Identification of lumichrome as a *Sinorhizobium* enhancer of alfalfa root respiration and shoot growth

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Edited by Sharon R. Long, Stanford University, Stanford, CA, and approved September 10, 1999 (received for review July 21, 1999)

Sinorhizobium meliloti **bacteria produce a signal molecule that enhances root respiration in alfalfa (*Medicago sativa* L.) and also triggers a compensatory increase in whole-plant net carbon assimilation. Nuclear magnetic resonance, mass spectrometry, and ultraviolet–visible absorption identify the enhancer as lumichrome, a common breakdown product of riboflavin. Treating alfalfa roots with 3 nM lumichrome increased root respiration 21% ($P < 0.05$) within 48 h. A closely linked increase in net carbon assimilation by the shoot compensated for the enhanced root respiration. For example, applying 5 nM lumichrome to young alfalfa roots increased plant growth by 8% ($P < 0.05$) after 12 days. Soaking alfalfa seeds in 5 nM lumichrome before germination increased growth by 18% ($P < 0.01$) over the same period. In both cases, significant growth enhancement ($P < 0.05$) was evident only in the shoot. *S. meliloti* requires exogenous CO_2 for growth and may benefit directly from the enhanced root respiration that is triggered by lumichrome. Thus *Sinorhizobium*–alfalfa associations, which ultimately form symbiotic N_2-reducing root nodules, may be favored at an early developmental stage by lumichrome, a previously unrecognized mutualistic signal. The rapid degradation of riboflavin to lumichrome under many physiological conditions and the prevalence of riboflavin release by rhizosphere bacteria suggest that events demonstrated here in the *S. meliloti*–alfalfa association may be widely important across many plant–microbe interactions.**

Bacteria in the *Rhizobiaceae* (i.e., rhizobia) affect fundamental processes in plants through powerful signal molecules. These compounds, known as nodulation factors, are complex lipochitooligosaccharides (LCOs) that are active in picomolar concentrations (1). The molecules help rhizobia usurp control of normal plant cell functions as they infect the root and eventually form a nodule, where the rhizobia reduce N_2 (2) and release N-containing compounds, such as alanine (3), directly to the plant. The easy availability of LCOs from bacterial cultures now offers reagents that can help define how plant cells function (4). Identification of other rhizobial signals that affect fundamental plant cell processes, likewise, will increase our understanding of both plant–microbe interactions and plant cell functions.

One long-neglected characteristic of rhizobial growth is its dependence on an exogenous source of carbon dioxide (5). Soil normally is viewed as a CO_2-rich environment (6), but microsites occupied by rhizobial colonies on the root surface may deplete CO_2 and create a condition where additional CO_2 would facilitate bacterial growth. Such a situation could favor the rhizobial production of molecular signals that promote root respiration. It is well known that pathogens, such a *Phytophthora* (7) and *Fusarium* (8), release factors that increase plant cell respiration. The active elicitor fractions from these organisms often contain cell wall fragments, but specific structures of such molecules have not been defined.

Previous experiments showed that *Sinorhizobium meliloti* increases CO_2 availability by enhancing alfalfa (*Medicago sativa* L.) root respiration with an extracellular compound (9). That structurally undefined molecule was termed compound D for its chromatographic mobility. Key characteristics of compound D established in ref. 9 included the following: (*i*) The enhancer is produced by *S. meliloti* in the absence of the plant. (*ii*) Increases in root respiration occur primarily in the root hair zone. (*iii*) Nodulation factors are not the active material because *S. meliloti* cells mutated in *nodC*, a gene required for synthesis of these lipochitooligosaccharides, still enhanced alfalfa root respiration. (*iv*) Compound D increases root respiration slowly through several hours rather than rapidly in minutes like respiratory elicitors from pathogens. The current investigation was initiated to define the structure of compound D.

Materials and Methods

Bacterial and Plant Growth. *S. meliloti* 1021 was grown in 2.8-liter flasks shaking at 150 rpm. The initial bacterial medium contained the major components (g/liter) K_2HPO_4 (1.0), KH_2PO_4 (1.0), KNO_3 (0.6), $MgSO_4$ (0.13), $FeCl_3 \cdot 6H_2O$ (0.01), $CaCl_2 \cdot 2H_2O$ (0.07), and mannitol (10.0) and the minor components (mg/liter) thiamin (1.0), biotin (1.0), $Na_2MoO_4 \cdot 2H_2O$ (0.24), H_3BO_4 (3.0), $MnSO_4 \cdot H_2O$ (1.83), $ZnSO_4 \cdot 7H_2O$ (0.29), $CuSO_4 \cdot 5H_2O$ (0.13), and $CoCl_2 \cdot 6H_2O$ (0.12). The final, improved medium for maximum production of compound D contained the major components (g/liter) K_2HPO_4 (1.0), KH_2PO_4 (1.0), KNO_3 (6.0), proline (5.0) $MgSO_4$ (0.26), $FeCl_3 \cdot 6H_2O$ (0.02), $CaCl_2 \cdot 2H_2O$ (0.07), and dextrose (10.0) and the minor components (mg/liter) thiamin (2.0), biotin (2.0), $Na_2MoO_4 \cdot 2H_2O$ (0.24), H_3BO_4 (3.0), $MnSO_4 \cdot H_2O$ (1.83), $ZnSO_4 \cdot 7H_2O$ (0.29), $CuSO_4 \cdot 5H_2O$ (0.13), and $CoCl_2 \cdot 6H_2O$ (0.24).

'Moapa 69' alfalfa seeds for all experiments were soaked 30 min in 70% ethanol, treated 5 min in concentrated H_2SO_4, rinsed with sterile water, soaked 30 min in commercial bleach, and then washed with sterile water. For respiration tests approximately 400 seeds (1 g) were germinated on a 10 × 10 cm screen above a hydroponic nutrient solution (9). In some hydroponic experiments the nutrient solution was supplemented to contain ampicillin (125 mg/liter) and rifampicin (10 mg/liter). Plant growth experiments were done in sterile pots (10 × 10 × 10 cm) containing vermiculite. The pots were watered with the hydroponic nutrient solution (9) supplemented to contain 1 mM NH_4NO_3 and various lumichrome treatments every second day. In all experiments, plants were grown under controlled conditions with a 12/12-h day/night photoperiod and a 25/20°C temperature regime with a photon flux density (400–700 nm) of 250 μmol/m^2·s.

Compound Isolation and Identification. Supernatant from bacterial cultures was collected by centrifugation and stirred 4 h with XAD-4 resin (Alltech; 10 g/liter). Compounds adsorbed to the resin were eluted in methanol and dried under reduced pressure with or without freezing. The dry pellet from 15 liters of bacterial

This paper was submitted directly (Track II) to the PNAS office.

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medium was solubilized in water and injected into a preparative HPLC column (Waters μBondapak C_{18}, 300 × 25 mm), which had been equilibrated in water. The column was eluted at 8 ml/min with water, 0–12 min; methanol/water (3:7, vol/vol), 12–15 min; methanol/water (35:65), 15–65 min; and methanol/water (1:1), 65–112 min. Under these conditions compound Y eluted at 55–62 min, and compound D eluted at 106–112 min. The samples were dried by lyophilization. Then compounds D and Y were individually purified through four additional HPLC separations: (*i*) A semipreparative column (Alltech Hypersil C_{18}, 199 × 10 mm) was equilibrated and eluted isocratically (2 ml/min) with methanol/water (2:8 or 35:65) for compounds Y and D, respectively. (*ii*) The same column was equilibrated and eluted isocratically (2 ml/min) with acetonitrile/water (1:9 or 2:8) for compounds Y and D, respectively. (*iii*) An analytical column (Alltech C_8/cation mixed-mode, 150 × 4.6 mm) was equilibrated in phosphate buffer (0.2 M K_2HPO_4, pH 4.5) and eluted at 0.5 ml/min with 100% phosphate buffer for 0–5 min; a gradient going from 0% to 35% acetonitrile with the remainder comprised of phosphate buffer for 5–40 min, and acetonitrile/phosphate buffer (35:65) for 40–50 min. Under these conditions compounds Y and D eluted at 32–35 and 42–46 min, respectively. (*iv*) Finally, samples were desalted on an analytical column (Alltech Lichrosorb C_{18}, 250 × 4.6 mm) by rinsing on the column for 30 min with water and then eluting compounds Y and D with a 70-min gradient going from methanol/water (3:7) to 100% methanol.

Thin-layer chromatography (TLC) on silica-gel-coated glass (Alltech 0.2 × 100 × 100 mm HPTLC Silica Gel 60 plates) separated compounds in the lipophilic fraction by using chloroform/methanol/water (17.5:12.5:1.5). TLC plates were viewed on a UV-light box (Sigma model T1202) and photographed with Polaroid 667 3000 ISO film.

UV–visible absorption spectra were recorded in methanol in a Perkin–Elmer Lambda 6 dual-beam spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) analyses were conducted at the University of California Davis NMR facility with a 600-MHz Bruker Avance DRX-600 operating on XWINNMR software 2.1. Tetramethylsilane was added as an internal standard to the deuterated methanol solvent. MS data were obtained at the University of California Davis Advanced Instrumentation Facility on a Finnigan LC-ion-trap MS, model LC-Q, using negative mode electrospray ionization running at 1 mtorr (0.133 Pa) of He after injecting samples in methanol/water (1:1). Tandem MS experiments were conducted by increasing the translational energy of selected ions to promote collisions with He and then detecting the major daughter ions.

Biological Tests with Lumichrome. Stock solutions of 100 μM lumichrome (Aldrich) were prepared in methanol/1 M HCl (49:1) by stirring for 2 h and storing at −20°C for 24–48 h before using once and discarding. In respiration tests, lumichrome was added to the root systems of intact plants 3 or 4 days after germination. Respiration was assessed 18–40 h later by using a gas chromatograph to measure the CO_2 evolved by replicate samples of root segments from which meristematic tips had been removed (9). A typical replicate from 5-day-old seedlings contained 1 g fresh weight of root segments from approximately 200 plants.

Lumichrome effects on plant growth were tested by treating roots or seeds. Surface-sterilized seeds were soaked 2 h in either 0 or 5 nM lumichrome. Then 50 seeds were planted in vermiculite in each pot. Plants germinating from untreated seeds received nutrient solution containing 0, 5, or 50 nM lumichrome. Plants developing from seeds soaked in 5 nM lumichrome were watered with lumichrome-free nutrient solution. Pots were covered initially with a clear, plastic wrap, which was removed after 6 days. Plants were harvested 12 days after germination, and roots and shoots were separated, dried, and weighed.

Results

Optimizing Production of Compound D. Purification and identification of compound D was facilitated by optimizing its production. First, in the initial bacterial medium (9), it was established that maximal amounts of compound D were present in the supernatant of stationary-phase cultures and that extracting the bacterial cells did not increase yield of compound D markedly (data not shown). Then the carbon source was modified to increase yields of the enhancer (Fig. 1*A*). Replacing mannitol with maltose, lactose, or dextrose at 10 g/liter increased compound D production by factors of 3, 16 ($P \leq 0.01$), and 19 ($P \leq 0.001$), respectively. All subsequent work used dextrose as the carbon substrate.

When bacteria were grown on dextrose, a bright yellow

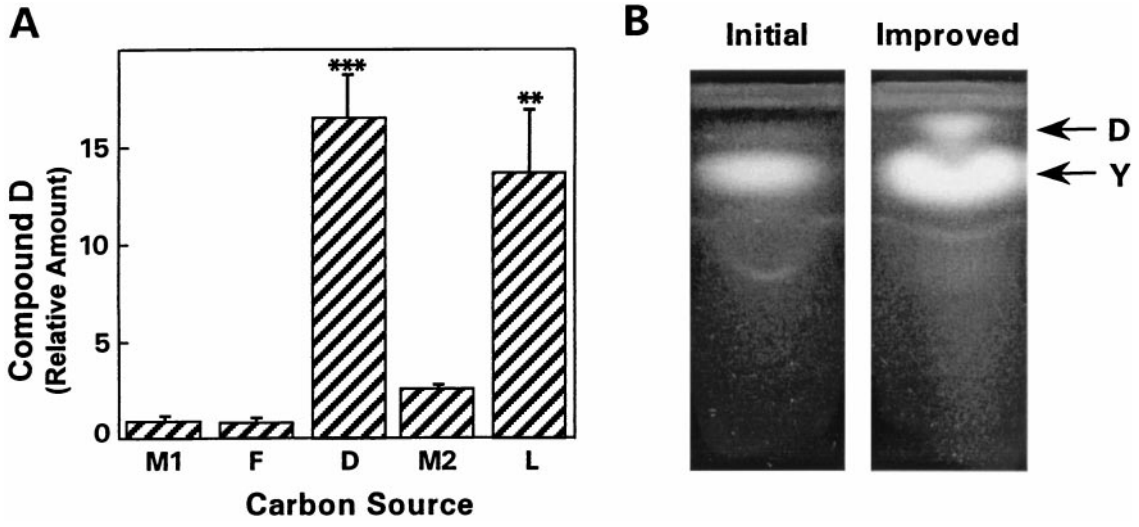

Fig. 1. Production of compound D by *S. meliloti* bacteria. (*A*) Cells were grown in medium containing mannitol (M1), fructose (F), dextrose (D), maltose (M2), or lactose (L), and the amount of compound D released was quantified by HPLC. ** and *** indicate treatment effect significant at $P \leq 0.01$ or 0.001, respectively, relative to the mannitol medium. (*B*) Compound D production in the dextrose medium was improved by modifying the medium as specified in the text. UV-illuminated TLC plates showed compounds D and Y clearly.

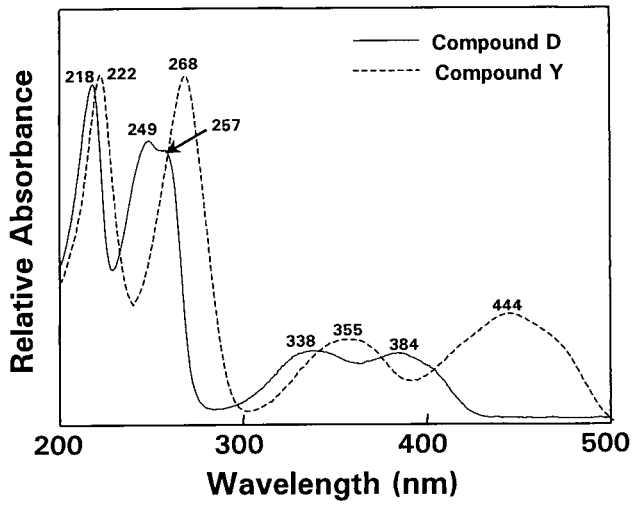

Fig. 2. UV-visible absorption traits of compounds D and Y in methanol. These spectra were later shown to match those of lumichrome and riboflavin, respectively.

Table 1. Proton NMR analyses of compounds D and Y

Assignment	Compound D	Compound Y
1	8.08 (<1H, s)	None
3	8.58 (1H, s)	8.55 (<1H, s)
6	7.75 (1H, s)	7.97 (1H, s)
7-Me	2.54 (3H, s)	2.59 (3H, s)
8-Me	2.52 (3H, s)	2.49 (3H, s)
9	7.94 (1H, s)	7.99 (1H, s)
1′	None	5.12 (1H, m)
1′	None	Not detected
2′	None	4.50 (1H, m)
3′	None	3.95 (1H, m)
4′	None	3.88 (1H, m)
5′	None	3.72 (1H, dd, J = 11.6, 5)
5′	None	3.84 (1H, dd, J = 11.4, 3.5)

Comparable signals were detected in lumichrome and riboflavin, respectively. Data are given as δ_H (ppm) relative to tetramethylsilane for samples in deuterated methanol. Peak morphologies are represented as dd, double doublet; m, multiplet; or s, singlet. Coupling constants (J) are given in Hz. Numbers of protons in each signal are represented as 1H or 3H. Positional assignments are shown in Fig. 6.

compound appeared in the culture supernatant of stationary-phase cells, and it copurified with compound D and other lipophilic components on XAD-4 resin. This material, which was termed compound Y for its yellow color, separated easily from compound D on C_{18} resin when methanolic gradients were used in HPLC analyses. When production of compounds D and Y was quantified by HPLC in cells grown at different temperatures, maximum yield of D was evident at 34°C (data not shown). Thus in all subsequent work cells were grown at 34°C.

TLC analyses of 2- to 5-ml cultures of medium containing dextrose (10 g/liter) further optimized production of compounds D and Y (Fig. 1*B*). Maximum production of compound D was observed in the dextrose medium with the following additions and modifications: proline (5.0 g/liter), KNO_3 (6.0 g/liter), thiamin (2.0 mg/liter), biotin (2.0 mg/liter), $MgSO_4$ (0.26 g/liter), $FeCl_3{\cdot}6H_2O$ (0.02 g/liter), and $CoCl_2{\cdot}H_2O$ (0.24 mg/liter).

Purification of Compounds D and Y. Purification of compounds D and Y was achieved by following the characteristic UV–visible absorption spectra of the molecules (Fig. 2) through nine HPLC steps. The initial preparative HPLC column separated compounds D and Y, and four additional purification steps were used for each compound before material was judged suitable for NMR and MS experiments. The blue and yellow fluorescence of compounds D and Y, respectively, facilitated the detection of trace amounts. Solubility problems with compound D in all solvents limited yields, and compound Y was only marginally better. Eventually milligram quantities of compound Y and microgram amounts of compound D were available for analysis.

Identification of Compounds D and Y. The ^{1}H NMR spectra of compounds D and Y indicated the molecules were structurally related (Table 1) and suggested both were relatively small. The ^{1}H NMR spectra showed several minor peaks which did not change proportionally to the major proton signals in samples purified at different times. The presence of these trace impurities required that exceptional care be taken in the MS analyses. Exploratory MS experiments tested positive and negative fast-atom-bombardment ionization, positive and negative electrospray ionization, matrix-assisted laser desorption ionization, and atmospheric pressure chemical ionization. From these MS evaluations it was determined that negative electrospray ionization measurements gave the most reproducible signals for different samples of compound Y (data not shown).

The negative electrospray ionization MS analyses of compound Y repeatedly gave an unexpectedly high molecular weight, apparent [M − H] ion at m/z 751 (Fig. 3). The validity of that conclusion was supported by tandem MS experiments demonstrating that the m/z 751 ion fragmented to produce an m/z 375 signal, which broke down to yield an m/z 255 fragment (data not shown). The difficulty of reconciling the simple NMR spectrum with molecular weight = 752 led us to consider a number of unsatisfactory structures. Finally, it was found that simple dilution of the sample essentially eliminated the m/z 751 ion and left m/z 375 as the dominant signal. Additional tandem MS experiments showed once again that the m/z 375 ion produced the m/z 255 signal, which yielded m/z 212 (Fig. 3).

Using the data available, we searched the Beilstein database with Beilstein CrossFire Minerva version 3.1 through http://www.library.wisc.edu:4001, which indicated riboflavin was a possible candidate for compound Y. Tests with authentic riboflavin gave data that were consistent with the observed values (Table 1; Figs. 2 and 3) and with the literature for ^{1}H NMR (10), MS (11), and UV–visible absorbance (12) data. Published MS analyses (13) supported the structures of the indicated ion fragments from riboflavin (Fig. 3).

Identification of compound D as lumichrome was facilitated by experience gained with compound Y. Initial MS analyses were confounded by an ion at m/z 769, which fragmented in tandem MS experiments to m/z 241, and ^{1}H NMR spectra seldom gave clear signals simultaneously for both the 1-N and 3-N protons. Nevertheless, direct comparisons with authentic lumichrome gave data consistent with the observed values (Table 1; Figs. 2 and 4) and with the literature for ^{1}H NMR (14), MS (13), and UV–visible absorbance (15) data.

Biological Activity of Lumichrome. Biological tests confirmed that lumichrome enhanced alfalfa root respiration (Fig. 5). Across several experiments, 3–50 nM lumichrome treatments of alfalfa roots produced significant increases in respiration ranging from 11% to 30% ($P \leq 0.05$). Values reported in Fig. 5 show 21% and 27% increases in root respiration associated with 3 nM lumichrome and *S. meliloti* treatments, respectively. Tests with riboflavin showed that it too enhanced root respiration, but generally higher concentrations were required to elicit a response comparable to lumichrome. For example, in one test 200

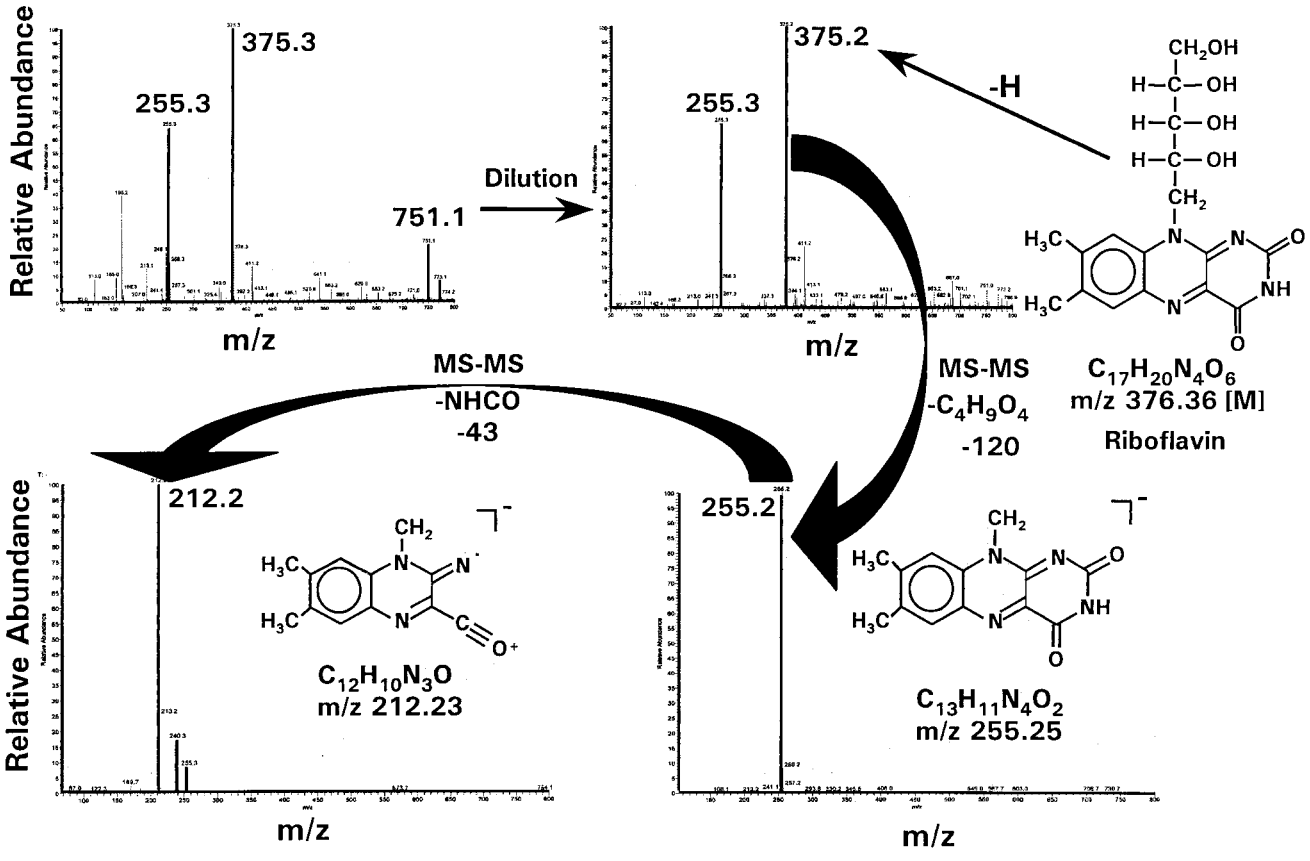

Fig. 3. Mass spectrometric analysis of compound Y. For both compound Y and riboflavin, negative electrospray ionization gave a pseudomolecular ion [M − H] at *m/z* 751, which was nearly eliminated by dilution. Sequential tandem MS tests fragmented the true [M − H] ion, *m/z* 375, to *m/z* 255 and to *m/z* 212, respectively, in both compound Y and riboflavin.

nM riboflavin produced approximately the same increase in root respiration as 30 nM lumichrome (data not shown). Such results presumably were produced by conversion of riboflavin to lumichrome (Fig. 6).

Longer term tests showed that, when roots were exposed to lumichrome or seeds were immersed in a lumichrome solution before germination, the treated seedlings contained significantly more dry matter than the controls (Fig. 7). Increases ranged from 8% to 18% ($P \leq 0.05$) on a whole-plant basis, and results from several experiments showed significant increases only in shoot dry weight. For example, the root drench (5 nM or 50 nM lumichrome) and the seed soaking (5 nM lumichrome) treat-

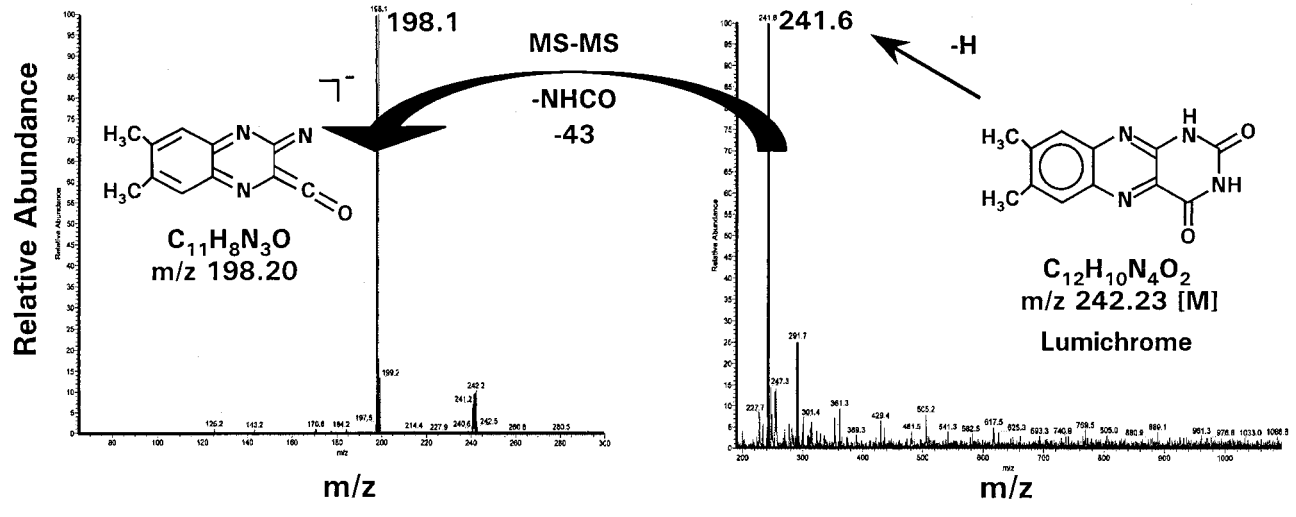

Fig. 4. Mass spectrometric analysis of compound D. For both compound D and lumichrome, negative electrospray ionization produced the [M − H] ion *m/z* 241. Tandem MS experiments fragmented the *m/z* 241 ion to produce the *m/z* 198 daughter ion.

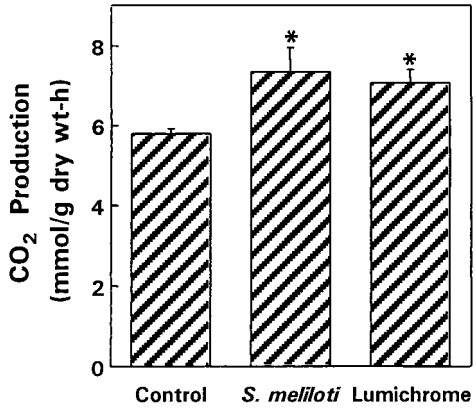

Fig. 5. Effect of lumichrome on root respiration. Respiration of alfalfa root segments was measured after exposing intact root systems for 39 h to *S. meliloti* cells (10^7 colony-forming units/ml) or 3 nM lumichrome. Mean values (+SE) are reported from four replicates, each of which contained several hundred root segments. *, Treatment effect significant at $P \leq 0.05$.

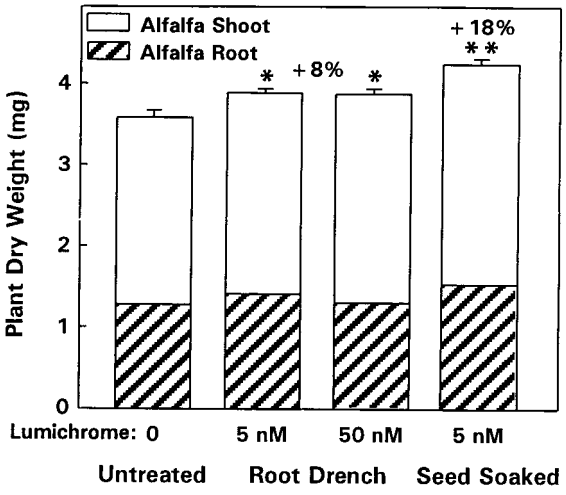

Fig. 7. Lumichrome enhancement of alfalfa seedling growth. Mean values (+SE) are reported from three replicates, each of which contained 30 plants. Percentages indicate changes relative to the untreated control. * and ** indicate treatment effects significant at $P \leq 0.05$ or 0.01, respectively.

ments increased shoot dry weight of the plants represented in Fig. 7 by 7% ($P \leq 0.05$), 12% ($P \leq 0.01$), and 18% ($P \leq 0.01$), respectively, relative to the control shoots. Root masses also were numerically, but not significantly, larger in lumichrome-treated plants.

Discussion

Both chemical and biological data reported here (Table 1; Figs. 2, 4, and 5) support the identification of lumichrome as the previously described (9) *S. meliloti* factor that increases alfalfa root respiration. Several traits of compound D delayed that conclusion: Purification was made difficult by its "sparingly" soluble nature in all solvents (18); aggregates that appeared as a pseudomolecular ion confounded the initial MS conclusion; and tautomeric forms of the pyrimidine ring, which are affected by pH (19), complicated ^{1}H NMR interpretations. Coproduction of compound Y by *S. meliloti* simplified the task somewhat, but even the identification of compound Y as riboflavin presented problems of solubility, tautomerism, and an artifactually high-molecular-weight MS ion. A previous report using negative ion MS to study a riboflavin derivative also noted the presence of peaks much larger than the molecular ion (20).

The close relationship between lumichrome and riboflavin (Fig. 6) complicates the issue of which compound actually functions in the rhizosphere, but all evidence points to lumichrome as being the active signal molecule. First, concentrations

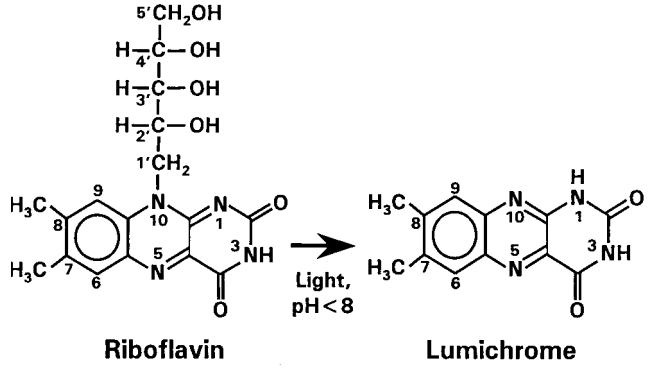

Fig. 6. Riboflavin conversion to lumichrome. Lumichrome is produced from riboflavin by photodegradation in neutral or acidic solutions (12) and by enzymes in bacteria (16) and plants (17).

of lumichrome required for activity were lower than those of riboflavin. Second, lumichrome was detected as compound D in the alfalfa rhizosphere when *S. meliloti* was added to the roots (9). Third, lumichrome is produced easily from riboflavin by both chemical and biological factors. Riboflavin is converted to lumichrome in light by a photochemical-induced cleavage of the ribityl group under neutral and acidic conditions (12). *Pseudomonas* bacteria enzymatically degrade riboflavin to lumichrome (16), and thus light, which may be absent in natural rhizosphere environments, is not required for production of lumichrome from riboflavin. Diverse species of pseudomonads are commonly found in the rhizosphere, and presumably other bacteria recover ribitol (adonitol) as a carbon substrate from riboflavin. This combination of facts strongly suggests that lumichrome is freely available to plants, if riboflavin is present in the rhizosphere.

In fact, riboflavin is well established as a rhizosphere molecule, but there has been no recognition that its easy degradation to lumichrome is ecologically relevant. Riboflavin occurs in soil (21), presumably because many soil microorganisms, including *Azospirillum* (22, 23), *Azotobacter* (24), *Rhizobium* (25, 26), and half of 63 bacterial isolates from pine roots (27), release this molecule. Some legumes, including alfalfa, also exude trace amounts of riboflavin from roots under certain conditions (28). Traditional views of how bacteria benefit from the presence of riboflavin are based on the early observation that riboflavin promoted bacterial growth (25). The fact that riboflavin auxotrophs in rhizobia form weakly effective root nodules on legumes (29) also focused past attention on riboflavin rather than lumichrome.

The primary benefit to rhizosphere bacteria of enhancing root respiration with lumichrome probably is the increased availability of CO_2. This conclusion certainly is supported by the proven growth requirement in rhizobia for exogenous CO_2 (5). Mycorrhizal fungi also grow more rapidly under elevated CO_2 (30) and thus could benefit from neighboring rhizobia or, possibly, from their own production of lumichrome. It is also conceivable that an increased flow of carbon substrates needed to support the additional respiration in the root cells results in enhanced exudation of plant compounds beneficial to rhizosphere bacteria. Previous results showing that nanomolar amounts of riboflavin stimulated alfalfa root colonization by *S. meliloti* (31) may have reflected both direct stimulation of bacterial growth by

riboflavin (25) and indirect stimulation through an increase in rhizosphere CO_2 availability (Fig. 5).

Several previous studies claimed that riboflavin stimulated plant growth (32, 33), but data reported here (Fig. 7) are, to our knowledge, the first indication that lumichrome is an active factor. Given the limited persistence of riboflavin in soil ($t_{1/2} \leq$ 72 h) (34) and its photoconversion to lumichrome in plants (35), lumichrome, rather than riboflavin, may actually be the primary stimulatory factor. The mechanism by which lumichrome increases plant growth (Fig. 7) remains to be clarified. Soil microorganisms increase net photosynthesis in diverse plant species (36, 37), and lumichrome could be one factor responsible for that effect. At this point, however, it is not known whether lumichrome operates primarily through the root or the shoot. One might postulate an initial effect on root respiration because that is where the compound was applied in most of our experiments. One report, however, claimed that riboflavin moves from soil to the shoot via the xylem stream (21). If lumichrome does the same, then it may have direct pleiotropic effects in both the root and the shoot.

Finally, it can be suggested that lumichrome represents a previously unrecognized mutualistic signal molecule in the *Sinorhizobium*–alfalfa association. Textbook examples of *Rhizobiaceae*–legume interactions (38) describe how the legume profits from N compounds supplied by the bacteria in the mature root nodule. Some workers have proposed that rhizobia benefit from an increase in their numbers during root colonization (39). Few workers, however, have speculated on the precise benefits to the plant that favored evolution of the intermediate stages of root nodule formation when no N_2 fixation is observed in modern *Rhizobiaceae*–legume interactions. The data reported here suggest that lumichrome produced from rhizobial riboflavin may benefit both organisms long before any N_2 reduction occurs. Bacteria can use the extra CO_2 from root respiration (Fig. 5) to grow (5), and plants respond to lumichrome with increases in net carbon assimilation (Fig. 7). In this sense, therefore, lumichrome apparently functions as a mutualistic signal benefiting both the bacteria and the plant. Because signals by definition transmit data, one can view lumichrome as containing the information that bacteria are present in the rhizosphere. The mechanism(s) by which lumichrome functions and its quantitative ecological significance remain to be established. However, the fact that riboflavin is released by 50% of the bacteria isolated from roots of pine (27) and rice (E.M.-R., unpublished data) supports the possibility that lumichrome is a widespread, and possibly quite primitive, mutualistic signal molecule in the rhizosphere.

We thank Dr. Roger Mercer for MS analyses. This work was supported by National Science Foundation Grant IBN-97-22988.

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