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Strain and species differences in rhizobial secretion of lumichrome and riboflavin, measured using thin-layer chromatography

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Abstract
Thin-layer chromatographic analysis of 16 bacterial isolates from root nodules of eight members of the tribe Psoraleae and 14 standard laboratory strains from the genera *Rhizobium, Bradyrhizobium, Mesorhizobium* and *Sinorhizobium* revealed significant differences in the synthesis and extracellular release of riboflavin and lumichrome into culture filtrate. The measured absorbances for both riboflavin and lumichrome were not only significantly different among the rhizobial isolates, but also sometimes between paired bacterial isolates from root nodules of the same *Psoralea* species. Isolates AP1 from *P. aphylla*, AS1 from *P. asarina*, M2 from *P. monophylla* and AC2 from *P. aculeata* respectively produced 9.7±2.6, 13.3±1.7, 13.7±3.1 and 13.3±0.3 ng.ml^{-1} riboflavin, and were therefore the most limited in the synthesis and release of this metabolite. The isolates from *P. aphylla*, *P. asarina*, *P. repens* together with M2 from *P. monophylla* also produced the lowest concentrations of lumichrome, ranging from 2.1±0.5 to 3.5±0.4 ng.ml^{-1} culture filtrate. The concentrations of lumichrome and riboflavin released by the 14 rhizobial strains belonging to the four genera (i.e. *Rhizobium, Bradyrhizobium, Mesorhizobium* and *Sinorhizobium*) showed strain and species differences in bacterial secretion of both molecules. From the measured absorbances and estimated levels of the two metabolites in culture filtrates, *Bradyrhizobium japonicum* Tal 110, *Sinorhizobium meliloti* RAKI, and *Sinorhizobium fredii* 6217 showed significantly greater release of riboflavin (ranging from 8.2±0.7 to 9.7±0.4 ng.ml^{-1}) compared to the rest (which varied from 3.4±0.8 to 7.8±0.3 ng.ml^{-1}). With lumichrome, however, *Sinorhizobium arboris* Ima 14919, *Bradyrhizobium* CB756, *Sinorhizobium meliloti* RAKI, and *R. leguminosarum bv. viceae* 30 exhibited the most limited production of this metabolite (13.5±2.2, 14.6±5.4, 20.6±6.7 and 23.4±2.3 ng.ml^{-1}, respectively) compared with the other strains (whose concentrations ranged from 24.4±3.9 to 37.7±4.0 ng.ml^{-1}). Future studies should explore the possibility of using lumichrome and riboflavin released by symbiotic rhizobia as a chemotaxonomic tool.

Keywords: Lumichrome, riboflavin, rhizobial strains, *Psoralea* isolates

1. Introduction

From much earlier studies (West and Wilson, 1938) some species of root-nodule bacteria ("rhizobia") have been reported to synthesize the vitamins riboflavin (Fig. 1) and thiamin for cell growth. Various studies have since shown that several other bacterial species, including *Sinorhizobium meliloti* (Yang et al., 2002), *Pseudomonas fluorescens* (Marek-Kozaczuk and Skorupska 2001), *Rhizobium leguminosarum bv. viceae* (Sierra et al., 1999), *Azospirillum brasilense* (Rodelas et al., 1993) and *Azotobacter vinelandii* (Gonzalez-Lopez et al., 1983) produce vitamins (e.g. thiamin, riboflavin, niacin, biotin, and panthothenic acid) and amino acids (e.g. glutamate, lysine, arginine, tryptophane, methionine, etc.) for use in their own growth. Whether from bacteria or other sources, the presence of riboflavin in soil has been established, and its uptake and translocation to shoots of plants demonstrated by Capenter (1943). But whether symbiotic rhizobia and other diazotrophic microbes can directly utilize riboflavin from soil, has not yet been determined, although it has been shown that the uptake and assimilation of this vitamin stimulates growth in higher plants (Rao, 1973).

In addition to these vitamins, various novel signal molecules have been found that stimulate seedling development and promote plant growth (Dakora, 2003). For example, a number of biologically-active novel molecules have been purified and identified from rhizobial exudates

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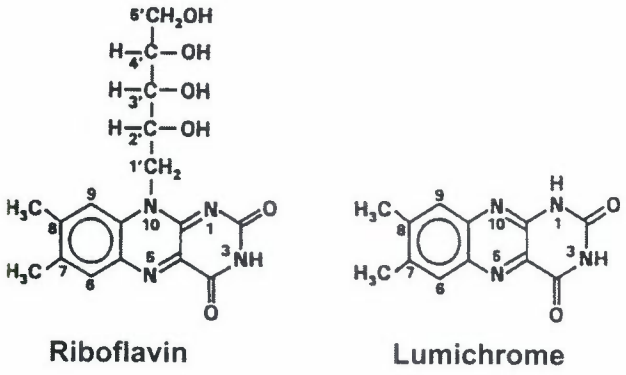

Figure 1. The structure of lumichrome and riboflavin, two signal molecules from rhizobia.

that act as morphogens in stimulating cell growth and plant development (De Jong et al., 1993; Daychok et al., 2000; Truchet et al., 1991; Philips et al., 1999; Dakora, 2003). More specifically, Phillips et al. (1999) have shown that lumichrome (Fig. 1) isolated from *Sinorhizobium meliloti* culture filtrate can increase root respiration and promote plant growth when applied to seeds or seedling roots. Matiru and Dakora (2005a) have also shown that at very low nanomolar concentrations, lumichrome can promote plant growth and seedling development in monocots and dicots such as soybean, cowpea, Bambara groundnut, sorghum and millet, but depresses growth at high doses in these species.

Lipo-chito-oligosaccharide (LCO) molecules isolated from rhizobia have similarly been shown to stimulate cell growth, nodule organogenesis (De Jong et al., 1993; Daychok et al., 2000; Truchet et al., 1991), seed germination (Zhang and Smith 2001), and seedling development in both monocots and dicots (Smith et al., 2002). Taken together, these findings show that soil bacteria are capable of producing various organic molecules that alter seed germination and plant development. This is in addition to their ability to synthesize and release the commonly known phytohormones such as auxins, gibberellins, cytokinins and abscicic acid (Phillips and Torrey, 1970; Dart, 1974; Dakora, 2003), which control plant growth.

Although we still do not understand how lumichrome and riboflavin molecules (Fig. 1) affect plant developmental processes, they are nevertheless important in rhizosphere ecology of both legume and non-legume species (Dakora et al., 2005) This is because riboflavin is easily converted to lumichrome enzymatically and photochemically under neutral and acidic conditions (Yagi, 1956; Yanagita, 1956). So microbial release of riboflavin into the rhizosphere can potentially increase soil concentration of lumichrome via enzymatic or photochemical degradation of riboflavin with the possibility of the former influencing rhizosphere processes, including stimulating plant growth and altering stomatal conductance and leaf transpiration (Dakora et al., 2005; Matiru and Dakora, 2005a,b). Despite the importance of lumichrome and riboflavin in the rhizosphere ecology of symbiotic rhizobia, no study (except for the one by Phillips et al., 1999) has so far assessed different strains and species of rhizobia for extracellular release of the two bacterial metabolites. This study quantitatively examines the secretion of lumichrome and riboflavin by different strains and species of symbiotic rhizobia, and measures the concentrations of the two metabolites released into bacterial culture filtrates.

2. Materials and Methods

Rhizobial strains

In all, 30 rhizobial strains were used in this study. The test strains included 16 isolates from 8 species of *Psoralea,* (namely, *Psoralea laxa* (L1 and L2), *P. aphylla* (AP1 and AP2), *P. asarina* (AS1 and AS2), *P. repens* (RP1 and RP2), *P. pinnata* (P1 and P2), *P. monophylla* (M1 and M2), *P. aculeata* (AC1 and AC2) and *P. restioides* (*RT1* and RT2)) together with 14 standard laboratory strains from the genera *Rhizobium, Bradyrhizobium, Mesorhizobium* and *Sinorhizobium.* The laboratory strains consisted of *Rhizobium* GHR2 (*Acacia cyanophylla*), *Rhizobium* NGR234 (*Lablab purpureus*), *R. leguminosarum bv. viceae* 30 (*Vicia faba*), *R. leguminosarum bv. viceae* Cn6 (lentil), *Mesorhizobium* (wild type) xhj 7, *Bradyrhizobium* (wild type) 14b, *B. japonicum* Tal 110, *Bradyrhizobium CB756 (Lablab purpureus), B. japonicum WB74, Sinorhizobium meliloti* 1 (*Medicago sativa*), *S. meliloti* RAKI, *S. fredii* 6217, *S. arboris* Ima 14919 and *S. kostiense* 19227.

Bacterial growth media

The bacterial broth culture was prepared as described by Phillips et al. (1999) for quantifying lumichrome and riboflavin released by *Sinorhizobium meliloti.* The components of the medium were (g/l): K_2HPO_4 (1.0), KH_2PO_4 (1.0), KNO_3 (6.0), proline (5.0), $MgSO_4$ (0.26), $FeCl_3.6H_2O$ (1.83), $CaCl_2.2H_2O$ (0.07), and dextrose (10.0) and the minor components (mg/l) thiamine (2.0), biotin (2.0), $Na_2Mo\ O_4.5H_2O$ (0.24), H_3BO_4 (3.0), $MnSO_4.H_2O$ (1.83), $ZnSO_4.7H_2O$ (0.29), $CuSO_4.H_2O$ (0.13), and $CoCl_2.6H_2O$ (0.24). Once prepared, the broth solution was autoclaved at 121°C for 15 min, and cooled to room temperature. The broth medium was then inoculated with the different rhizobial strains. In all instances, 4 replicate broth cultures were used for each strain and the culture incubated at 30°C with continuous shaking in the dark for 7 d to reach stationary phase.

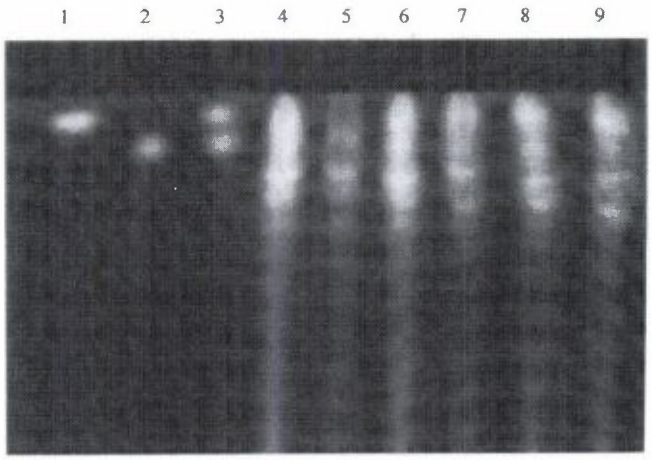

Figure 2. HPLC plate showing runs of lumichrome and riboflavin in standards, as well as separation of methanolic extracts of 10 ml cell free culture filtrates from different rhizobial strains. Lane 1: 24 ng lumichrome; lane 2: 12 ng riboflavin; lane 3: 24 ng lumichrome (top spot) and 12 ng riboflavin (bottom spot); lanes 4, 5 and 6: *Sinorhizobium meliloti* strain RAKI (in three replicates); lanes 7, 8 and 9: *Bradyrhizobium japonicum* Tal 110 (in three replicates).

Extraction of lumichrome and riboflavin from culture filtrate

The cultures were centrifuged at 10,000 × g for 10 min to pellet the bacterial cells; 5 ml of the supernatant was then passed through a C18 cartridge, rinsed three times with deionised water to remove salts, and the lumichrome and riboflavin eluted with methanol. The eluates were dried down, and then resolubilized in 30 µl methanol for thin-layer chromatography as described by Phillips et al. (1999). All experimental procedures were conducted under low light conditions to avoid degradation of riboflavin.

Thin-layer chromatographic separation of riboflavin and lumichrome

The separation of lumichrome and riboflavin in culture filtrates was carried out as described by Phillips et al. (1999). The resolubilized lumichrome and riboflavin were spotted on silica- gel-coated glass plates (Alltech 0.2 × 100 × 100 mm HPTLC silica gel plates). The compounds in the lipophilic fraction were separated using chloroform/ methanol/water (17.5:12.5:1.5) mixture. The TLC plates were viewed on a UV-light box (Ultra-Violet Products Ltd, Science Part, Milton Road, Cambridge, UK) and photographed with Polaroid film (Thermal paper, High density type, Kyoto, Japan) as shown in Fig. 2.

Standards of known concentrations of riboflavin and lumichrome were also spotted, ran on the TLC plates, and photographed as described above. Once more, all operations were done under low light conditions.

Quantification of lumichrome and riboflavin concentration and rates of release

The spots indicating the positions of lumichrome and riboflavin were located under a UV lamp for both the standards and compounds extracted from the bacterial culture filtrates and marked on the TLC plates. The spots were then scraped off the plates, eluted and their absorbances measured at 444 nm for riboflavin and 249 nm for lumichrome using a spectrophotometer (DU-64 Beckman Instruments Inc., Fullerton, Canada). For each strain, three independently grown bacterial broth cultures were used, with each assay being a replicate. For visual comparison, the three replicates of each strain were run side by side on the TLC plates. Because this method proved to be tedious, a modified version was adopted, tested, and used for quantifying the bacterial metabolites.

A modified method for extraction and quantification of lumichrome and riboflavin from culture filtrate (D.A Phillips, pers. comm.)

The broth culture for each bacterial strain or isolate was centrifuged at 10,000 × g for 10 min to pellet the bacterial cells, and the supernatant collected. Samples (5 ml) of the supernatant were passed through C18 cartridges, to trap the two molecules out of water. The 3 ml C18 cartridges were each rinsed three times with deionised water (2 ml each time) to remove all salts and the riboflavin and lumichrome eluted with 300 µl methanol as described by Phillips et al. (1999). The absorbances of eluates were measured at 444 nm for riboflavin and 249 nm for lumichrome using a standard spectrophotometer (DU-64 Beckman Instruments Inc., Fullerton, Canada). Incubation of broth culture for riboflavin production was done in the dark, and all other experimental procedures conducted under very low light conditions in order to avoid degradation of this metabolite into lumichrome (see Fig. 1).

Statistical analysis

Statistical analysis was done on the absorbances, the concentrations and rates of release of riboflavin and lumichrome from cultured rhizobia using one-way ANOVA on STATISTICA 2004 package.

3. Results and Discussion

Thin-layer chromatographic analysis of 16 bacterial isolates from root nodules of 8 members of the tribe Psoraleae and 14 standard laboratory strains revealed

significant differences in the synthesis and extracellular release of riboflavin and lumichrome into culture filtrate (Fig. 2; Tables 1 and 2). The measured absorbances for both riboflavin and lumichrome were not only significantly different among the bacterial isolates, but also sometimes between two paired bacterial strains from root nodules of the same *Psoralea* species. Because the absorbances differed, the estimated concentrations of the two metabolites as well as their rates of release were also significantly different for the test rhizobial isolates (Table 1). The bacteria isolated from *P. repens* (RP1 and RP2) and *P. restioides* (RT1 and RT2) showed much greater concentrations of riboflavin in culture filtrate as a result of their higher rates of extracellular release (Table 1). With lumichrome, however, the two isolates from *P. aculeata* (AC1 and AC2), showed significantly greater concentrations in culture filtrate, followed by the isolate RT1 from *P. restioides* and L2 from *P. laxa* (Table 1).

Because the number of bacterial isolates used in this study was rather small, the taxonomic value of quantifying lumichrome and/or riboflavin from rhizobia may not be obvious. However, from the limited data obtained here for the 16 isolates from *Psoralea* species, it appears that measuring the levels of naturally released lumichrome and riboflavin from culture filtrate could prove to be a useful tool for rhizobial taxonomy. For example, except for *P. monophylla,* whose isolates produced significantly different levels of lumichrome, data from paired bacterial strains isolated from each *Psoralea* species were generally similar. With riboflavin, however, there was generally greater variation between pairs of bacterial isolates from the same *Psoralea* species (Table 1).

Measuring the concentrations of lumichrome and riboflavin released by 14 standard laboratory strains from four rhizobial genera (namely, *Rhizobium, Bradyrhizobium, Mesorhizobium* and *Sinorhizobium*) also showed strain and species differences in bacterial secretion of these metabolites (Table 2). From the measured absorbances and estimated levels of lumichrome and riboflavin in culture filtrate, *Bradyrhizobium japonicum* Tal 110, *Sinorhizobium meliloti* RAKI and *Sinorhizobium fredii* 6217 showed significantly greater production and release of riboflavin relative to the rest (Table 2). In contrast, *Rhizobium leguminosarium bv. viceae* 30, *Bradyrhizobium* CB756, *Sinorhizobium meliloti* RAKI and *S. arboris* Ima 14919 showed the most limited production of lumichrome in culture filtrate compared to the other strains (Table 2). It is interesting to note that *Sinorhizobium meliloti* RAKI, which was one of the strongest producers of riboflavin (Table 1), proved to be a weak producer of lumichrome (Table 2).

It is important to note that with the bacterial isolates from *Psoralea* nodules (Table 1), lumichrome release was much lower at 30°C incubation temperature relative to the standard laboratory strains (Table 2). This difference could be due to the fact that these species as well as their associated rhizobia, and the nodulation process are adapted to the lower (10–15°C) winter temperatures of the Cape when there is adequate rainfall for the symbiotic establishment. The synthesis and release of symbiotic signals are therefore likely to be optimal at such lower temperatures for mediterranean legumes. We have, in fact, shown elsewhere (Boone et al., 1999) that *Aspalathus* rhizobia from the Western Cape of South Africa produce more LCOs at 15°C than at 28°C, while their counterparts from hotter tropical environments produce more LCOs at 28°C than at 15°C. Our observation with lumichrome release is therefore consistent with the finding by Boone et al. (1999).

The amounts of lumichrome produced by the test strains in this study were within the range of those found for *S. meliloti* (Phillips et al., 1999). The concentrations of riboflavin released by individual strains and species of the rhizobia used in this study were also similar to that of a wild-type *S. meliloti* strain, but lower relative to the *S. meliloti* mutant carrying extra copies of riboflavin biosynthesis gene *rib*BA (Yang et al., 2002). Although the ecological significance of low or high production of lumichrome and riboflavin have not yet been properly assessed, the *Sinorhizobium meliloti* mutant carrying extra copies of the riboflavin biosynthesis gene *rib*BA was found to release 15% more riboflavin than the wild-type, resulting in 55% more efficient rhizobial colonization of alfalfa roots (Yang et al., 2002). The enhanced root colonization by the mutant strain was, no doubt, a first step towards better nodulation of the host plant. But whether the strain and species differences obtained here for riboflavin production by rhizobial bacteria also influence root colonization and nodule formation of their respective homologous hosts, remains to be determined.

Riboflavin and lumichrome released by soil bacteria, as shown in this study, serve many functions in the rhizosphere (Dakora et al., 2005). Both molecules elicit CO_2 production by plant roots, although riboflavin is less efficient than lumichrome in inducing root respiration (Phillips et al., 1999). So the accumulation of riboflavin in the legume's rhizosphere and its subsequent degradation into lumichrome could indirectly promote an increase in the concentration of rhizosphere CO_2, which is essential for growth of rhizobial bacteria (Lowe and Evans, 1962). Furthermore, such an increase in rhizosphere CO_2 concentration from lumichrome and riboflavin could potentially stimulate growth of vesicular-arbuscular fungi (Becard and Piche, 1989; Becard et al., 1992), and thus promote the establishment of mycorrhizal symbiosis. These indirect benefits of lumichrome and riboflavin to the legume symbiosis via their effects on root respiration of host plant are essential for enhanced N and P nutrition. Also, the mere fact that i) most root-colonizing bacteria commonly produce and release riboflavin and lumichrome (Strzetezyk and Rozycki, 1985; Phillips et al., 1999),

Table 1. Absorbances, concentrations and rates of production of lumichrome and riboflavin extracted from culture filtrates of different bacterial isolates from root nodules *of Psoralea* species. Values (Mean±S.E.) followed by dissimilar letters in a column are significantly different at P<0.05. The bacterial isolates were obtained from root nodules of *P. laxa* (L1 and L2), *P. aphylla* (AP1 and AP2), *P. asarina* (AS1 and AS2), *P. repens* (RP1 and RP2), *P. pinnata* (P1 and P2), *P. monophylla* (M1 and M2), *P. aculeata* (AC1 and AC2) and *P. restioides* (RT1 and RT2).

Host plant	Bacterial isolates	Riboflavin			Lumichrome		
		Absorbance (444 nm)	Concentration (ng ml^{-1})	Rate of release (pg ml^{-1} h^{-1})	Absorbance (249 nm)	Concentration (ng ml^{-1})	Rate of release (pg ml^{-1} h^{-1})
Psoralea laxa	L1	0.15±0.02a	17.6±1.83a	105.0±11.0a	0.89±0.19a	4.1±0.9a	25.0±5.0a
P. laxa	L2	0.15±0.03a	18.2±4.17a	108.0±25.0a	1.28±0.39a	6.0±1.8a	35.0±1.1a
P. aphylla	AP1	0.08±0.02b	9.7±2.66b	58.0±16.0b	0.67±0.11b	3.1±0.5b	19.0±3.0b
P. aphylla	AP2	0.16±0.04a	19.3±4.30a	115.0±26.0a	0.61±0.18b	2.8±0.8b	17.0±5.0b
P. asarina	AS1	0.11±0.02b	13.2±1.79b	78.0±11.0b	0.62±0.17b	2.9±0.8b	17.0±5.0b
P. asarina	AS2	0.14±0.02a	16.8±2.21a	100.0±13.0a	0.72±0.14b	3.4±0.6b	20.0±4.0b
P. repens	RP1	0.18±0.02a	22.4±2.19a	133.0±13.0a	0.67±0.09b	3.1±0.4b	19.0±2.0b
P. repens	RP2	0.23±0.01a	27.7±1.04a	165.00±6.0a	0.76±0.09b	3.5±0.4b	21.0±3.0b
P. pinnata	P1	0.13±0.01a	16.3±1.25a	97.0±7.0a	0.87±0.15a	4.0±0.7a	24.0±4.0a
P. pinnata	P2	0.17±0.03a	18.6±3.87a	111.0±23.0a	0.94±0.04a	4.4±0.2a	26.0±1.0a
P. monophylla	M1	0.17±0.02a	20.3±2.50a	121.0±15.0a	1.00±0.22a	4.7±1.0a	28.0±6.0a
P. monophylla	M2	0.11±0.03b	13.7±3.18b	81.0±19.0b	0.45±0.11b	2.1±0.5b	12.0±4.9b
P. aculeata	AC1	0.14±0.02a	17.1±1.88a	102.0±11.0a	1.37±0.08a	6.4±0.4a	38.0±2.0a
P. aculeata	AC2	0.11±0.00b	13.3±0.32b	79.0±2.0b	1.12±0.10a	5.2±0.5a	31.0±3.0a
P. restioides	RT1	0.20±0.04a	24.1±4.42a	144.0±26.0a	0.99±0.07a	6.4±1.2a	38.0±7.0a
P. restioides	RT2	0.18±0.05a	21.5±6.50a	128.0±39.0a	0.99±0.06a	4.6±0.3a	38.0±7.0a

Table 2. Absorbances, concentrations and rates of production of lumichrome and riboflavin extracted from culture filtrates of different rhizobial strains. Values (Mean±S.E.) followed by dissimilar letters in a column are significantly different at P<0.05.

Rhizobial strain	Riboflavin			Lumichrome		
	Absorbance (444 nm)	Concentration (ng ml^{-1})	Rate of release (pg ml^{-1} h^{-1})	Absorbance (249 nm)	Concentration (ng ml^{-1})	Rate of release (pg ml^{-1} h^{-1})
Rhizobium leguminosarum bv viceae 30	0.67± 0.16b	3.4±0.8b	20.0±5.0b	1.44±0.14b	23.4±2.3b	139.0±14.0b
R. leguminosarum bv viceae Cn 6	1.11± 0.07b	5.6±0.3b	33.0±2.0b	1.75±0.37a	28.3±6.0a	169.0±36.0a
Bradyrhizobium japonicum Tal 110	1.70±0.13a	8.5±0.7a	51.0±4.0a	2.25±0.11a	36.4±1.8a	217.0±11.0a
Sinorhizobium meliloti 1	0.91±0.20b	4.6±1.0b	27.0±6.0b	2.04±0.50a	33.0±8.1a	197.0±48.0a
Bradyrhizobium CB756	1.40±0.24b	7.0±1.2b	42.0±7.0b	0.90±0.33b	14.6±5.4b	87.0±32.0b
Sinorhizobium meliloti RAK1	1.64±0.13a	8.2±0.7a	49.0±4.0a	1.27±0.45b	20.6±6.7b	122.0±40.0b
Sinorhizobium fredii 6217	1.93±0.07a	9.7±0.4a	58.0±2.0a	1.50±0.24a	24.4±3.9a	145.0±23.0a
Rhizobium GHR2	1.37±0.19b	6.9±0.9b	41.0±6.0b	1.53±0.56a	24.8±9.0a	147.0±54.0a
Rhizobium NGR234	1.31±0.08b	4.6±0.4b	39.0±2.0b	1.57±0.14a	25.5±2.0a	151.0±13.0a
Sinorhizobium arboris Ima 14919	0.91±0.20b	6.6±0.5b	27.0±3.0b	0.84±0.13b	13.5±2.2b	81.0±12.0b
Bradyrhizobium japonicum WB74	1.55±0.06b	7.8±0.3b	46.0±2.0b	2.32±0.24a	37.7±4.0a	224.0±24.0a
Sinorhizobium kostiense 19227	1.43±0.29b	7.2±1.5b	43.0±9.0b	1.67±0.16a	27.1±2.6a	161.0±16.0a
Bradyrhizobium (wild type) 14b	1.19±0.19	6.0±1.0b	34.0±7.0b	1.94±0.10a	31.4±1.6a	187.0±10.0a
Mesorhizobium (wild type) xhj 7	1.40±0.06b	7.1±0.3b	42.0±2.0b	2.02±0.12a	32.8±1.9a	195.0±11.0a

ii) adding trace amounts of riboflavin to the rhizosphere increases alfalfa root-colonization by sinorhizobia (Streit et al., 1996), and iii) some bacteria even produce 8 times more extracellular riboflavin in exudates relative to internal cellular concentration (Wilson and Pardee, 1962), suggest that these molecules might have evolved directly or indirectly as rhizosphere signals influencing the outcomes of plant-bacterial interactions. Additionally, the observation that stomatal conductance and transpiration in some plant species are decreased with root application of lumichrome or infective rhizobial cells (which produce both lumichrome and riboflavin) suggests that these metabolites serve as rhizosphere signals for environmental sensing by plant roots (Dakora et al., 2005). For example, when drought occurs in soil, these bacterial metabolites can influence plant roots and cause a decrease in leaf stomatal conductance and transpiration, thus leading to water saving in the plant.

In conclusion, in this study, many symbiotic rhizobia and unauthenticated bacterial isolates from root nodules of *Psoralea* could release into their culture filtrates biologically significant concentrations of riboflavin and lumichrome, two organic molecules that have evolved as major players in the rhizosphere of plants hosting bacterial interactions.

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