Plant Physiol. (1993) 102: 967–973

Iron Deficiency Induced by Chrysobactin in *Saintpaulia* Leaves Inoculated with *Erwinia chrysanthemi*

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In this communication, we examine the fate of iron during soft rot pathogenesis caused by *Erwinia chrysanthemi* on its host, *Saintpaulia ionantha*. The spread of soft rot caused by this enterobacterium was previously shown to depend on a functional genetic locus encoding a high-affinity iron assimilation system involving the catechol-type siderophore chrysobactin. Leaf intercellular fluid from healthy plants was analyzed with regard to the iron content and its availability for bacterial growth. It was compared to the fluid from diseased plants for the presence of strong iron ligands, using a new approach based on the iron-binding property of an ion-exchange resin. Further characterization allowed the identification of chrysobactin in diseased tissues, thus providing the first evidence for the external release of a microbial siderophore during pathogenesis. Competition for nutritional iron was also studied through a plant-bacterial cell system: iron incorporated into plant ferritin appeared to be considerably reduced in bacteria-treated suspension soybean cells. The same effect was visualized during treatment of soybean cells with axenic leaf intercellular fluid from *E. chrysanthemi*-inoculated saintpaulia leaves or with chrysobactin.

Prokaryotic and eukaryotic organisms utilize elaborate mechanisms to acquire iron from their natural environments, even when this essential metal is not readily available. Excretion of siderophores and the specific transport of their ferric complexes is one of the routes commonly used by bacteria and grasses (Marschner et al., 1986; Neilands, 1987). Dicotyledonous and nongrass monocotyledonous plants respond to iron deficiency by stimulating powerful reductive mechanisms for ferric chelates from root epidermal cells (Bienfait, 1985; Römheld, 1987). The importance of iron in the interactions among saprophytic microorganisms, pathogens, and plants has long been recognized (Swinburne, 1986). For instance, among the diverse members of the rhizosphere, according to the efficiency and the specificity of iron uptake systems involved, a competitive or a collaborative relationship may occur between plant roots and bacteria (Loper and Buyer, 1991). With regard to pathogenic interactions, the problem of competition for iron is less well documented in plant than in animal infections (Bullen and Griffiths, 1987). The iron-complexing proteins responsible for transport of the metal within body fluids of vertebrates may prevent proliferation of a pathogen through a bacteriostatic effect, i.e. by depriving it of nutritional iron (Weinberg, 1984). Because ferric ion is insoluble at physiological pH [at pH 7, the solubility product constant of $Fe(OH)_3$ is 10^{-17} M], a similar nutritional problem may occur during pathogenesis by a systemic phytopathogen. In other words, whichever synthesizes the most stable iron ligand, the host or the parasite, should be the winner.

Particular attention has been drawn to this question in the case of the soft rot caused by *Erwinia chrysanthemi* 3937 on saintpaulia plants (Enard et al., 1988). The bacterial cells invade the intercellular spaces of leaf parenchymatous tissues and may move to other aerial parts of the plant through xylem vessels. A functional genetic system responsible for the production of the siderophore chrysobactin (Persmark et al., 1989) and utilization of its ferric complex is assumed to be required for dissemination of the pathogen. However, it is still unknown whether this high-affinity iron transport system is expressed during bacterial colonization of plant tissue. It is interesting that *E. chrysanthemi* cannot use ferric citrate, i.e. the major iron carrier in plant vessels (Brown, 1978), as an iron source (Expert and Gill, 1991). Iron levels in intercellular fluids must be abundant enough for plant growth, but we question whether there is enough of the metal available for bacterial growth.

The purpose of this study was to analyze intercellular fluids from diseased saintpaulia plants, which are expected to be the sites of competition for nutritional iron, using a plant-bacterial cell system designed for this purpose. Biochemical changes, measured primarily as a reduction of iron incorporation into plant ferritin, were shown to occur in bacteria-treated suspension soybean cells. Such an iron deficit was also induced by intercellular fluids from inoculated saintpaulia leaves or by chrysobactin.

MATERIALS AND METHODS

Bacterial Strains and Synthetic Culture Media

The pathogenic *Erwinia chrysanthemi* strain 3937 was isolated from *Saintpaulia ionantha* (Kotoujansky et al., 1982). The chrysobactin biosynthetic mutants 3937 *cbs*-29 (Cbs^-) and *fct-18* (Fct^-Cbs^-) are impaired in the catechol pathway, the latter being also unable to transport the ferri-chrysobactin

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Abbreviations: Chrysobactin, *N*-(N^2-(2,3-dihydroxybenzoyl)-D-lysyl)-L-serine; EDDA, ethylene-diamine-*N*-*N*′-bis(2-hydroxyphenyl)acetic acid; HIF, intercellular fluid from healthy plants; IIF, intercellular fluid from inoculated plants.

complex (Franza et al., 1991). Rich medium and chemicals (EDDA) were used as described previously (Enard et al., 1988). Tris medium was used as an iron-depleted medium (Franza et al., 1991).

Plant Leaf Intercellular Fluid Preparation and Iron Content Estimation

The intercellular spaces of leaves were washed by infiltrating the entire leaflet with distilled water in vacuo, and then centrifuging them according to the method of de Witt and Spikman (1982). Two-month-old potted plants (*S. ionantha* cv Blue Rhapsody) were used. Intercellular fluid was taken up from healthy plants or from plants inoculated 24 h previously by infiltration of the bacterial suspension within the leaf (about 3×10^7 colony-forming units per leaf) as previously described (Expert and Toussaint, 1985). By 24 h, a translucent spot was visible at the site of inoculation with the wild-type pathogen. When required, intercellular fluid was filter sterilized and concentrated 5-fold by vacuum centrifugation. Intercellular fluid iron was quantitatively measured with ferrozine as described by Carter (1971).

Chemical and Biological Assays for Detecting Chrysobactin

Catechol was assayed according to the method of Arnow (1937). Siderophore activity was detected by the Chrome Azurol S assay of Schwyn and Neilands (1987), which is based on the removal of ferric ion from a pigmented complex by a competing ligand. Production of chrysobactin was bioassayed as described previously (Persmark et al., 1989).

Outer Membrane Preparation and Analysis

Triton-insoluble walls (outer membranes) of *E. chrysanthemi* were prepared and analyzed by SDS-PAGE as described previously (Expert and Toussaint, 1985).

Chelex Treatment and Titration of Strong Ligands

A 25% (w/v) stock suspension of Chelex 100 resin (H form, Bio-Rad) was prepared in distilled water. For treatment, 1 mL of the stock suspension was centrifuged in a microfuge, and, after the supernatant was eliminated, the pellet was drained, suspended in 1 mL of the sample to be analyzed, and shaken for 5 min. The samples analyzed were each 1 mL of a serial dilution of intercellular fluid containing 1 μL of 2.2 mM ^{59}Fe (0.2 mCi mL^{-1} of iron [III] chloride in 0.1 M HCl; Amersham). Samples were centrifuged at 3000 rpm for 3 min, and the strongly chelated iron remaining in the supernatant fraction was quantified by counting an aliquot in a scintillation counter. Soluble ^{59}Fe was plotted as a function of intercellular fluid dilution. The amount of strong free ligand was determined using the dilution that retained 50% of added radioiron in the supernatant fluid. Several iron ligands including citrate, EDTA, and the siderophore deferrioxamine B (Desferal; Ciba-Geigy, Inc.) were used as controls.

Spectrophotometric Analysis of Intercellular Fluids

Potential iron chelators were examined in intercellular fluids by spectrophotometric analysis of the samples after addition of 0.1 up to 100 μM $FeCl_3$. Ferric complexes were studied over a range of pH values as described previously (Persmark and Neilands, 1992). Spectra were recorded from 400 to 800 nm, using a Perkin-Elmer apparatus. Chrysobactin concentrations were estimated spectrophotometrically at 570 nm from ΣFe(bis) = 3.8 mM (pH 6.5) (Persmark et al., 1989).

In Vivo Labeling of Soybean Cell Suspensions Exposed to Various Treatments

Soybean cells (*Glycine max*) were cultured in B5 medium as described previously (Proudhon et al., 1989) and maintained in iron-free medium for 24 h before use. Equal aliquots of cell suspension were drained, and cells were suspended in 2 mL of iron-free B5 medium inoculated with bacteria or supplemented with the aqueous siderophore solution to be tested. Bacterial cultures grown overnight in Tris medium were used. Chrysobactin was synthesized previously (Persmark et al., 1989). Samples of drained cells were also suspended in 2 mL of filter-sterilized intercellular fluids from inoculated or noninoculated saintpaulia plants. Iron labeling was started by adding a ferric citrate complex solution (4 μM ^{59}Fe:40 μM citrate final ratio) to the treated plant cell suspension. After incubation with gentle shaking at 30°C, in the dark, for indicated times, plant cells were collected by centrifugation over a nylon cloth (50-μm pore size) and washed three times with the same plant culture medium containing unlabeled iron citrate. Bacteria were harvested from the washing fluids by centrifugation.

Detection of Ferritin-Bound ^{59}Fe Iron

Plant and bacterial cell pellets were treated and analyzed as reported previously (Laulhère et al., 1992), with minor modifications. Cells were suspended in a minimum volume of lysis solution (50 mM Tris/maleate buffer, pH 7, containing 0.1% Triton X-100, 1% PVP, and 0.2 mg of PMSF per mL). Plant cells were ground with a cell-breakage apparatus (Tissue Tearor; Biopec Products, Bartlesville, OK), and bacteria were broken by sonication and syringe shearing. After centrifugation in a microfuge, 20 to 30 μL of supernatant were analyzed by native PAGE, using 4 to 20% polyacrylamide gradient gels. ^{59}Fe incorporated into ferritin and low molecular mass molecules was visualized by autoradiography. Ferritin-bound radioiron was quantified from the suspension obtained by homogenizing the ferritin band from the dried gel in scintillation medium.

RESULTS

Characteristics of the Leaf HIF: Is Intercellular Fluid Sensed as an Iron-Restricted Environment by Bacterial Cells?

Because *E. chrysanthemi* 3937 cells invade the intercellular spaces of leaf parenchymas (Temsah et al., 1991), we first looked at the possibility of growing bacterial cells of the wild-

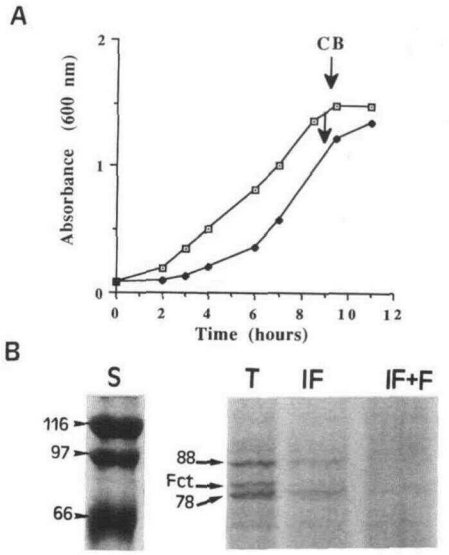

Figure 1. Iron stress response of *E. chrysanthemi* strain 3937 grown in 5-fold concentrated HIF. A, Growth of bacteria after inoculation of a 100-fold diluted stationary-phase culture in Luria broth into HIF (open squares) compared to Tris medium (filled squares). Arrows indicate when chrysobactin (CB, identified as described in the text) became apparent in the culture supernatant. B, SDS-PAGE analysis of *E. chrysanthemi* outer membrane proteins harvested at an A_{600} of 0.8 from HIF (IF) or HIF supplemented with 10 μM FeCl3 (IF + F) compared to Tris medium (T). The chrysobactin receptor (Fct) is indicated; other iron-regulated proteins and standard proteins (SDS-PAGE low molecular mass standards from Bio-Rad) (S) are referred to by their apparent molecular masses, in kD.

type strain in the HIF diluted with wash water. Diluted intercellular fluid is believed to contain the main water-soluble nutrients necessary for bacterial multiplication and could represent the environmental conditions encountered during pathogenesis. Even supplemented with Glc (0.2%) as a carbon source, wild-type cells grew poorly in this fluid, indicating that one or several nutrients were lacking. Indeed, their growth was not stimulated by supplementing the fluid with the different salts required for bacterial multiplication, including iron chloride. In contrast, when the fluid was concentrated 5-fold, bacteria grew to a level similar to that in minimal Tris medium (Fig. 1A). Such a concentration of the intercellular fluid might reflect the situation in vivo. The 5-fold concentrated HIF contained less than 2 μM iron, which corresponds to restrictive conditions for the growth of *E. chrysanthemi* 3937 cells (Enard et al., 1988). As shown in Figure 1A, catechol (10–20 μM 2,3-dehydroxybenzoic acid equivalent) and Chrome Azurol S-reacting material appeared to be released during the stationary phase. A bioassay specific for chrysobactin revealed the presence of the bacterial siderophore in the culture supernatant. In addition, PAGE analysis showed that the three iron-regulated proteins, including the chrysobactin receptor Fct (molecular mass = 80 kD) and the receptors for enterobactin (molecular mass = 88 kD) and ferrichrome (molecular mass = 78 kD), were present in the outer membrane (Fig. 1B). All of these effects, typical of the iron-stress response, were prevented by the addition of 10 μM $FeCl_3$ in the culture (Fig. 1B).

Changes Occurring in Leaf Intercellular Fluid during Pathogenesis

Diluted leaf IIF from saintpaulia plants inoculated 24 h previously was harvested and analyzed for the presence of iron-free ligands. Because many molecules such as organic acids, amino acids, or nucleotides can bind iron, we devised a method allowing selection of strong ligands. In preliminary experiments, we found that Chelex, which is an iron-binding resin, removes iron complexed to relatively weak ligands such as citrate, whereas stronger chelators, including EDTA and deferrioxamine B, resisted deferration. In addition, when supplemented with 2.2 μM ^{59}Fe, diluted intercellular fluids failed to be deferrated by Chelex treatment, which indicated the occurrence of strong iron-free ligands. IIF and HIF were compared for their content of strong iron-free ligands by volumetric titration: the amounts of ^{59}Fe remaining in the soluble fraction of serial dilutions were determined for both fluids, after treatment with the resin (Fig. 2). The analysis, which was carried out twice on independent batches of diluted intercellular fluids, revealed that both intercellular fluids contained unsaturated strong ligands, but the levels in IIF were much higher than those found in HIF, i.e. 44 versus 1.1 μM iron equivalents. We suspect that this difference might be at least partially due to the presence of chrysobactin, which is absent from fluids harvested from noninoculated plants.

Evidence for the Presence of Chrysobactin in IIF

Unlike the monocatecholate-free ligand, ferric chrysobactin has a typical spectrum in the 500-nm range, which depends on stoichiometry of the complex. In aqueous solution, the proportion of bis complex relative to tris complex varies with pH and metal-to-ligand ratio and is concentration dependent (Persmark and Neilands, 1992). Therefore, the presence of

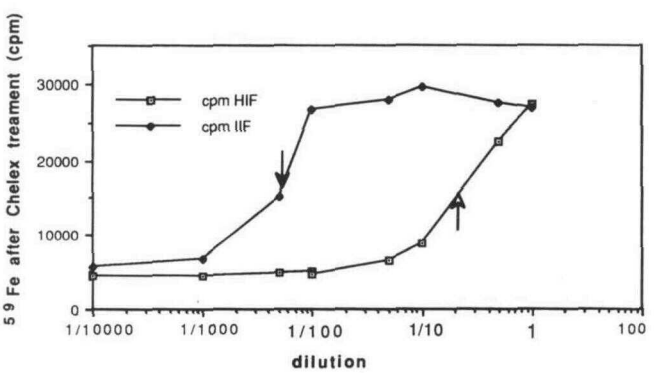

Figure 2. Titration of strong free ligands in intercellular fluids. Serial dilutions of HIF and IIF were supplemented with 2.2 mM ^{59}Fe and treated with iron-binding Chelex resin as described in "Materials and Methods." Arrows indicate dilutions allowing 50% of the initial label to be maintained in the supernatant fluid, which correspond to 1.1 mM iron equivalents of strong chelates.

the siderophore in IIF will be detected by this spectral shift when ferric iron is added.

As presented in Figure 3, the addition of Fe(III) to IIF (100 μM $FeCl_3$) led to the appearance of a typical catecholate complex not visible in HIF. Absorbance maxima proved to be dependent on pH values: at pH 8.7, IIF became red with an absorption maximum at 493 nm. These results resemble the spectrum and color expected in the presence of the tris complex of chrysobactin (Persmark and Neilands, 1992). The solution turned blue as the pH was lowered to pH 5.6, concomitant with a shift to a new maximum at 567 nm. These data strongly suggested the presence of chrysobactin in IIF at concentrations of about 100 μM. A bioassay (Table I) confirmed the spectrophotometric study: increasing amounts of IIF were tested for their ability to enhance the growth of a chrysobactin biosynthetic mutant, unlike a transport mutant, under iron-restrictive conditions. IIF amounts equal to or higher than 5 μL led to the formation of halos of growth only with the chrysobactin nonproducer.

Does Chrysobactin Affect the Iron Metabolism of Plant Cells during Pathogenesis?

The production of chrysobactin during infection may disturb the nutritional iron flow and even the iron metabolism of plant cells. To investigate this question, we designed a plant-bacterial cell system that allows accurate control of the iron flux in both partners. Ferritins are one of the first iron-binding molecules to be labeled with ^{59}Fe in soybean cells as well as in bacterial cells (Laulhère et al., 1991). In this context, we used soybean cells as the plant partner. These cells

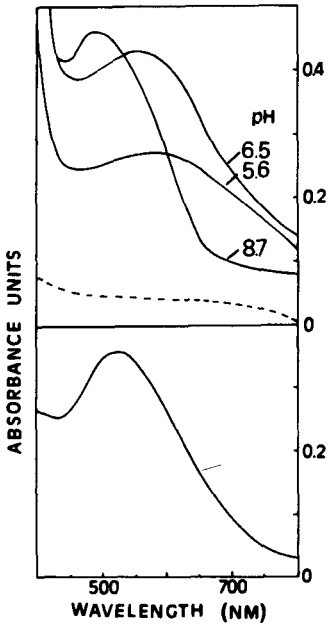

Figure 3. Absorption spectrum of ferric chelates in intercellular fluid from *E. chrysanthemi*-inoculated saintpaulia leaves (IIF) as a function of pH (solid lines) compared to spectra of HIF, (pH 6.8; dotted line) (A) and pure ferric chrysobactin (0.3 mM, pH 6.5) (B). Spectra at selected pH values are shown.

Table I. *Stimulation of growth of biosynthetic and transport mutants by iron sources*

Various amounts of HIF and IIF spotted on sterile paper discs (5 mm diameter) were tested for their ability to stimulate the growth of a chrysobactin biosynthetic mutant (Cbs^-) when cultured in the presence of EDDA. Numbers refer to the diameter (mm) of the growth zones surrounding the discs; —, no growth. Like chrysobactin, IIF failed to enhance the growth of the ferri-chrysobactin transport mutant (Fct^- Cbs^-). This assay was performed at least three times, with independent batches of plant extracts.

Iron Source	Mutant	
	Cbs^-	Fct^- Cbs^-
Chrysobactin (nmol)		
1.2	7	—
IIF (μL)		
<5	6	—
6	7	—
7	8	—
8	9	—
9	10	—
10	10	—
HIF (μL)		
10	—	—
$FeCl_3$ (nmol)		
10	15	15

appeared to be susceptible to the bacterial lytic attack: they collapsed 24 h after inoculation, as visualized by the lysed appearance of inoculated cultures.

We looked at the level of ^{59}Fe incorporation into plant ferritins in inoculated soybean cell suspensions by comparison to axenic suspensions. ^{59}Fe was supplied with ferric citrate, and the labeling was carried out for a short period (3 h) to avoid plant cell lysis. We expect that during this period the iron concentration of the culture medium will influence the loading of iron into ferritin but not the ferritin content (Lescure et al., 1990). Cell protein extracts were analyzed by PAGE under native conditions, and ferritin iron from bacterial and plant cells was visualized by autoradiography (Fig. 4A) and quantified (Fig. 4B). Our results confirm that ferritin from *E. chrysanthemi* cells is a protein migrating in a manner similar to plant ferritin. The addition of increasing numbers of bacterial cells to plant cells resulted in a progressive decrease in plant ferritin iron. At the same time, an increase in incorporation of ^{59}Fe into bacterial ferritin was apparent. These data indicate that the bacteria were responsible for the reduction in plant cell-mobilized iron. To test whether this effect was caused by a compound released by the bacteria during pathogenesis, we studied the influence of IIF compared to HIF on the level of ferritin iron (Fig. 5): HIF allowed the labeling that was found to be associated with ferritin and low molecular mass molecules, whereas chrysobactin or IIF prevented it. This strongly suggests that chrysobactin was responsible for this shift. Treatment of soybean cells with chrysobactin or HIF supplemented with chrysobactin for 1 h appeared to be as efficient as a treatment with deferrioxamine B for preventing iron incorporation into plant ferritin and low molecular mass molecules (Fig. 5). None of the treatments irreversibly affected the flux of nutritional iron into soybean

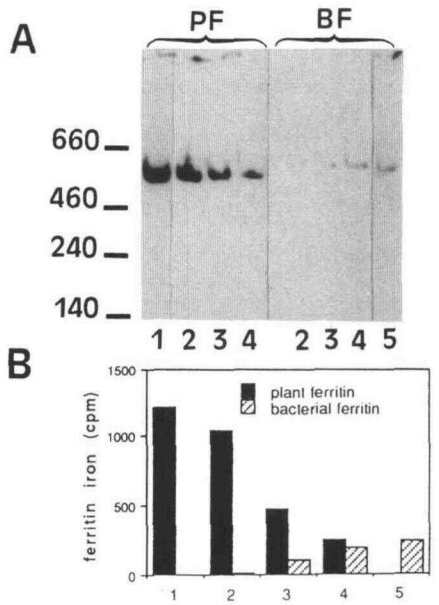

Figure 4. Native PAGE analysis of plant and bacterial protein extracts isolated from *E. chrysanthemi*-inoculated soybean cell suspension after labeling with ^{59}Fe for 3 h. ^{59}Fe bound to plant ferritin (PF) or to bacterial ferritin (BF) was visualized by autoradiography (A) and quantified (B) by scintillation counting of the excised bands from the gels. Lane 1, Extract from 2 mL of axenic soybean cell suspension. Lanes 2 to 4, Extracts of PF or BF after inoculating 2 mL of plant cell suspension with 10^7 (2), 2×10^7 (3), or 10^8 (4) colony-forming units. Lane 5, Labeling in a suspension of 10^8 colony-forming units of *E. chrysanthemi*. Migration of standard proteins is indicated by their masses in kD to the left.

cells. After exposure to IIF for 3 h or to chrysobactin, washed cells were able to incorporate ^{59}Fe from iron citrate as well as untreated cells (Fig. 5).

DISCUSSION

Although much information has been collected concerning diverse physiological aspects of iron metabolism in plants (Chaney et al., 1972; Bienfait, 1989), the question of iron availability for pathogenic bacteria, which can potentially move throughout the vascular system of their host, remained to be addressed. In this regard, strain 3937 of *E. chrysanthemi* is of particular relevance because iron emerges as a signal controlling not only bacterial iron nutrition but also additional factors involved in the systemic spread of the pathogen and the amount of damage to the host plant (Sauvage et al., 1990). The localized response induced by the mutants deficient in the chrysobactin biosynthetic or transport pathways led us to consider this siderophore to be critical for dissemination (Enard et al., 1988). Furthermore, the ability to restore normal pathogenesis by inoculating a pair of mutants consisting of a chrysobactin transport mutant that was able to cross-feed a chrysobactin biosynthetic mutant under condition of iron deprivation (Enard, 1990) suggested that chrysobactin was produced in vivo.

Because strain 3937 grows intercellularly, a simple approach was to investigate the iron status and the presence of potential iron chelators in intercellular spaces of saintpaulia leaves before and after inoculation. Analysis of intercellular fluids as reported previously proved to be a valuable tool to assess in vivo microbial growth conditions (De Witt and Spikman, 1982) and substrate availability (Klement, 1965). The low iron content found in HIF (this study) and the inability of *E. chrysanthemi* to use ferric citrate as an iron source (Expert and Gill, 1991) strengthened the hypothesis that chrysobactin must be produced in vivo. In intercellular fluid from noninoculated plants, bacteria behave as in any iron-depleted synthetic medium: they induce their high-affinity iron transport systems. However, the analysis of IIF turned out to be more complex. Indeed, the high levels of catechol detected after inoculation (data not shown) might not be due solely to the presence of chrysobactin. They were much higher than those produced by the bacterium when grown in a synthetic medium (i.e. 50 μM DHBA equivalent for 5×10^8 cells). Plants are known to produce a variety of phenolics in response to various stresses (Davies et al., 1984; Metraux et al., 1990; Scalbert, 1991), and a portion of the catechols identified might be of host origin. Thus, we devised a simple approach based on the iron-binding property of Chelex, currently used for deferration, that allows differentiation of strong ligands from weak ones. We showed that a strong ligand occurs in IIF. Furthermore, only IIF displayed the typical spectrum as well as the biological activity of the chrysobactin complex. In addition, taking into account the relative concentrations of iron and chrysobactin in intercellular fluids, one can conclude that chrysobactin, when detected in inoculated plants, was essentially free of iron (about 98%). Chrysobactin must allow the pathogen to compete

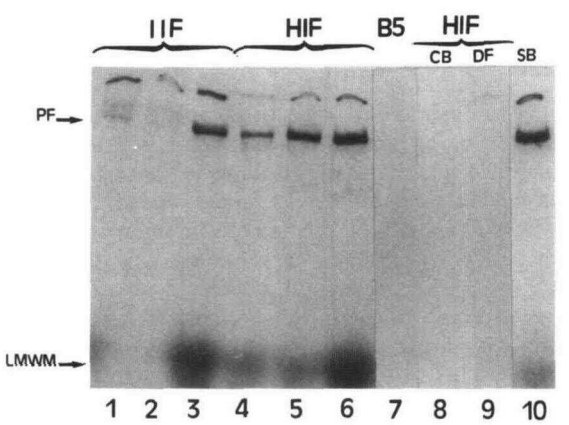

Figure 5. Autoradiogram of a native PAGE gel of protein extracts from soybean cells treated or not treated with saintpaulia leaf extracts, chrysobactin, or deferrioxamine. ^{59}Fe-citrate labeling was performed in leaf fluids from inoculated saintpaulia plants (IIF, lanes 1, 2, and 3), in fluids from noninoculated control plants (HIF, lanes 4, 5, and 6), in B5 medium (lane 7), or in HIF supplemented with 500 μM chrysobactin (CB, lane 8) or 500 μM deferrioxamine B (DF, lane 9). Labeling was carried out for 1 h (lanes 1 and 4) or 3 h (lanes 2, 5, 7, 8, and 9). Lanes 3, 6, and 10 are controls showing that, after treatment for 3 h with IIF, HIF, or chrysobactin, respectively, washed soybean cells (SB) were still able to incorporate ^{59}Fe from iron citrate into plant ferritin. PF, Plant ferritin; LMWM, low molecules mass molecules.

efficiently for Fe(III), which is readily released from ligands with stability constants toward the metal related to that of citrate (log FeL = 11.8). A catechol-type siderophore such as chrysobactin, which can remove iron from EDDA (log FeL = 34), must be a stronger ligand than the various potential plant iron chelators. By comparing the relative amounts of iron and free ligands present in intercellular fluids, we found that IIF can potentially bind 50 times the iron content of HIF. The high chelating capacity of chrysobactin detected in vivo may create an important iron deficit for the host.

Does the release of such strong chelating molecules by bacteria during pathogenesis affect the plant's iron metabolism? Is there a competition for iron between the host and colonizing bacteria? To explore these questions, we looked at the distribution of iron supplied to cultured plant cells inoculated with bacteria. Cells in suspension have often been used as model systems for investigation of plant responses to stress (Marton et al., 1979; Tong et al., 1986; Feistner, 1988). We also noticed that soybean cells were susceptible to the bacterial lytic attack, which is likely correlated with plant cell electrolyte loss (Brisset and Paulin, 1992) and may, therefore, be considered a representative system with which to pursue this analysis. We should also note that host specificity in pectinolytic erwinias is loose and that the problem of pathogenic iron acquisition may not be of special importance in the early stage of host recognition. The choice of the iron metabolites to be studied as a relevant label of iron availability for each partner has proven to be difficult. Total iron was not considered because plant cells cannot be easily separated from bacterial cells, especially because of the lytic process. Apoplastic iron (retained in cell walls) was poorly defined, but it represented 40% of the total iron in our experimental conditions. Among iron-related proteins, ferritins retained our attention because they represent one of the first molecules to bind iron in soybean cells (Laulhère et al., 1991). In this regard, we found that the bacterial cells and the IIF that contained iron-free chrysobactin completely prevented the binding of iron to ferritins as well as significantly decreased the low molecular mass plant cell metabolites also associated with iron. The same effect was produced with chrysobactin and deferrioxamine B, a siderophore commonly used in human iron overload therapy. In summary, this indicates that chrysobactin, when present in inoculated plants, is required for bacterial iron nutrition but is deleterious to plant cells because it denies them essential iron. Chrysobactin may be considered a toxic compound that can potentially reduce the amount of iron present in tissues colonized or inoculated with *E. chrysanthemi*.

ACKNOWLEDGMENTS

We thank Magnus Persmark and J.B. Neilands for providing chrysobactin and helpful discussions.

Received January 29, 1993; accepted April 6, 1993.
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