Supplementary information:

Natural Trojan horse inhibitors of aminoacyl-tRNA synthetases

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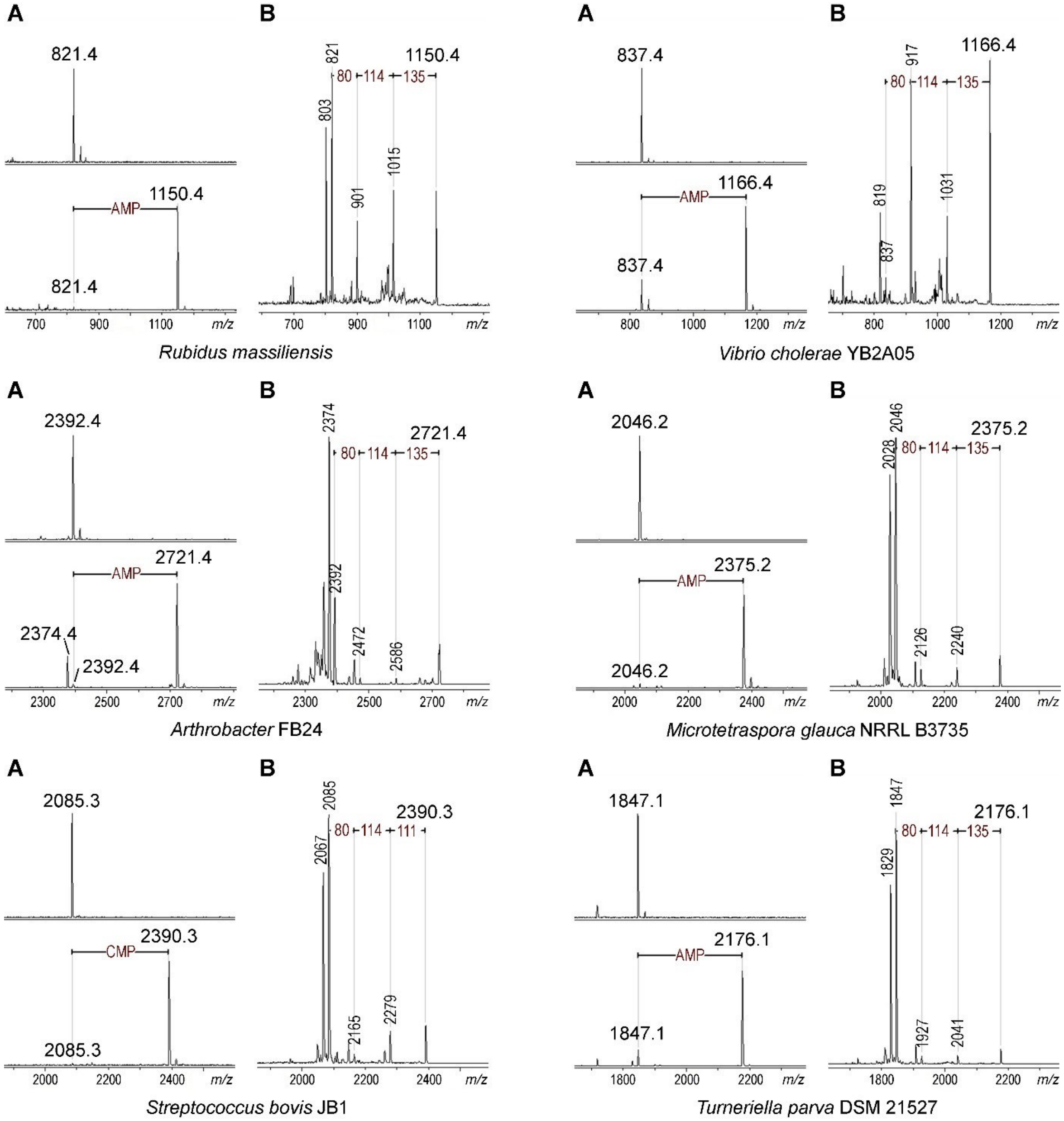

Figure S1. ***In vitro*** **biosynthesis of selected McC-like peptidyl-nucleotides.**

(A) MALDI MS spectra of the reaction products of chemically synthesized MccA peptides (sequences are listed in **Table S1)** combined with their cognate recombinant MccB homologs in the absence (top) and the presence (bottom) of NTPs. Reaction conditions were as described in [1]. **(B)** MALDI-ToF MS/MS analysis of the modified peptides. Differences in masses between the parental ions and the first daughter ions equal to 135 and 111 Da correspond to adenine and cytidine nucleobase, respectively. Mass differences in 114 and 80 Da between the daughter ions match the masses of ribose and phosphate moiety, respectively.

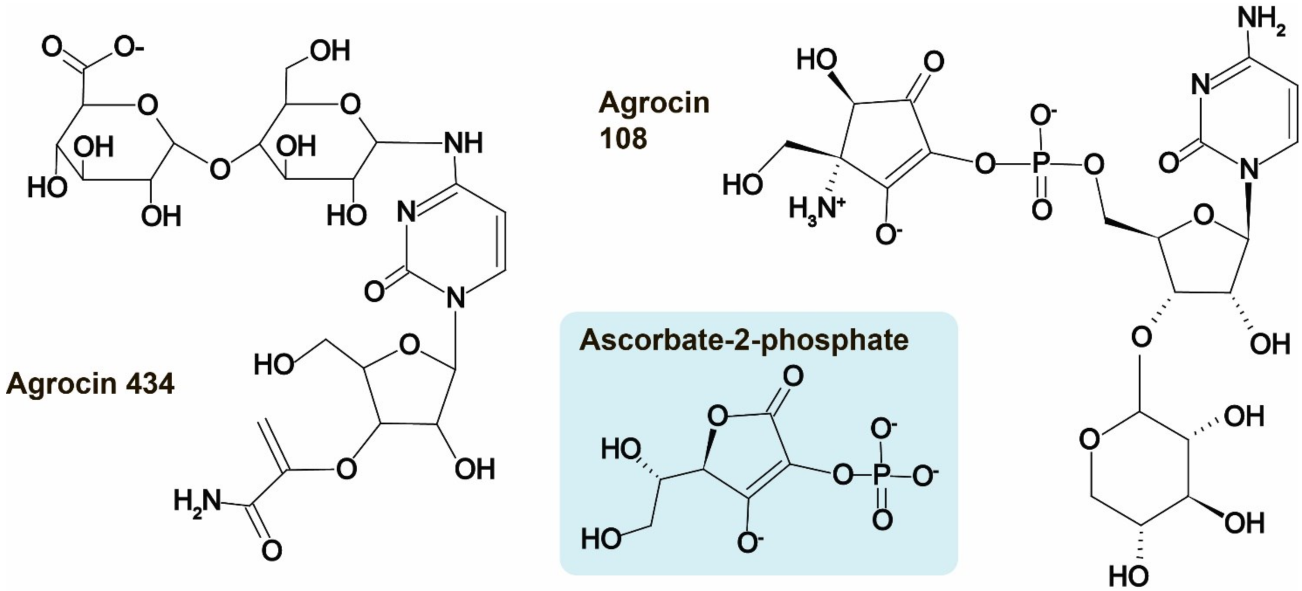

Figure S2. Chemical structures of agrocin 434 (proposed), agrocin 108 and ascorbate-2-phosphate.

Table S1. The characteristics of selected *mcc*-like BGCs

Group #	Organism	Protein ID for MccB	MccA length	MccA sequence	Reference
1	*Helicobacter pylori* HPP12 and pHPM8 plasmids	WP_001149949.1	7	MKLSYRN*	[1]
1	*Lactobacillus johnsonii* NCC 533	WP_011161263.1	7	MHRIMKN*	[1]
1	*Bartonella washoensis* Sb944nv	WP_006922866.1	7	MDHIGFN*	[1]
1	*Streptococcus thermophilus* LMD-9	WP_011227610.1	7	MKGTILN*	[1]
2	*Rubidus massiliensis*	CDZ81242.1	7	MNTITKN*	This study
3	*Escherichia coli* pMccC7 plasmid	WP_012262109.1	7	MRTGNAN*	[2]
4	*Dickeya sp.* NCPPB 569	WP_042873199.1	7	MISVSSN*	
5	*Turneriella parva* DSM 21527	WP_014804506.1	17	MKIEKTAKKITRTGGAN*	This study
6	*Bacillus subtilis* RU36A	WP_076458000.1	22	MKVKKIKKKKPIKIGFDGPV MN*	
7	*Marinomonas sp.* QM202	TBR37457.1	7	MIARLLN*	
8	*Enterobacter cloacae* NFPP41	PPL04129.1	31	MTVKVASRIKSNKVQTVTMA NLKVVVETASN*	
9	*Arthrobacter sp.* FB24	WP_156810615.1	19	MLKLKRLTKRRSKLNFVFN*	This study
10	*Microtetraspora glauca* NRRL B-3735	IH35_RS0121550	19	MLKIIKVVTAKAVSGRAFN*	This study
11	*Vibrio cholerae* YB6A06	WP_057557102.1	8	MNAFAVAN*	This study
12	*Hyalangium minutum* DSM 14724	WP_044187426.1 ; WP_157231975.1	36,46	MNDKATIEIKKDEKKAEPKK VVVVKTSIKAGPAAFN*; MNEKTAQESQKTESPKAETP AKKAVIVKTRIKAGPGGGGL VHPIAN*	[3]
13	*Synechococcus*	WP_156783136.1	56	MTQPNDRQLSNEELSDVAAG	[1][4]

	sp. CC9605			LFRRTFFKPRTSRKTLLQPKR LDKVAKNQLWADMMN*	
14	*Bacillus amyloliquefaciens* DSM 7	WP_013352374.1	19	MLKIRKVKIVRAQNGHYTN*	[5]
15	*Yersinia pseudotuberculosis* IP32953	WP_011192338.1	42	MHQSEIKLTKRLKIKRVDVN KVKEQQKKVLECGAATCGG GSN*	[1][6]
16	*Lysinibacillus sp.* YR326	WP_134026785.1	19	MLKIRKVSGVKGFSLPVFN*	
17	*Staphylococcus delphini* 14S03318-1	WP_096546674.1	19	MLEIQEVKEIEGRSAHVSN*	
18	*Nocardia vaccinii* NBRC 15922	WP_067901914.1	21	MKIVLKLKRIVRGAGPIIVSN*	
19	*Nocardiopsis sp.* JB363	WP_160049295.1	21	MRRISMTPVRKVKGSTTVVA N*	
20	*Streptococcus equinus* JB1	WP_039696479.1	19	MLKIKKVKVVAASSTRVLN*	This study

Supplementary references

[1] O. Bantysh, M. Serebryakova, K. S. Makarova, S. Dubiley, K. A. Datsenko, and K. Severinov, "Enzymatic synthesis of bioinformatically predicted microcin C-like compounds encoded by diverse bacteria.," *MBio*, vol. 5, no. 3, pp. e01059-14, May 2014.

[2] R. F. Roush, E. M. Nolan, F. Löhr, and C. T. Walsh, "Maturation of an Escherichia coli ribosomal peptide antibiotic by ATP-consuming N-P bond formation in microcin C7.," *J. Am. Chem. Soc.*, vol. 130, no. 11, pp. 3603–9, Mar. 2008.

[3] E. Yagmurov *et al.*, "Histidine-Triad Hydrolases Provide Resistance to Peptide-Nucleotide Antibiotics.," *MBio*, vol. 11, no. 2, Apr. 2020.

[4] J. Paz-Yepes, B. Brahamsha, and B. Palenik, "Role of a microcin-C-like biosynthetic gene cluster in allelopathic interactions in marine Synechococcus.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 110, no. 29, pp. 12030–5, Jul. 2013.

[5] M. Serebryakova *et al.*, "A Trojan-Horse Peptide-Carboxymethyl-Cytidine Antibiotic from Bacillus amyloliquefaciens.," *J. Am. Chem. Soc.*, vol. 138, no. 48, pp. 15690–15698, 2016.

[6] D. Tsibulskaya *et al.*, "The Product of Yersinia pseudotuberculosis mcc Operon Is a Peptide-Cytidine Antibiotic Activated Inside Producing Cells by the TldD/E Protease.," *J. Am. Chem. Soc.*, vol. 139, no. 45, pp. 16178–16187, 2017.