Urease-Null and Hydrogenase-Null Phenotypes of a Phyloplane Bacterium Reveal Altered Nickel Metabolism in Two Soybean Mutants

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ABSTRACT

Mutation at either of two genetic loci (Eu2 or Eu3) in soybean (Glycine max [L.] Merr.) results in a pleiotropic elimination of the activity of both major urease isozymes. Surprisingly, the phenotype of a phyloplane bacterium, Methylobacterium mesophilicum, living on the leaves of eu2/eu2 or eu3-e1/eu3-e1 mutants is also affected by these plant mutations. The bacteria isolated from leaves of these soybean mutants have transient urease- and hydrogenase-deficient phenotypes that can be corrected by the addition of nickel to free-living cultures. The same bacterium growing on wild-type soybeans or on urease mutants eu1-sun/eu1-sun or eu4/eu4, each deficient in only one urease isozyme, are urease-positive. These results suggest that the bacterium living on the eu2/eu2 or eu3-e1/eu3-e1 mutant is unable to produce an active urease or hydrogenase because it is effectively starved for nickel. We infer that mutations at Eu2 or Eu3 result in defects in nickel metabolism but not in Ni2+ uptake or transport, because eu2/eu2 and eu3-e1/eu3-e1 mutants exhibit normal uptake of 65NiCl2. Moreover, wild-type plants grafted on mutant rootstocks produce seeds with fully active urease, indicating unimpeded transport of nickel through mutant roots and stems.

Mutations in the expression of the urease isozymes in soybean fall into three general classes (12). Class I mutations result in the loss or modification of the abundant embryospecific urease. All class I mutations isolated thus far identify a single genetic locus, Eu1, of which eu1-sun is the best-studied allele. Because Eu1 alleles affect either the level of urease transcripts or the nature of urease protein, it was concluded that Eu1 contains the gene encoding the embryospecific urease (15).

The sole class III mutation, eu4 (20), affects the so-called ubiquitous isozyme normally present in all tissues of the plant. Co-segregation between eu4 and a restriction fragment length polymorphism marker revealed by a urease genomic subclone is consistent with Eu4 being the structural gene of the ubiquitous urease (J.D. Griffin, J.C. Polacco, unpublished observations).

Unlike class I and class III mutations, each of which affects the expression of a single urease isozyme, class II mutations are pleiotropic, resulting in the loss of all urease activities in the plant. Class II mutations define two genetic loci, Eu2 and Eu3 (16). Although the production of inactive ureases in eu2 and eu3 mutants suggests that they are defective in maturation processes common to both isozymes, the function of the two genes is not known.

A study of urease mutants made us aware of the association of Methylobacterium mesophilicum with soybean tissues (13). Maternal tissues of eu4/eu4 plants are generally urease-negative but show 15 to 40% of the wild-type activity in whole tissue assays of callus (20) and expanding unfoliated leaves (our unpublished observation). This activity is absent in class II mutants and does not appear to be due to "leaky" expression of the eu4 allele, because the urease activity in eu4/eu4 callus and leaf does not resemble the ubiquitous isozyme by several biochemical and immunological criteria. Nor does it appear to be due to low-level Eu1 expression in nonembryogenic tissue, because eu4/eu4, eu1-sun/eu1-sun double mutants also express it. To test the possibility that a bacterium is responsible for this basal eu4/eu4 urease activity, we ground leaf tissue and callus in sterile water and streaked the macerates on rich (LB2) and defined (AMS) media (ref. 8, formulations 1065 and 784, respectively). All plates showed bacterial growth after several weeks at 28°C. LB plates inoculated with leaf macerate, as expected, showed a variety of bacterial and fungal contaminants. Surprisingly, LB plates inoculated with apparently axenic callus and AMS plates inoculated with either leaf or callus shared what appeared to be a single bacterial species. This PPFM has been assigned to the genus Methylobacterium (10), formerly Pseudomonas spp. (2, 5, 6, 14) among other designations.

In soybean, PPFMs are present on mature leaves in populations as high as 1 × 105 colony-forming units/g fresh weight (J. Dunleavy, personal communication). In young leaves, we have observed up to 4 or 5 × 105 colony-forming units/g fresh weight. The bacterium is difficult to remove by normal treatments for surface sterilization, e.g. soaking in 10% bleach or 70% ethanol. Indeed, every leaf and leaf-derived callus sample tested contained the bacterium.

In work presented here, we examined whether PPFMs

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2Abbreviations: LB, Luria broth; AMS, ammonium mineral salts; PPFM, pink-pigmented facultative methylophroph.
contribute to urease activity in eu4/eu4 tissues and, if so, why their activity is absent in the class II (eu2 and eu3) mutants. Our findings indicate that the bacteria do contribute to the plant’s urease profile and that bacteria on eu2 and eu3 mutants do not display urease activity because they are effectively deprived of the urease cofactor, nickel.

MATERIALS AND METHODS

Plant Material

Urease mutants used in this study have been previously characterized. The eu2/eu2 (16), eu3-3/eu3-3 (16), and eu4/eu4 (20) genotypes, recovered from an ethyl methanesulfonic acid-derived M2 population of cv Williams, were outcrossed once to Williams 82. The eu1-sun/eu1-sun genotype (seed-urease-null phenotype) was originally identified in PI229324 (15) and was introgressed into the Williams genetic background by five backcrosses by Dr. R. Bernard (Department of Agronomy, University of Illinois, Urbana, IL).

Isolation of PPFMs from Soybean Leaf

Leaves harvested from growth chamber-grown soybean (Glycine max [L.] Merr.) seedlings were washed in sterile distilled water, then ground in a sterile mortar in sterile water. The resulting slurry was diluted in series and 100-μL aliquots of each dilution were plated on AMS medium solidified with agar (8). Plates were incubated at 28°C for 1 week before bacterial colonies were isolated from them. Liquid cultures of the bacterium were grown in AMS medium or in Tryptic Soy Broth (Difco). In cultures supplemented with nickel, 10 μM NiSO₄ was added in the presence of 10 mM K citrate, pH 6.0 (19).

Urease Assays

Urease was assayed as previously described (15). Briefly, an assay chamber was constructed from two 7-ml plastic scintillation vials connected by a short length of latex tubing. An assay mix of soybean tissue (leaf, callus, or seed extract) or bacterial culture (200 μL) in 1 mL of 0.1 M Tris-maleate, 1 mM EDTA buffer, pH 7.0, 5% 1-propanol (included in assays of whole tissue to facilitate permeabilization), and 10 mM [14C]urea occupied one of the vials, and a 1 cm² glass fiber wick saturated with 50 μL of 9 M ethanolamine was affixed to the wall of the second vial. After incubating the apparatus at 37°C for 2 h, 0.5 mL of 2 N H₂SO₄ was injected into the assay mix through the latex tubing, stopping the enzyme-catalyzed reaction and driving 14CO₂ out of solution to be captured on the base-saturated wick. Scintillation fluid was then added to the wick-containing vial of the disassembled chamber, and 14CO₂ trapped on the wick was quantified by scintillation counting. These data were used to calculate urease activity (1 unit = 1 nmol urea hydrolyzed·min⁻¹ at 37°C).

Hydrogenase Assay

Hydrogenase activity in bacterial colonies was assayed by the method of Haugland et al. (11). Colonies were grown overnight in a H₂ and CO₂ atmosphere (Gas Pak; BBL Microbiology Systems, Cockeysville, MD) on filter papers saturated with 10 mM methylene blue dye in 50 mM KH₂PO₄, 2.5 mM MgCl₂ with or without 10 μM NiSO₄ in the presence of 10 mM K citrate. Hydrogenase activity was detected by the hydrogen-dependent ability of the bacteria to reduce (i.e. clear) the dye surrounding the colony.

Nickel Uptake

Plants were grown hydroponically without supplementary nickel and were then pulsed with 63NiCl₂. Seeds were germinated in Petri dishes lined with moist germination paper (Anchor Paper Co., St. Paul, MN). Emerging radicles of these seeds were inserted through the perforated lid of a black plastic box containing half-strength Murashige and Skoog salts (18). The solution was aerated by an aquarium pump and was changed three times weekly. Seedlings thus planted were kept in a growth chamber for 14 d, after which time 63NiCl₂ was added to the nutrient solution to a final concentration of 10 μM (specific activity: 4 μCi/μg Ni). After a 24 h pulse-labeling period, the seedlings were incubated for 24 h in nutrient solution containing unlabeled equimolar nickel. Tissue harvested from these plants was lyophilized and assayed for nickel content by scintillation counting as described by Cataldo et al. (3).

Grafts

Scions, usually cut below the cotyledons, were taken from seedlings of Williams 82, eu2/eu2, and eu3-1/eu3-1 and were grafted onto rootstocks of the same three genotypes in all possible combinations. Thus, control plants contained scion and rootstock of the same genotype (not necessarily from the same individual). The grafted plants were grown to maturity in the greenhouse and were allowed to self-pollinate. Dry seeds harvested from these plants and homogenized in distilled water were assayed for urease activity as described above.

RESULTS

PPFM Bacteria Contribute to the Urease Profile of eu4/eu4 Mutant Tissue

Table I shows the results of biochemical comparisons made among the urease activities in wild-type and eu1-sun.eu4 double mutant soybean tissues, and in free-living cultures and extracts of PPFMs isolated from soybean. We have established (15) that the ratio of urease activities at pH 7 and pH 9 is a useful diagnostic for the identification of urease isozymes. In permeabilized whole tissues or in extracts, the ubiquitous isozyme shows higher activity at pH 9 than at pH 7; the embryo-specific urease is more active at pH 7 than at pH 9. In assays of powdered, permeabilized callus and permeabilized bacterial cells, the pH 7/9 activity ratio of soybean double mutant tissue more closely resembles that of bacterial cells than of wild-type soybean callus. Antibodies raised against the soybean embryo-specific urease precipitate 80 and 95% of the activity in extracts of soybean double mutant callus and of bacterial extracts, whereas only 65% of an equivalent
amount of activity of wild-type soybean callus extracts is precipitated by the same antiserum. Because the soybean double mutant produces inactive ubiquitous urease (20), more urease antigen was present in extracts of the double mutant callus than in either of the other two extracts. This disparity in antigen levels between extracts of the double mutant and the bacterium probably explains the lower level of precipitation (80%) of active urease in the double mutant compared with that (95%) in the bacterium. However, it is significant that double mutant callus activity shows more affinity for anti-seed urease than does the (mainly) ubiquitous urease of wild-type callus. The level of immunoprecipitation of the wild-type callus urease (65%) agrees with previously reported values (21). Finally, activities in the permeabilized leaf tissue of soybean double mutant and permeabilized bacterial cells are similarly inhibited (81 and 85%, respectively) by 0.6 M borate compared with the resistant activity in permeabilized wild-type leaf.

To demonstrate further that microbial activity is responsible for the urease measured in callus and leaves of the eu4 mutant, we attempted to cure these tissues of the bacterium. Callus maintained for 2 weeks on R3 medium (19) with 100 μg/mL cefotaxime showed no decrease in growth, but 30% lower urease activity than control callus (Table II). In separate experiments, callus maintained on 100 μg/mL cefotaxime showed a 25 to 34% reduction in PPFM population compared with untreated controls. Soybean seeds were cured of bacteria by two different heat treatments (22), followed by normal germination in soil and growth in a growth chamber for 2 weeks. The extent to which bacterial populations were reduced by the heat treatments was assessed by the method of Corpe (5), by pressing the abaxial surfaces of excised leaves onto plates of AMS and/or rich media and incubating the resulting "leaf prints" for 1 week at 28°C. From 70 to 87.5% of leaves from treated seed show no growth of PPFMs. Figure 1 compares leaf prints on AMS plates from control and heated leaves. Leaves of the same plants were assayed for urease activity and, as seen in Table II, a reduction in the number of PPFMs in the tissue results in a commensurate loss of urease activity.

Because the urease of the bacteria resembles that of eu4/eu4 soybean tissue biochemically and immunologically, and because the level of eu4/eu4 urease activity is correlated with the size of its associated bacterial population, we conclude that phylloplane microbes, of which M. mesophilicum is representative, are responsible for the urease activity detected in callus and in young leaves of the urease structural gene mutant eu4/eu4. We have reported recently that this activity plays a significant role in hydrolysis of urea in developing and germinating seed (23).

**Bacteria from Mutants eu2/eu2 and eu3-e1/eu3-e1 Are Urease-Negative**

Little or no urease activity is detectable in callus (16) or leaves (16, 20) of the class II soybean mutants eu2/eu2 and eu3-e1/eu3-e1, although PPFMs can be isolated from them. PPFMs newly isolated on AMS medium from Williams 82 (wild type), eu2/eu2, eu3-e1/eu3-e1, and eu4/eu4 leaves were used to inoculate liquid cultures in Tryptic Soy Broth. After overnight incubation at 28°C, bacteria isolated from the class II mutants exhibited only 20 to 25% of the urease activity of bacteria from both wild-type and eu4/eu4 plants (Table III). Interestingly, over a period of days to weeks, cultures of the PPFMs isolated from class II mutants eventually become fully

### Table I. Comparisons of Urease Activities from Soybean Callus and Leaf with the Urease Activity of PPFMs

<table>
<thead>
<tr>
<th>Source of Activity</th>
<th>Activity at pH 7*</th>
<th>% Activity Precipitated by Anti-Urease Antibodya</th>
<th>Inhibition % by 0.6 M Boratea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type soybean</td>
<td>0.78/1.03 = 0.8</td>
<td>62</td>
<td>100</td>
</tr>
<tr>
<td>eu1-sun, eu4</td>
<td>1.83/0.94 = 1.7</td>
<td>80</td>
<td>81</td>
</tr>
<tr>
<td>double mutant</td>
<td>1.49/0.81 = 1.7</td>
<td>95</td>
<td>85</td>
</tr>
</tbody>
</table>

* Soybean samples were lyophilized callus tissue, rehydrated in assay buffer (0.1 M Tris-maleate, 1 mM EDTA, 5% 1-propanol). Bacterial samples were liquid cultures diluted in AMS medium. Similar activities (1.5–3.0 units) were assayed for each sample as described in the text. pH values are buffer values; pH in the assay mixes measured 7.0 and 8.8. Soybean extracts were from lyophilized callus that was ground in assay buffer and clarified by centrifugation; bacterial extracts were from pelleted cells disrupted in a French press at 18,000 psi and clarified by centrifugation. Similar activities (0.5–1.5 units) were employed in each sample. Nonimmune serum was included in the assay as a control. Preparation of anti-seed urease serum and details of the immunoprecipitation have been described previously (21). Soybean samples were leaf discs of tissue punched from leaves. Bacterial samples were liquid cultures. Urease was assayed as described in the text except that the assay buffer was Tris-borate (0.6 M in borate), pH 7.0, 5% 1-propanol.

### Table II. Urease Activities in Soybean Tissues Cured of PPFMs by Treatment with Antibiotic or Heat

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>Urease Activity* (% of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type callus</td>
<td>None</td>
<td>7.2 ± 0.5 (100%)</td>
</tr>
<tr>
<td></td>
<td>Cefotaximeb</td>
<td>7.0 ± 0.07 (97%)</td>
</tr>
<tr>
<td>eu1-sun/eu1-sun, eu4/eu4 callus</td>
<td>None</td>
<td>2.5 ± 0.02 (100%)</td>
</tr>
<tr>
<td>eu4/eu4 leafa</td>
<td>Cefotaximeb</td>
<td>1.8 ± 0.02 (72%)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>2.5 ± 1.6 (100%)</td>
</tr>
<tr>
<td></td>
<td>Heata</td>
<td>0.48 ± 0.3 (18%)</td>
</tr>
<tr>
<td></td>
<td>Heatb</td>
<td>0.48 ± 0.3 (18%)</td>
</tr>
</tbody>
</table>

* Urease activity expressed as nmol urea hydrolyzed min⁻¹ mg⁻¹ fresh weight. b Cefotaxime was grown on cefotaxime-containing medium (100 μg/mL) for 2 weeks. In separate experiments cefotaxime at this level was observed to reduce the PPFM population by 25 to 34% in callus tissue. c Leaf samples were from 14-d-old seedlings. d Dry seed was heated to 40°C for 4h before planting. Of the leaves of plants treated this way 87.5% showed no growth of PPFMs on AMS medium. e Imibed seeds were heated in a water bath to 52°C for 10 min before planting. Nearly 70% of leaves of plants treated this way showed no growth of PPFMs on AMS medium.
urease-positive. Because a likely functional impairment in class II mutants is the emplacement of a nickel cofactor on the urease apoenzymes (16), we tested the possibility that the PPFMs isolated from class II mutants might be urease-null because they are effectively nickel-starved. When parallel liquid cultures of the four PPFMs isolated were supplemented with nickel and incubated overnight at 28°C, the urease-null phenotype of the PPFMs isolated from the class II mutants was corrected relative to that of eu4/eu4- and Williams 82-derived bacteria (Table III).

Additional evidence that availability of nickel is responsible for the urease-null phenotype of the PPFMs on class II mutants is the observation that these bacteria are also hydrogenase-deficient. Hydrogenase, like urease, requires a nickel cofactor for activity (1, 24). Figure 2 shows by a qualitative, but reproducible, hydrogenase assay that PPFMs isolated from class II, but not from class III mutants or from Williams 82, were stimulated in their ability to reduce methylene blue in a hydrogen atmosphere (11) when supplied with nickel.

Class II Mutants Accumulate Nickel

Uptake of 63Ni by Williams 82, eu1-sun/eu1-sun, eu2/eu2, eu3-e1/eu3-e1, and eu4/eu4 seedlings was compared. Nickel uptake was comparable to that reported by Cataldo et al. (3), whose methods were employed, and no major differences were observed among the four genotypes (Table IV). The possible differences in nickel content among senescing cotyledons is questionable; the low level of 63Ni accumulation by eu4 cotyledons (0.12 µg/g dry weight) belies its fully active embryo-specific urease. Whereas eu3-e1 cotyledons accumulate half the nickel of eu4 cotyledons, urease activity in eu3-e1 cotyledons is at least three orders of magnitude lower (16, 20).

Wild-Type Scions Produce Urease-Positive Seeds When Grown on Class II Mutant Rootstocks

Urease activity in seeds developed on grafted plants is indicative of the availability of nickel to those seeds during

Table III. Urease Activity in PPFMs Isolated from Different Urease Mutant Soybean Plants

<table>
<thead>
<tr>
<th>Source of PPFM</th>
<th>Urease Activitya</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minus nickel</td>
</tr>
<tr>
<td>Williams 82</td>
<td>20.1 ± 0.8</td>
</tr>
<tr>
<td>eu2/eu2</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>eu3-e1/eu3-e1</td>
<td>4.6 ± 1.1</td>
</tr>
<tr>
<td>eu4/eu4</td>
<td>16.3 ± 4.2</td>
</tr>
</tbody>
</table>

*a Urease activity expressed as nmol urea hydrolyzed min⁻¹ unit⁻¹ of optical density at 550 nm of the liquid culture. Cultures were initiated from single colonies that appeared on AMS plates 5 d after plating leaf macerates. b Added as 10 µM NiSO₄₃ in the presence of 10 mM K citrate, pH 6.0.

Figure 1. Leaf prints of soybean on AMS plates (8) produced by the method of Corpe (5). The control (left) shows substantial growth of PPFMs after 1 week of incubation at 28°C. The leaf print from a seedling grown from heat-cured seed (right) shows no growth of PPFMs.
development. For example, nickel-deprived soybean plants exhibited a much sharper reduction in seed urease than in leaf urease (9). Cataldo et al. (4) showed that nickel taken up by soybean plants is eventually accumulated in the seeds. Table V shows that seeds developed on Williams 82 scions were urease-positive regardless of the genotype of the rootstock on which they developed. This was not the case for seeds developing on scions of either of the class II mutants. Seeds developed on either of the mutant scions were urease-negative regardless of rootstock.

The ability of nickel to traverse class II maternal tissue to activate urease in embryos of wild-type scions agrees with previously reported genetic analyses of the class II mutations. In crosses of "recessive class II mutant" × wild type, F1 seeds that develop on urease-null maternal tissue are themselves fully urease-positive (16).

Table IV. Nickel Accumulation (μg/g Dry Weight) in Various Tissues of Different Soybean Genotypes after Uptake of 65NiCl2

Fourteen-day-old seedlings, grown in a nutrient solution without added nickel, were exposed to 10 μM 65NiCl2 (4 μCi/μg Ni) for 24 h followed by a 24-h chase of equimolar unlabelled nickel. Data are the means of at least two determinations.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Roots</th>
<th>Cotyledons</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Williams 82</td>
<td>0.18</td>
<td>0.31</td>
<td>0.22</td>
</tr>
<tr>
<td>eu1-sun/eu1-sun</td>
<td>0.26</td>
<td>0.37</td>
<td>0.35</td>
</tr>
<tr>
<td>eu2/eu2</td>
<td>0.20</td>
<td>0.31</td>
<td>0.35</td>
</tr>
<tr>
<td>eu3-e1/eu3-e1</td>
<td>0.17</td>
<td>0.05</td>
<td>0.27</td>
</tr>
<tr>
<td>eu4/eu4</td>
<td>0.13</td>
<td>0.12</td>
<td>0.27</td>
</tr>
</tbody>
</table>

DISCUSSION

Association between PPFMs and Soybean

The association between plants and M. mesophilicum has not been studied extensively. However, the bacterium has been isolated from more than 70 species of plants representing a wide range of families (7). In our laboratory, we have isolated PPFMs from Arabidopsis, tobacco, barley, clover, rice, wheat, and Lotus, in addition to soybean. The bacterium is seed-transmitted (J Dunleavy, personal communication). Our observation that the bacterial population is not fully reestablished on plants grown from heat-treated seeds, even when they are grown in unsterilized soil, suggests that leaf bacteria are likely the descendants of seed-borne bacteria rather than colonists from air or water, from other plants, or from the soil. If the PPFMs were a mobile population, then they would not be nickel-starved on the class II mutants.

The precise location of the bacteria on (or in) plant leaves is unknown, although the observation that the population can...
be sampled by taking impressions of leaves on agar plates suggests that at least some of them are associated with the cuticle of the leaf. Further supporting this idea are scanning electron microscope micrographs (7) that show bacteria with PPFM morphology to be buried in the leaf cuticle. If the PPFMs were embedded in cuticle, their ability to withstand treatments normally employed to surface-sterilize leaf tissue for tissue culture would be more understandable. We expect that knowing the leaf microenvironment of the PPFMs will contribute to our understanding of how the bacteria are starved for nickel on the class II mutants.

**Nature of the Nickel Defect in Class II Soybean Mutants**

Given that soybean mutants at *Eu2* or *Eu3* take up and transport nickel (Tables IV and V), the inability of the mutant-associated PPFMs to use it is puzzling. Studies of xylem fluid (4, 25) suggest that nickel is transported as a complex with organic acids (citrate or malate) or with asparagine. The specific and drastic effect on urease by *eu2* and *eu3-el*, and the existence of a dominant mutant allele *Eu3-e3* (16), make it unlikely that mutation at *Eu2* or *Eu3* somehow affects complexation or that either of the mutations drastically reduces the levels of complexing agents.

A model based on our results requires that nickel be taken up and transported by the mutant plant, but that it not be appropriately incorporated into the plant ureases and it not be accessible to bacteria living on the leaf. We are not aware of any mechanism in plants that explains these results, but our observations parallel those for bacterial urease synthesis. The urease operon of *Klebsiella aerogenes* includes three open reading frames that do not encode subunits of the urease protein, but that are required for urease activity (17). One of these open reading frames encodes a metal-binding domain. None of these accessory proteins is thought to be involved in nickel uptake. By analogy, we suggest that *Eu2* and *Eu3* gene products participate in the chain of events that takes nickel from the transport stream and results in its emplacement on the urease apoenzyme. Although the details of these processes are not known, they might include receptor binding; decomplexation of nickel, possibly involving change of valence or binding to specific protein carriers; intracellular transport; and emplacement of nickel on the apoenzyme.

Loss or alteration of *Eu2* or *Eu3* function results in PPFMs not “seeing” nickel in the plant. If *Eu2* or *Eu3* affect complexation, then nickel complexes accumulating in class II mutants must not be accessible to or utilizable by the PPFMs, because PPFMs in free-living culture can use nickel complexed with citrate (Table III). Because the PPFMs are fully urease- and hydrogenase-positive when cultured away from the plant, soybean proteins do not play a direct role in bacterial metabolism of nickel. However, alteration of the affinity of a soybean nickel-binding protein(s) for the metal could result in the sequestration of nickel delivered to soybean cells and would explain the mechanism by which mutation, whether dominant or recessive, affects the activities of both plant and bacterial enzymes.

The transient urease- and hydrogenase-null phenotypes of the PPFMs on class II soybean mutants remain our only evidence that nickel metabolism is altered in these pleiotropic mutants. Nevertheless, we consider this evidence to be compelling. This work points out that metal utilization in plants is a poorly understood area of plant physiology. Perhaps one of the reasons it is poorly understood is that many mutations affecting the metabolism of metals are probably lethal. Because soybean urease and nickel mutants are not lethal, they may serve as a model system in which to investigate the metabolism of metals in plants.

**ACKNOWLEDGMENTS**

The authors thank J. Dunleavy (Iowa State University) and W.A. Corpe (Columbia University) for their helpful discussions and insights concerning the PPFMs. We acknowledge the advice and criticism of our colleagues at the University of Missouri, in particular Judy Wall and Roberto Borghese.

**LITERATURE CITED**

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