Supplementary Information for

Metallophore profiling of the nitrogen-fixing genus *Frankia* spp. (Actinobacteria) towards the understanding of metal acquisition and detoxification in the rhizosphere

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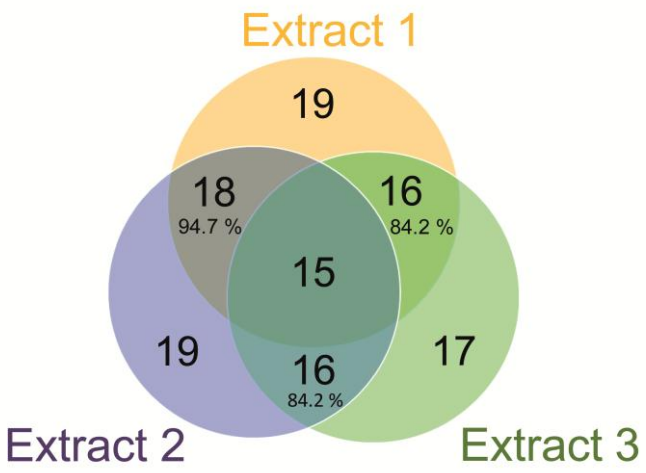
Figure S1: Venn diagram classification of UHPLC-MS *m/z* signals from three biologically independent replicates (Extract 1-3) of *Frankia* sp. strain CH37. The high recovery rate > 80% of the up to 19 identified siderophore candidates indicate the robustness of the applied methodology.



Figures S2: Change of the isotopic signature of the metallophores upon addition of (**A**) ^{56}Fe, (**B**) ^{63}Cu or (**C**) ^{66}Zn. The comparison of the mass spectra (positive ion mode) shows the decrease in the intensity of free ligand and an increase in the intensity of the respective complex upon addition of the metal. Arrows indicate the change in intensity of the molecular ion peak of the free ligand (down) and a metal complex (up) upon addition of the metal.

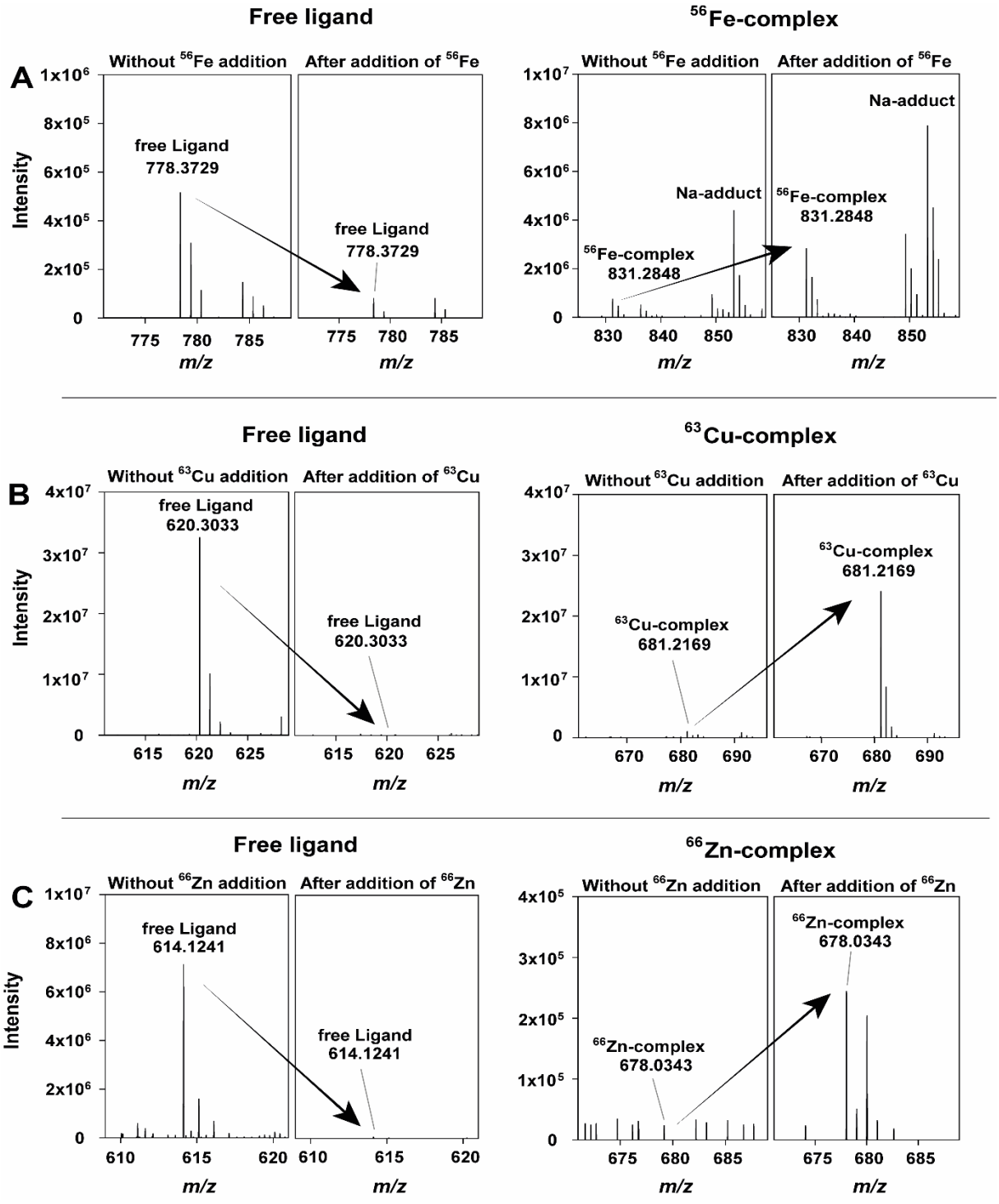


Table S1: Exclusion criteria used for formula assignment; example *m/z* = 782.3679

$[M+H]^+$ ligand without metal; *m/z*: 782.3679										
H	**C**	**O**	**N**	**S**	**DBE**	**DBE - O**	**O/N**	**Calc. Mass**	**Δm (ppm)**	**Comment**
52	33	13	9	0	13	0	1.44	782.367912	0.0153	confirmed by fragmentation
62	48	1	0	4	18.5	17.5		782.3678	-0.1278	non-integer DBE
56	41	10	3	1	16	6	3.33	782.368094	0.248	possible
60	26	10	11	3	3	-7	0.91	782.368128	0.2914	low DBE number
62	25	4	14	5	2.5	-1.5	0.29	782.367652	-0.317	non-integer DBE
58	40	4	6	3	15.5	11.5	0.67	782.367618	-0.3604	non-integer DBE
58	33	2	12	4	11.5	9.5	0.17	782.368303	0.5151	non-integer DBE
64	34	7	5	4	6	-1	1.4	782.36831	0.524	too much S
60	33	12	5	2	7	-5	2.4	782.367443	-0.5841	possible
54	32	7	12	2	12.5	5.5	0.58	782.367436	-0.5931	non-integer DBE
56	25	15	11	1	4	-11	1.36	782.367261	-0.8168	DBE – O < -10
52	26	11	15	1	9	-2	0.73	782.368597	0.8909	possible
66	40	3	2	5	9.5	6.5	1.5	782.367149	-0.9599	non-integer DBE
56	34	8	9	2	12	4	0.89	782.368779	1.1235	possible
62	35	13	2	2	6.5	-6.5	6.5	782.368786	1.1325	non-integer DBE
68	33	11	1	4	1	-10	11	782.366974	-1.1836	low DBE number
62	32	6	8	4	6.5	0.5	0.75	782.366967	-1.1925	non-integer DBE
56	31	1	15	4	12	11	0.07	782.36696	-1.2015	DBE – O > +10
58	47	6	0	2	19.5	13.5		782.366933	-1.236	non-integer DBE
58	24	9	14	3	3.5	-5.5	0.64	782.366785	-1.4252	non-integer DBE
54	39	9	6	1	16.5	7.5	1.5	782.366751	-1.4686	non-integer DBE
50	31	12	12	0	13.5	1.5	1	782.366569	-1.7012	non-integer DBE
48	34	9	13	0	18	9	0.69	782.369248	1.723	possible
54	35	14	6	0	12.5	-1.5	2.33	782.369255	1.7319	non-integer DBE
60	46	0	3	4	19	19	0*	782.366457	-1.8444	DBE – O > +10
62	39	8	2	3	10.5	2.5	4	782.366282	-2.0681#	non-integer DBE
56	38	3	9	3	16	13	0.33	782.366275	-2.077#	DBE – O > +10
58	31	11	8	2	7.5	-3.5	1.38	782.3661	-2.3007#	non-integer DBE
52	30	6	15	2	13	7	0.4*	782.366093	-2.3097#	too low O/N
54	46	11	0	0	20.5	9.5		782.366066	-2.3442#	non-integer DBE
54	23	14	14	1	4.5	-9.5	1	782.365918	-2.5333#	non-integer DBE
64	38	2	5	5	10	8	0.4*	782.365806	-2.6765#	too low O/N

*O/N ratio was too low

Δm, mass error (ppm) = [(m/z (experimental mass) – calc mass)/calc mass] × 1,000,000

$|\Delta m| > 2$ ppm

Table S2: Gradient for the metallophore separation using the UHPLC-HRMS system. Eluent A: 1 mmol L^{-1} ammonium acetate in water and 2% (v/v) acetonitrile, eluent B: 1 mmol L^{-1} ammonium acetate in acetonitrile and 10% (v/v) water.

Time [min]	**Eluent A [%]**	**Eluent B [%]**
0	100	0
0.20	100	0
8.00	0	100
9.00	0	100
9.10	100	0
10.0	100	0

Table S3: Fe-complexes were determined in the growth medium (MIM or BAP) of various *Frankia* strains using metal isotope-coded profiling. The positive (+) or negative (-) ionisation mode for determination of the metallophores is stated ([1]: MS-adducts in positive mode; [2]: MS -adducts in the negative mode were also found).

Mass of $^{56}Fe^{III}$-complex [*m/z*]	Strains	Retention time [min]	MS -polarity mode	Medium
803.2897[1]	CH37, Ea1-12	2.23	+	MIM
817.2701[1] / 815.2541	CH37	2.30	+/-	MIM
831.2848[1,2] / 829.2678	CH37, Cj1-82, BCU 110501, Arl3	2.34	+/-	MIM/BAP
831.8723	Ea1-12	2.45	+	BAP
835.2785[1] / 833.2626	CH37	2.28	+/-	MIM
846.6564	CH37	0.72	-	BAP
849.2961[1,2] / 847.2801	CH37, Ea1-12	2.49	+/-	MIM/BAP
853.2891 / 851.2733	CH37	2.36	+/-	MIM/BAP
854.6967	Cj1-82	4.18	-	MIM
863.3119[1] / 861.2961	CH37, Cj1-82, Ea1-12, DC12, BCU 110501	2.62	+/-	MIM/BAP
865.2925	CH37	1.53	-	MIM/BAP
869.2976[1]	BCU 110501	2.50	+	MIM
870.2924	CH37	2.28	+	MIM
877.0607	CcI3 (Lab. Boyer)	2.67	+	MIM/BAP
890.3297	BCU 110510	2.17	+	MIM
898.3605[1] / 896.3442	ACN14a, Cg70.4, Cg70.9, Cj1-82, CcI3 (Lab. Boyer), BCU 110501, Arl3	2.27	+/-	MIM/BAP
901.3270[1]	CH37	2.69	+	MIM
906.3259	BCU 110501	2.19	-	MIM
909.3307	CH37	2.41	+	MIM
915.3424[1,2] / 913.3277	CH37	2.61	+/-	MIM
932.1395[1]	BCU 110501	3.47	+	MIM/ BAP
933.3542[1]	CH37	2.32	+/-	MIM
947.3725[1]	CH37	2.73	+	MIM
950.1489[1]	BCU 110501	3.47	+	BAP
950.1511[1]	CcI3 (Lab. Boyer)	3.26	+	MIM
956.3180	CcI3 (Lab. Boyer)	2.35	+	MIM/BAP
958.3239[1,2] / 956.3077	CH37, ACN14a, Ea1-12, CcI3 (Lab. Boyer), CcI3 (Univ. Laval)	2.82	+/-	MIM/BAP
974.3188	ACN14a	2.5	-	MIM
996.3013	ACN14a	2.5	-	MIM
998.3575[1]	Arl3	2.25	+	BAP
999.3254	CH37	2.66	-	MIM
1002.4498	ACN14a	2.84	+	MIM
1019.3456	CH37	2.29	-	MIM
1062.4498	CH37	3.60	+	MIM
1089.2165	BCU 110501	3.78	+	MIM

Table S4: Cu-complexes were determined in the growth medium (MIM) of various *Frankia* strains using metal isotope-coded profiling. The positive (+) or negative (-) ionisation mode for determination of the metallophores is stated.

Mass of $^{63}Cu^{II}$- complex [*m/z*]	Strains	Retention time [min]	MS-polarity mode
361.0021	CH37	2.50	+
382.0817	CH37	3.47	+
401.0625	CH37, ACN14a	1.36	+
440.0984	CH37	0.98	+
444.0757	CH37	5.12	+
471.1294	CH37	0.84	+
475.0316 / 473.0154	ACN14a	2.21	+/-
497.1197	CH37	0.84	+
499.1358	CcI3 (Univ. Laval)	0.82	+
513.1539	CcI3 (Lab. Boyer)	1.44	+
519.1057	CH37	3.16	+
529.1826	CH37	2.58	+
544.1011	CH37	3.08	+
551.1046	ACN10a, ACN12a, ACN14a, CcI3 (Lab. Boyer), CH37, Cg70.4	1.18	+
560.1687 / 558.1529	CH37	3.98	+/-
563.1993 / 561.1831	CcI3 (Univ. Laval), CcI3 (Lab. Boyer)	1.93	+/-
573.0861	DSMZ 44251	1.19	+
574.1304	CH37	1.15	+
579.1010 / 577.0852	ACN14a	2.27	+/-
591.0737	CJ1-82, CcI3 (Lab. Boyer)	1.35	+
604.1234	CH37	1.36	+
604.1790	CcI3 (Lab. Boyer)	0.88	+
605.1619 / 603.1468	CH37	1.15	+/-
607.1306	CH37	2.91	+
619.1816 / 617.1656	CH37, DC12	3.28	+/-
627.1338	Cg70.9, CcI3 (Univ. Laval)	1.80	+
631.1522	CH37	1.15	+
643.0822 / 641.0662	CH37	2.17	+/-
657.1443	Ea1-12	1.86	+
659.0753	CH37	2.48	+
660.0863	CcI3 (Univ. Laval)	1.04	+
664.9652 / 662.9492	CH37	5.11	+/-
681.2169	CH37	3.53	+
689.1834 / 687.1673	CH37	3.47	+/-
693.1803 / 691.1647	ACN14a	3.04	+/-
714.1779	Cg70.4	6.11	+
716.1899	CcI3 (Univ. Laval), CH37, BCU 110501	6.04	+
719.1725	CH37	3.53	+
732.1871 / 730.1789	CPI1	5.21	+/-
824.2749	CH37	3.19	+
825.2225 / 823.2068	CH37	5.11	+/-
857.2962	CH37	3.47	+
950.3170	ACN14a	2.64	+

Table S5: Zn-complexes were determined in the growth medium (MIM) of various *Frankia* strains using metal isotope-coded profiling. The positive (+) or negative (-) ionisation mode for determination of the metallophores is stated.

Mass of $^{66}Zn^{II}$- complex [*m/z*]	**Strains**	**Retention time [min]**	**MS -Polarity**
664.1540	CcI3 (Lab. Boyer)	2.76	+
678.0343	BCU 110501	1.82	+
696.1789 / 694.1629	ACN14a	3.10	+/-
707.3463	ACN12a	4.75	+
719.1829 / 717.1669	ACN12a, CcI3 (Lab. Boyer), CH37	4.07	+/-