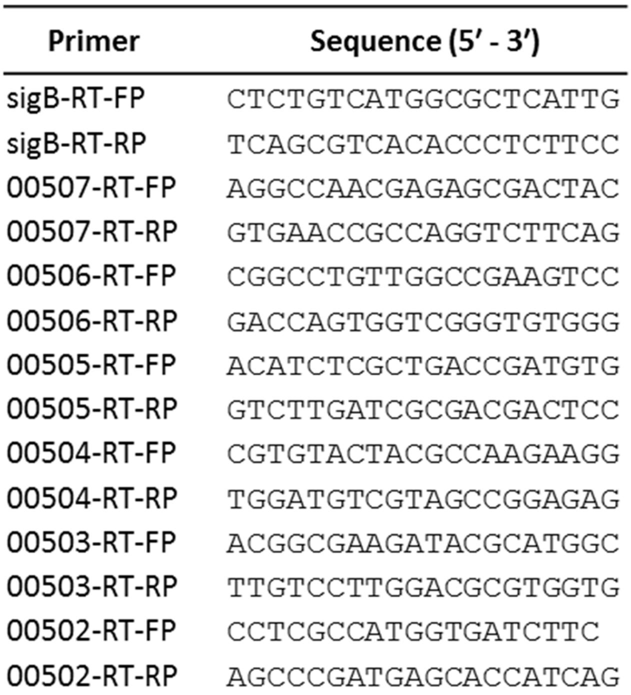
Supplementary file

Identification of a novel aminopolycarboxylic acid siderophore gene cluster encoding the biosynthesis of ethylenediaminesuccinic acid hydroxyarginine (EDHA)

Table S1

Primer list.



Primer	Sequence (5′ - 3′)
sigB-RT-FP	CTCTGTCATGGCGCTCATTG
sigB-RT-RP	TCAGCGTCACACCCTCTTCC
00507-RT-FP	AGGCCAACGAGAGCGACTAC
00507-RT-RP	GTGAACCGCCAGGTCTTCAG
00506-RT-FP	CGGCCTGTTGGCCGAAGTCC
00506-RT-RP	GACCAGTGGTCGGGTGTGGG
00505-RT-FP	ACATCTCGCTGACCGATGTG
00505-RT-RP	GTCTTGATCGCGACGACTCC
00504-RT-FP	CGTGTACTACGCCAAGAAGG
00504-RT-RP	TGGATGTCGTAGCCGGAGAG
00503-RT-FP	ACGGCGAAGATACGCATGGC
00503-RT-RP	TTGTCCTTGGACGCGTGGTG
00502-RT-FP	CCTCGCCATGGTGATCTTC
00502-RT-RP	AGCCCGATGAGCACCATCAG

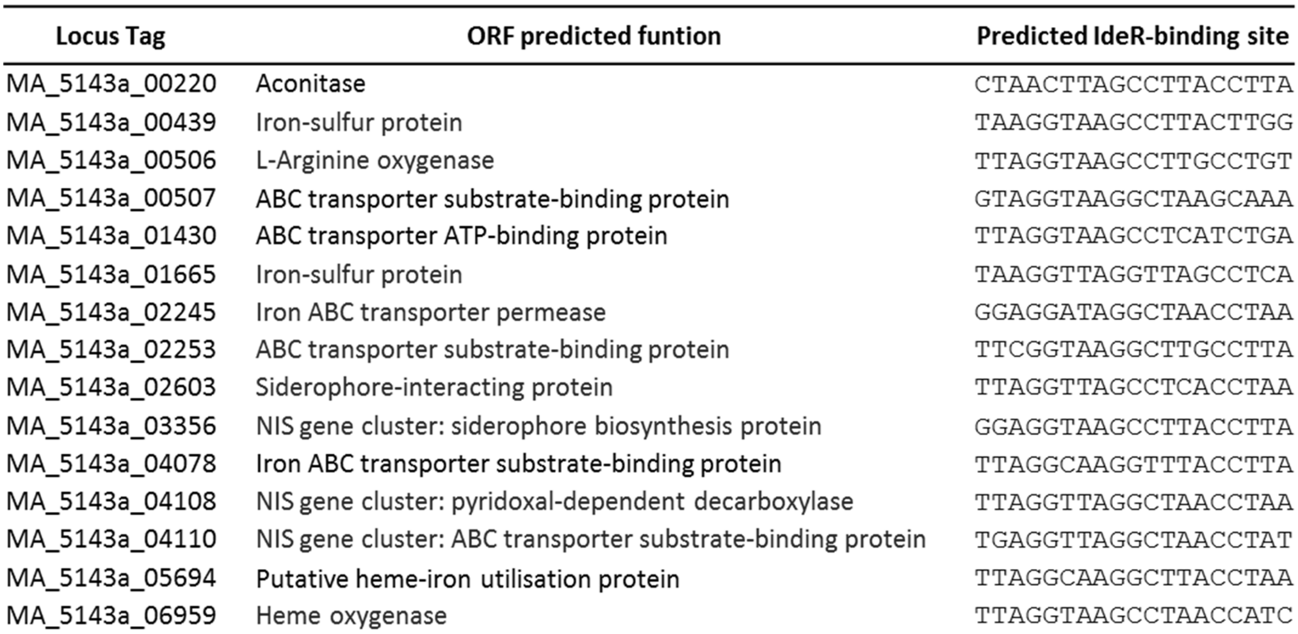
Table S2

List of 50 genes used for the multilocus sequence analysis.

Model ID	Description
Ribosomal_S8	Ribosomal protein S8
TIGR00020	peptide chain release factor 2
TIGR00060	ribosomal protein uL18
TIGR00065	cell division protein FtsZ
TIGR00092	GTP-binding protein YchF
TIGR00263	tryptophan synthase, beta subunit
TIGR00302	phosphoribosylformylglycinamidine synthase, purS protein
TIGR00343	pyridoxal 5'-phosphate synthase, synthase subunit Pdx1
TIGR00431	tRNA pseudouridine(55) synthase
TIGR00482	nicotinate (nicotinamide) nucleotide adenylyltransferase
TIGR00615	recombination protein RecR
TIGR00647	DNA-binding protein WhiA
TIGR00959	signal recognition particle protein
TIGR00962	ATP synthase F1, alpha subunit
TIGR00981	ribosomal protein uS12
TIGR01009	ribosomal protein uS3
TIGR01011	ribosomal protein uS2
TIGR01021	ribosomal protein uS5
TIGR01029	ribosomal protein uS7
TIGR01032	ribosomal protein bL20
TIGR01039	ATP synthase F1, beta subunit
TIGR01044	ribosomal protein uL22
TIGR01049	ribosomal protein uS10
TIGR01050	ribosomal protein uS19
TIGR01059	DNA gyrase, B subunit
TIGR01067	ribosomal protein uL14
TIGR01134	amidophosphoribosyltransferase
TIGR01137	cystathionine beta-synthase
TIGR01162	phosphoribosylaminoimidazole carboxylase, catalytic subunit
TIGR01164	ribosomal protein uL16
TIGR01169	ribosomal protein uL1
TIGR01171	ribosomal protein uL2
TIGR01393	elongation factor 4
TIGR01632	ribosomal protein uL11
TIGR01737	phosphoribosylformylglycinamidine synthase I
TIGR01795	chorismate mutase
TIGR01919	bifunctional HisA/TrpF protein
TIGR01966	ribonuclease PH
TIGR01978	FeS assembly ATPase SufC
TIGR01980	FeS assembly protein SufB
TIGR02012	protein RecA
TIGR02013	DNA-directed RNA polymerase, beta subunit
TIGR02075	UMP kinase
TIGR02729	Obg family GTPase CgtA
TIGR03188	phosphoribosyl-ATP diphosphatase
TIGR03631	ribosomal protein uS13
TIGR03632	ribosomal protein uS11
TIGR03687	ubiquitin-like protein Pup
TIGR03689	proteasome ATPase
TIGR03800	pyridoxal 5'-phosphate synthase, glutaminase subunit Pdx2

Table S3

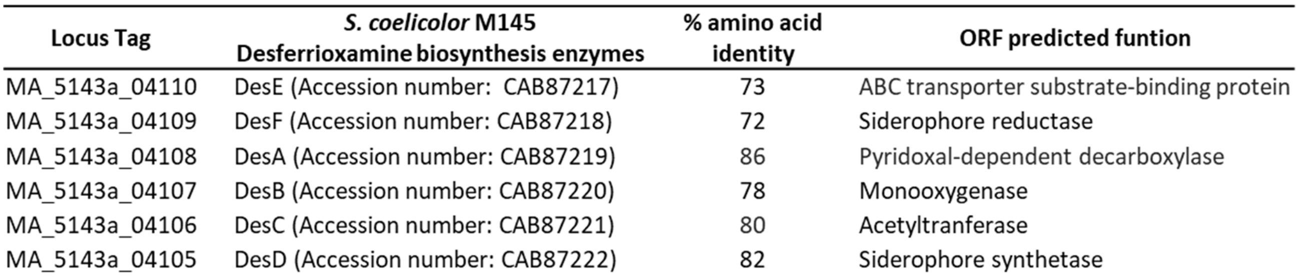
Predicted member genes of the IdeR regulon in *Streptomyces* sp. MA5143a.



Locus Tag	ORF predicted funtion	Predicted IdeR-binding site
MA_5143a_00220	Aconitase	CTAACTTAGCCTTACCTTA
MA_5143a_00439	Iron-sulfur protein	TAAGGTAAGCCTTACTTGG
MA_5143a_00506	L-Arginine oxygenase	TTAGGTAAGCCTTGCCTGT
MA_5143a_00507	ABC transporter substrate-binding protein	GTAGGTAAGGCTAAGCAAA
MA_5143a_01430	ABC transporter ATP-binding protein	TTAGGTAAGCCTCATCTGA
MA_5143a_01665	Iron-sulfur protein	TAAGGTTAGGTTAGCCTCA
MA_5143a_02245	Iron ABC transporter permease	GGAGGATAGGCTAACCTAA
MA_5143a_02253	ABC transporter substrate-binding protein	TTCGGTAAGGCTTGCCTTA
MA_5143a_02603	Siderophore-interacting protein	TTAGGTTAGCCTCACCTAA
MA_5143a_03356	NIS gene cluster: siderophore biosynthesis protein	GGAGGTAAGCCTTACCTTA
MA_5143a_04078	Iron ABC transporter substrate-binding protein	TTAGGCAAGGTTTACCTTA
MA_5143a_04108	NIS gene cluster: pyridoxal-dependent decarboxylase	TTAGGTTAGGCTAACCTAA
MA_5143a_04110	NIS gene cluster: ABC transporter substrate-binding protein	TGAGGTTAGGCTAACCTAT
MA_5143a_05694	Putative heme-iron utilisation protein	TTAGGCAAGGCTTACCTAA
MA_5143a_06959	Heme oxygenase	TTAGGTAAGCCTAACCATC

Table S4

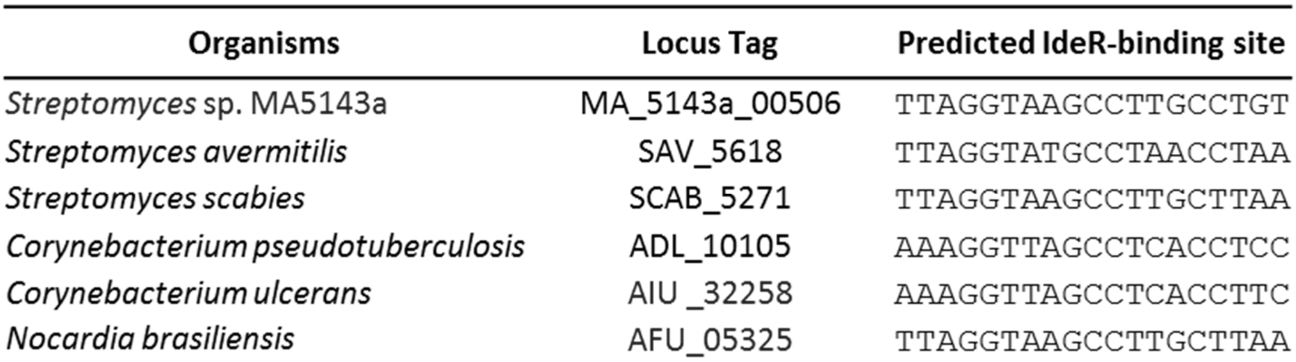
Comparison of the NIS gene cluster MA_5143a_04105 to MA_5143a_04110 and the desferrioxamine gene cluster (MiBiG entry BGC0000940) of *Streptomyces coelicolor*.



Locus Tag	***S. coelicolor* M145 Desferrioxamine biosynthesis enzymes**	**% amino acid identity**	**ORF predicted funtion**
MA_5143a_04110	DesE (Accession number: CAB87217)	73	ABC transporter substrate-binding protein
MA_5143a_04109	DesF (Accession number: CAB87218)	72	Siderophore reductase
MA_5143a_04108	DesA (Accession number: CAB87219)	86	Pyridoxal-dependent decarboxylase
MA_5143a_04107	DesB (Accession number: CAB87220)	78	Monooxygenase
MA_5143a_04106	DesC (Accession number: CAB87221)	80	Acetyltranferase
MA_5143a_04105	DesD (Accession number: CAB87222)	82	Siderophore synthetase

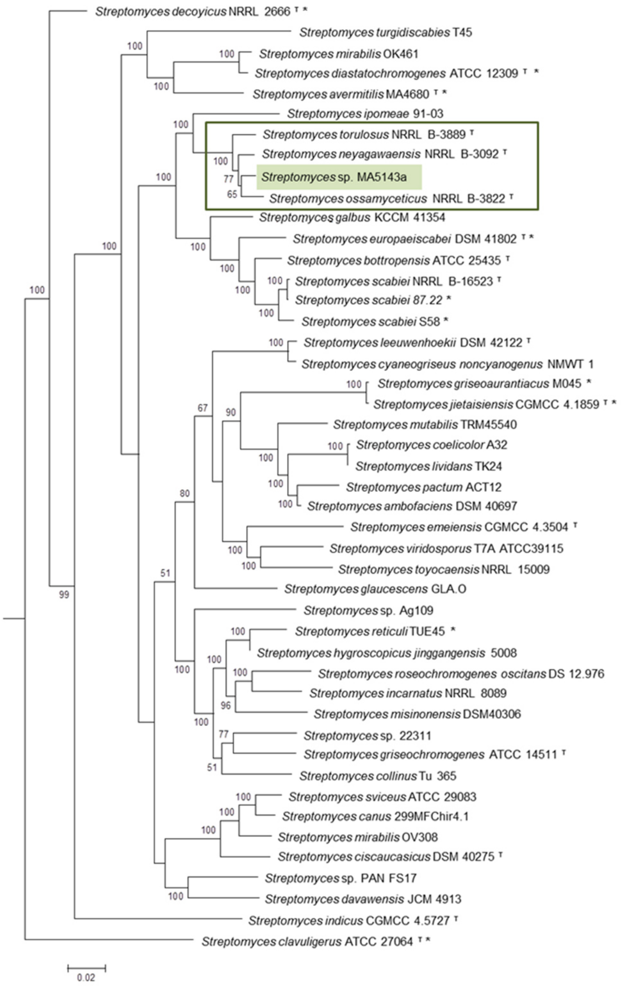
Table S4

Predicted IdeR-binding motifs upstream of *vioC*-like genes.



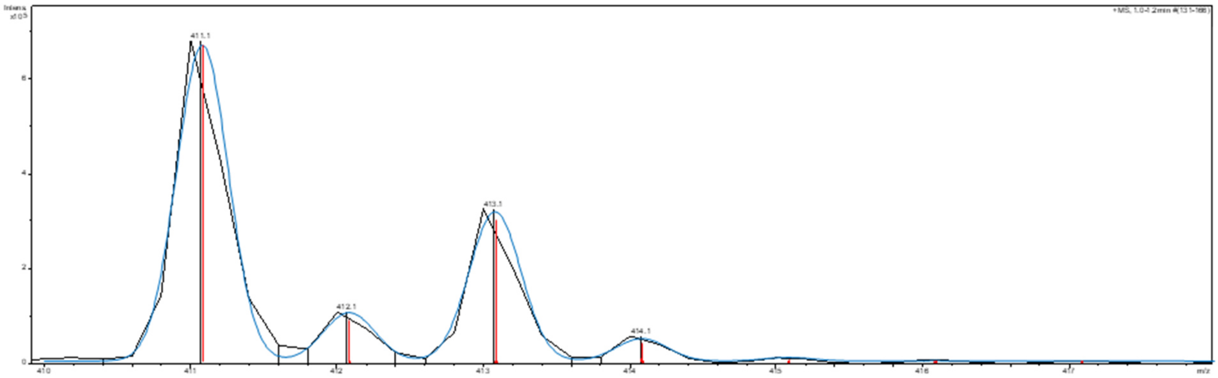
Organisms	Locus Tag	Predicted IdeR-binding site
Streptomyces sp. MA5143a	MA_5143a_00506	TTAGGTAAGCCTTGCCTGT
Streptomyces avermitilis	SAV_5618	TTAGGTATGCCTAACCTAA
Streptomyces scabies	SCAB_5271	TTAGGTAAGCCTTGCTTAA
Corynebacterium pseudotuberculosis	ADL_10105	AAAGGTTAGCCTCACCTCC
Corynebacterium ulcerans	AIU _32258	AAAGGTTAGCCTCACCTTC
Nocardia brasiliensis	AFU_05325	TTAGGTAAGCCTTGCTTAA

Figure S1



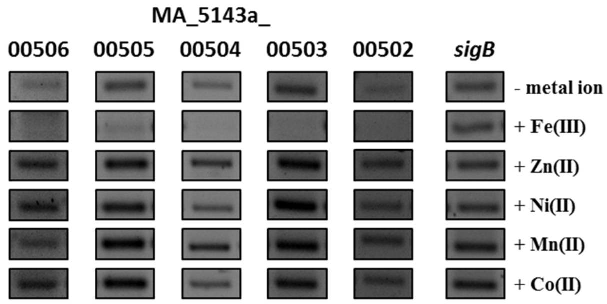
Phylogenetic tree allocating *Streptomyces* sp. MA5143a in the *Streptomyces* genera. Maximum likelihood tree based on 50 housekeeping genes showing *Streptomyces* sp. MA5143a (marked in green) and its relatives. Strains with verified EDHA cluster in their genome are marked by asterisks. Numbers at branch points indicate bootstrap percentages (based on 1000 replicates); only values >50 % are shown. Bar, 0.02 substitutions per nucleotide position; T, indicates type strains.

Figure S2



Overlay of measured isotope pattern of the putative EDHA copper adduct and calculated isotope pattern for $C_{12}H_{22}N_5O_7Cu$. The calculated (blue) and the measured (black) isotope pattern match well, confirming the presence of Cu in the complex. Due to the limited mass resolution of the ion trap used, isotope peaks arising from ^{65}Cu/$^{13}C_2$/$^{13}C^{15}N$ etc. cannot be distinguished. The isotope pattern has been calculated using the software Universal Mass Calculator 3.7.

Figure S3



Trace metal-dependent transcriptional pattern of MA_5143a_00502 – MA_5143a_00506. The gene names are indicated at the top of the gels. Cultures were grown in production media supplemented with no additional ion, 60 mg L^{-1} Fe(III)citrate or 25 µM $ZnSO_4$, $NiSO_4$, $MnSO_4$ or $CoSO_4$ and samples were taken after 72 h of incubation to isolate RNA. *sigB* was used as a housekeeping gene to normalize the cDNA.