. Values are the mean of three independent experiments, and brackets represent the standard error. One unit (U) of activity was defined as the amount of enzyme that caused an increase of 1.0 absorbance unit per minute at 232 nm and 20 °C.
produced by strain CY901 in the medium containing CaCl₂ (or SrCl₂) was excreted into the culture fluid. In contrast, a large proportion of PL produced by this strain in the media containing other divalent salts was retained within the cells. Also, Fe²⁺ appeared to have an adverse effect on PL production. Strain CY901 produced approximately 75% less PL in the medium containing 0.2 mM FeSO₄ than in that containing no FeSO₄ (Table 3).

**Inhibitory effect of EDTA on bacterial growth and on tissue maceration.** *P. fluorescens* was highly susceptible to the bactericidal activity of EDTA. The minimal inhibitory concentration of EDTA required for inhibiting the growth of strain CY901B in Luria broth was determined to be 4 mM (Table 4). When this rich broth was supplemented with 2 mM CaCl₂, the amount of EDTA required for inhibition of bacterial growth remained about the same (in the range of 4–8 mM). This indicates that the bactericidal activity of EDTA is not simply due to its Ca⁴⁺ chelating property. When assayed on potato tuber disks, EDTA at a concentration of 0.13 mM (approximately 40 ppm) was sufficient to prevent strain CY901B from growing and causing maceration in potato tuber tissue. However, when potato tuber disks were submerged in water supplemented with 2 mM CaCl₂, the EDTA concentration required for prevention of tissue maceration in potato tuber disks increased to 4 mM, the level that was required for inhibition of bacterial growth in rich broth.

Although the bactericidal activity of EDTA in rich medium could not be overcome by the addition of CaCl₂, the inhibitory effect of EDTA on soft rot development could be reversed by adding 2 mM CaCl₂.

**DISCUSSION**

The data presented here and elsewhere (11) demonstrate that Ca⁴⁺ is an important factor in the regulation of PL production in *P. fluorescens*. In pectin-inducible strains, Ca⁴⁺ as an enzyme捐助者 involved in the initial steps of pectin degradation required by the bacteria to generate unsaturated oligouronides needed for PL induction (2). In pectin-nondetectable strains (such as the four strains identified in this study), Ca⁴⁺ appears to act as an environmental factor regulating PL synthesis and/or PL export in the bacteria. Since PL synthesis and PL export are two independent events, it is presently unclear whether the effect of Ca⁴⁺ on PL production occurs in transcription, translation, or protein secretion. Previously, it was shown that Ca⁴⁺ is essential for maintaining the structural integrity and functional activity of proteases produced by *P. fluorescens* strains associated with raw milk spoilage (19). We also found that heat stability of PL is greatly enhanced in the presence of Ca⁴⁺ or other positively charged molecules such as polylysine (L.-J. Wong and C.-H. Liao, unpublished). The possibility that the lower PL activities detected in Ca⁴⁺-deficient media (Table 2) may result from formation of inactive PL needs to be further investigated. Recently, we found that protease production in strain CY901 is also regulated by Ca⁴⁺ (16). Moreover, we found that partially purified proteases from strain CY901 were unable to digest PL proteins (16). These results imply that lower PL activities detected in Ca⁴⁺-deficient media (Table 2) are probably not due to degradation of PL.

Divalent cations have been shown to inhibit extracellular enzyme production in two other microbial systems. McQueen-Ross et al. (20) reported that esterase production in Streptomyces scabies was inducible by Zn²⁺. Reverson et al (25) showed that *Escherichia coli* cells carrying Erwinia PL genes produced higher levels of PL in a medium containing 0.05–0.50 mM CaCl₂. Since neither of these studies included experiments to determine the location of the enzyme in the subcellular fractions, it is not known whether the increase in enzyme activity was due to a defect in the enzyme synthesis or to enzyme export. In this study, we found that over 87% of total PL produced by four *P. fluorescens* strains grown in Ca⁴⁺-deficient media remained cell-bound. It is possible that the outer membrane of *P. fluorescens* grown under Ca⁴⁺-deficient conditions may form an incompetent configuration (8) unsuitable for protein translocation. Recently, we identified and cloned a genomic DNA fragment (designated *rep*) from *P. viridiflava*, which appears to contain a cluster of genes required for PL, protease, and exopolyasaccharide (alginic) production (13). The exact function of *rep* genes and its relation to the Ca⁴⁺ effect on PL production as presented in this paper is presently obscure. We suspect that Ca⁴⁺ may serve as an environmental signal, which is directly or indirectly involved in the activation of *rep* genes required for the synthesis and/or export of extracellular enzymes and exopolysaccharide.

The presence of Ca⁴⁺ in the pectic polysaccharide matrix of plant cell walls is essential for maintaining the strength and flexibility of the walls (24). A high Ca²⁺ content in plant tissue has been shown to reduce internal breakdown of pectic polysaccharides in plant cell walls (9) and to inhibit the galacturonanase activities of plants (9) and pathogens (4,23,32). Based on these findings, it has been suggested that the disease resistance mechanism of plants may be enhanced by raising the Ca²⁺ content in plant tissue. So far, this approach has proven useful for controlling pre- and postharvest diseases caused by polygalacturonase-pro-
ducting fungal pathogens, such as *Penicillium expansum* (4) and *Botrytis cinerea* (4,32). It is not totally certain, however, whether the same approach can be employed to control diseases caused by PL-producing pathogens, such as *Erwinia* and *Pseudomonas*. McGregor and Kelman (17,18) previously reported that potato tubers with high Ca\(^{2+}\) content were more resistant to *Erwinia* rot. However, a recent study by Tzeng et al (31) showed that the Ca\(^{2+}\) content alone cannot entirely account for resistance or susceptibility of a specific potato cultivar to *Erwinia*. Pagel and Heitelfuss (22) also found a slight correlation between the Ca\(^{2+}\) content and the resistance of potato cultivars to bacterial rot. For pathogens that produce PL as the principal disease factor, the presence of readily available Ca\(^{2+}\) in the plant environment apparently favors PL production. Pagel and Heitelfuss (23) showed that the presence of 0.05–0.50 mM CaCl\(_2\) in potato tubers infected with *Erwinia* stimulates PL activities and increases the rate of tissue maceration. The results (Table 4) presented in this study show that 1) the presence of free Ca\(^{2+}\) in plant tissue is essential for the development of *Pseudomonas* rot, 2) prevention of *Pseudomonas* rot can be achieved by treating potato tuber disks with the Ca\(^{2+}\) chelator EDTA, and 3) the suppressive effect of EDTA on soft rot development can be reversed by adding 2 mM CaCl\(_2\). Previously, it was shown that the presence of Ca\(^{2+}\) at levels higher than the PL activity optimum (0.5 mM) reduces the release of neutral sugars from potato cell walls (27) and slightly inhibits PL activities in potato tubers infected with *Erwinia* (23). There is no conclusive evidence, however, that total inhibition of PL activities and cell wall degradation can be achieved by raising the Ca\(^{2+}\) content to a level that is technically and commercially feasible (4).

In this study, we demonstrated that the addition of the ion-chelating agent EDTA at a low concentration (0.13 mM) is sufficient to prevent *P. fluorescens* from growing and causing tissue maceration in potato tuber disks. The effectiveness of EDTA as a disease control agent is mainly due to its bactericidal activity and its ability to bind Ca\(^{2+}\) required for PL activity and for bacterial growth in plants. The bactericidal effect of EDTA was previously demonstrated with *P. aeruginosa* (6) and *P. fluorescens* (34). Despite the finding that EDTA may be potentially useful for control of *Pseudomonas* rot, the possibility of undesirable effects that may arise from iTA EDTA treatment need to be considered. The Ca\(^{2+}\) deficiency in plant tissue as a result of the prolonged EDTA treatment may cause release of pectic fragments from cell walls (26), stimulate plant and microbial polygalacturonase activities (9,22,23), and interfere with plant physiological functions related to senescence and disease resistance (30). To avoid all these undesirable effects, a treatment method that does not require a prolonged EDTA exposure needs to be devised.

**LITERATURE CITED**

