PNAS COMMENTARY

Chemical symphony of coumarins and phenazines in rhizosphere iron solubilization

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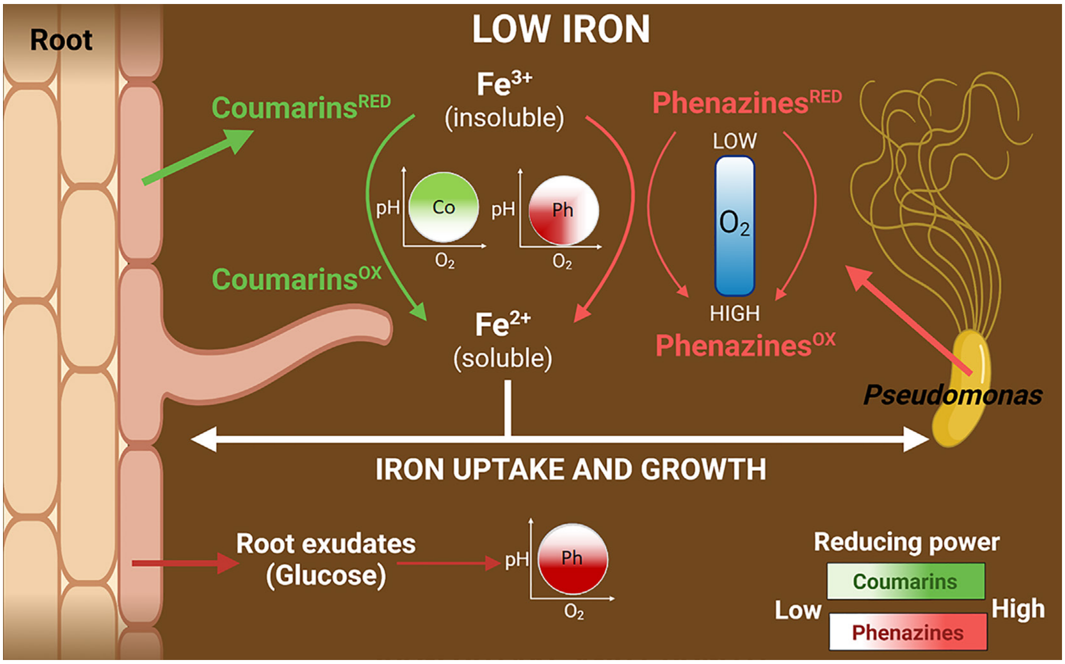

Fig. 1. Model for the role of the plant-derived coumarins (Co) and bacterial phenazines (Ph) in Fe reduction and solubilization. Coumarins and phenazines are redox-active metabolites (RAMs) that under Fe-limiting conditions are secreted in the soil environment where they reduce insoluble Fe^{3+} to soluble Fe^{2+}, which can then be taken up and stimulate growth of both plants and bacteria. The reducing power of coumarins and phenazines is differentially affected by pH, oxygen levels, and carbon sources in the rhizosphere. It is proposed that the differential redox activity of bacterial and plant-derived RAMs under a range of rhizosphere-relevant environmental conditions may contribute to safeguarding Fe bioavailability for both microbes and their plant host (created with https://www.BioRender.Com).

Iron (Fe) is an essential mineral nutrient for virtually all life on Earth. It serves as an important redox catalyst for many cellular processes steering growth, development, and survival. Although Fe is abundantly present in the soil, its bioavailability is often very low, especially in alkaline soils, which represent 30% of the world's cultivated land. In the soil, Fe is mainly present as ferric (Fe^{3+}) oxide, which is poorly soluble at neutral and high (alkaline) pH. Plants and microbes have developed ingenious strategies to increase Fe bioavailability for their own or their neighbor's advantage (1). One strategy is acidification of the soil environment, which leads to a higher solubility of Fe. A second strategy is the secretion of (phyto)siderophores into the soil environment. These low-molecular-weight organic compounds chelate Fe, which can then be taken up by the secreting organism via specialized transporters. A third strategy is the secretion of redox-active-metabolites (RAMs), which reduce relatively insoluble Fe^{3+} to soluble ferrous Fe (Fe^{2+}), therewith increasing bioavailability of Fe. Coumarins are well-studied plant-derived RAMs that are crucial for plant growth under conditions of low Fe availability (2, 3). They are specifically secreted by plant roots when plants experience Fe shortage. Phenazines are well-studied bacterial RAMs that can help to solubilize Fe and stimulate bacterial growth (4). Under low Fe conditions, many soil-dwelling bacteria, especially pseudomonads, produce and secrete phenazines. The rhizosphere is a hotspot of microbial activity, in which plant roots and a large diversity of microbiota compete for the same resources. Among these rhizosphere microbiota are plant-beneficial microbes, many of which are pseudomonads that can promote plant growth and stimulate the plant's immune system (5, 6). Hence, it is in the plant's interest to shape its root microbiome in favor of microbes with profitable functions, while limiting the impact of deleterious and pathogenic ones. Fe is among the highly wanted mineral nutrients, thus the crusade for this critical cofactor, for example, via the release of coumarins

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Author contributions: C.M.J.P. and I.A.S. wrote the paper.

The authors declare no competing interest.

See companion article, "The chemical ecology of coumarins and phenazines affects iron acquisition by pseudomonads," 10.1073/pnas.2217951120.

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Published April 24, 2023.

and phenazines, is constantly ongoing. In the paper by McRose et al. (7) in PNAS, the authors hypothesized that the seemingly redundant role of plant- and bacteria-derived RAMs in Fe solubilization in the rhizosphere may in fact have so-far unrecognized distinct functions in the solubilization of Fe under the environmental conditions that occur in the diversity of niches in the rhizosphere. Moreover, considering the fact that both coumarins (8) and phenazines (9) also possess selective antimicrobial activities, they may play a dual role in shaping root–microbiome interactions. To date, RAM activity of coumarins and phenazines has been mainly studied in isolation. McRose et al. took the challenge to investigate side by side the redox activities, Fe solubilization capacities, and bacterial growth-promoting potential of plant coumarins and bacterial phenazines under Fe-limiting conditions. They did this in the context of variations in the chemical microenvironment that are likely of importance in the rhizosphere and may tune RAM redox state: oxygen level, pH, and carbon sources as released in root exudates.

> **"McRose et al. provided a fascinating peek into the complexity of the abiotic and biotic aspects of plant–microbe and microbe–microbe interactions in the rhizosphere."**

In the rhizosphere, oxygen levels are highly variable and after heavy rainfall can even become very low for the whole root system. In contrast to coumarins, reduced phenazines are highly reactive with oxygen (10). In their reduced, electron-carrying state RAMs can solubilize Fe through electron transfer to Fe^{3+}, resulting in water-soluble Fe^{2+}, which can then be taken up by plants or microbes. However, under oxic conditions, phenazines rapidly lose their reducing power and consequently their ability to solubilize Fe. Reduced coumarins are much less reactive with oxygen. Hence, the authors hypothesized that in contrast to phenazines, they keep their Fe-solubilizing capacity under oxic conditions. To investigate this, the authors tested four plant-derived coumarins (coumarin, daphnetin, fraxetin, and scopoletin) and the bacterial phenazine phenazine-1-carboxylic acid (PCA). While coumarin was not redox active and scopoletin only very mildly, daphnetin, fraxetin, and PCA were clearly redox active and consequently capable of solubilizing Fe and promoting growth of Fe-limited *Pseudomonas* bacteria under anoxic conditions. Oxygen completely blocked Fe^{3+} reduction and bacterial growth stimulation by PCA, but that of the coumarins daphnetin and fraxetin was largely unaffected. Hence, depending on the oxygen availability in an Fe-limited rhizosphere, either bacterial phenazines and/or plant-derived coumarins can dynamically contribute to Fe solubilization and uptake (Fig. 1).

Soils can vary in pH, and especially neutral and alkaline soils display very low bioavailability of Fe. Under such conditions, plant roots secrete protons to acidify the rhizosphere, therewith increasing Fe availability (11). Soil pH not only influences Fe solubility, it can also affect the redox activity of RAMs. To investigate this, McRose et al. (7) tested the range of coumarins and PCA for their redox potential, Fe solubilization capacity, and promotion of bacterial growth under low (5.5) and high (7.5) pH levels. The coumarins daphnetin and fraxetin performed better at pH 7.5, while PCA was most active at pH 5.5. The authors thus concluded that bacterial phenazines are most beneficial for Fe solubilization under hypoxic, acidic conditions, while plant coumarins provide most benefits under oxic, mildly alkaline conditions (Fig. 1). What this means in terms of plant–microbiome interactions was not investigated. It is, however, tempting to speculate that the differential redox activity of bacterial and plant-derived RAMs under a range of rhizosphere-relevant environmental conditions may contribute to safeguarding Fe bioavailability for both microbes and their plant host in this dynamically changing environment.

It is well established that plants secrete a significant proportion of their photosynthetically fixed carbon sources into the rhizosphere, where microbiota feast on these root exudates (12). Glucose can promote phenazine reduction (making it capable of solubilizing Fe), either due to its direct reducing power or by changes in respiration rate in the system, drawing down oxygen levels and consequently preventing oxygen-mediated phenazine oxidation. The authors hypothesized that this may potentially restore the Fe-solubilizing capacity of phenazines under oxic conditions. By adding different root exudate–relevant carbon sources (including glucose) into the experimental equation, McRose et al. (7) provide evidence that indeed glucose has a positive effect on the reduction rate of PCA under oxic conditions at low pH and that root exudates, thus, may expand the environmental niche in which bacterial phenazines can promote Fe acquisition (Fig. 1).

Plant–microbe and microbe–microbe interactions in the rhizosphere are highly complex. By ironing out parts of the chemical ecology of bacterial and plant-derived RAMs in mobilization of the crucial common good Fe, McRose et al. provided a fascinating peek into the complexity of the abiotic and biotic aspects of plant–microbe and microbe–microbe interactions in the rhizosphere. While oxygen level, pH, and root exudates are enlightened players in the tunability of the system, others are still in the dark. For instance, besides their Fe-solubilizing capacity, both coumarins and phenazines have been demonstrated to possess selective antimicrobial activity and through this activity shape rhizosphere microbiome composition (4, 8, 13–15). In *Arabidopsis thaliana*, it was shown that coumarin-mediated changes in the microbiome result in enrichment for microbiota with Fe-mobilizing capacity (16, 17). Future studies are expected to shed light on how spatial and temporal dynamics of plant and bacterial RAM production affect their microenvironment, not only chemically, for example, via the solubilization of Fe, but also biologically through changes in root–microbe and microbe–microbe interactions in different root niches. A detailed understanding of the biological mechanisms involved will provide a firm knowledge base for future microbiome-assisted agricultural systems in which microbiota help improve plant nutrition and health, while reducing the use of agrochemicals.

ACKNOWLEDGMENTS. We were supported by the Dutch Research Council Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) through the Gravitation program MiCRop (grant no. 024.004.014; C.M.J.P. and I.A.S.), NWO-XL program "Unwiring beneficial functions and regulatory networks in the plant endosphere" (grant no. OCENW.GROOT.2019.063; C.M.J.P.), and the Spinoza prize awarded to C.M.J.P.

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