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Appendix I

Supplementary materials for Chapter 2

Table S1. A selection of studies examining microbial divisions of labor across different model systems. Prokaryotic examples are shown above while eukaryotic examples are shown below in gray.

* Check the section of References in this book

Species	Colony-level benefits	Cooperative cell types	Interactions between cells	References*	Evidence: Benefits of division of labor
Bacillus subtilis	Flagellum-independent migration on solid surfaces	Surfactin-producing cells Matrix-producing cells	Surfactin-producing cells lubricate cells and substrate to allow matrix-producing cells to form van Gogh bundles for colony expansion	(*121*)	Experimentally confirmed
Pseudomonas fluorescens	Mobility and population size	D-cells (Dry) M-cells (Mucoid)	D-cells push M-cells during colony spreading while M-cells produce a mucoid polymer to reduce resistance to movement	(*58*)	Experimentally confirmed
Anabaena spp.	Growth under depletion of usable nitrogen	Vegetative cells Heterocysts	Heterocysts fix nitrogen while vegetative cells fix carbon. These products are shared.	(*62*, *99*)	Experimentally confirmed in certain conditions.

Salmonella typhimurium	Cooperative virulence/Antibiotic-tolerance	Virulent cells Avirulent cells	Host colonization/antibiotic tolerance	(*242*, *243*)	Experimentally confirmed
Myxococcus xanthus	Reproduction/ Dispersal	Spore cells Peripheral rod cells Lysed cells	Stalk cells are assumed to undergo PCD, thereby potentially facilitating dispersal and/or spore survival.	(*56*, *60*)	Widely cited as a clear division of labour, but colony-level benefits remain to be quantified. Possible dispersal benefits.
Pseudomonas aeruginosa	Biofilm/Microcolony structure	Motile cells Non-motile cells	Non-motile cells form a stalk, while motile cells migrate to the top of the microcolony where they form a mushroom-like cap. Non-motile cells produce quorum-sensing signals, siderophores, surfactants and polysaccharides to support motile cells.	(*49*, *244*)	Colony-level benefits of phenotypic heterogeneity remain uncertain. Increased individual nutrient access.
Pseudomonas aeruginosa	Dispersal/Virulence/ Iron acquisition	Dispersing cells Lysed cells	Localized cell lysis and siderophore production facilitates microcolony formation and cell dispersal.	(*245*)	Colony-level benefits remain to be clarified. Possible dispersal benefits.
Streptomyces spp.	Nutrient acquisition /Dispersal	Vegetative hyphae Aerial hyphae Spores	Vegetative hyphae grow into the substrate and secrete enzymes to break down inorganic nutrients. Under nutrient depletion, these hyphae produce antibiotics and undergo PCD that are believed to provide nutrients for the subsequent growth of aerial hyphae, which differentiate into spore chains.	(*9*, *59*)	Colony-level benefits remain to be clarified. Mechanisms of PCD unclear.

Aspergillus spp.	Nutrient acquisition /Dispersal	Vegetative hyphae Aerial hyphae Conidia	Vegetative hyphae absorb nutrients from the environment and grow into the air as aerial hyphae which sporulate as conidia for dispersal.	(*246*, *247*)	Colony-level benefits remain to be quantified.
Cryptococcus gattii	Pathogenicity/ Virulence	Tubular mitochondrial morphology Non-tubular mitochondrial morphology	A sub-population of cells undergoes mitochondrial tubularisation which enhances the proliferation of remaining cells within macrophages.	(*223*)	Mechanisms confirmed, but the population benefits of increased virulence are uncertain.
Dictyostelium discoideum	Reproduction/ Dispersal	Stalk cells Spore cells	Stalk cells undergo PCD and elevate spores above the substrate.	(*92*)	Widely cited as a clear division of labour, but colony-level benefits remain to be confirmed. Possible dispersal benefits.

Appendix II

Supplementary materials for Chapter 3

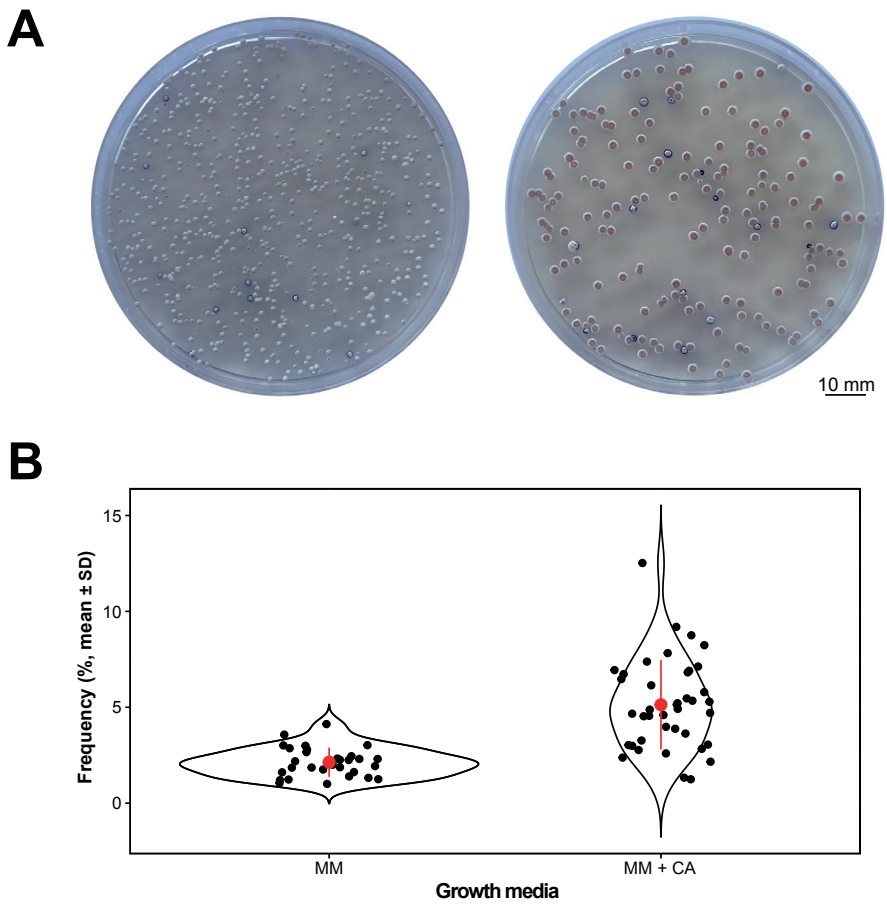

Fig. S1. Mutant frequencies in different media. Phenotypes (**A**) and mutation frequencies (**B**) of colonies grown on minimal media (MM) or minimal media supplemented with casamino acids (MM + CA). (**A**) Examples of mutant and wild-type colonies growing on minimal media (MM) (Left) or minimal media supplemented with casamino acids (MM + CA) (Right). (**B**) The frequency of mutants emerging from WT colonies on both media types.

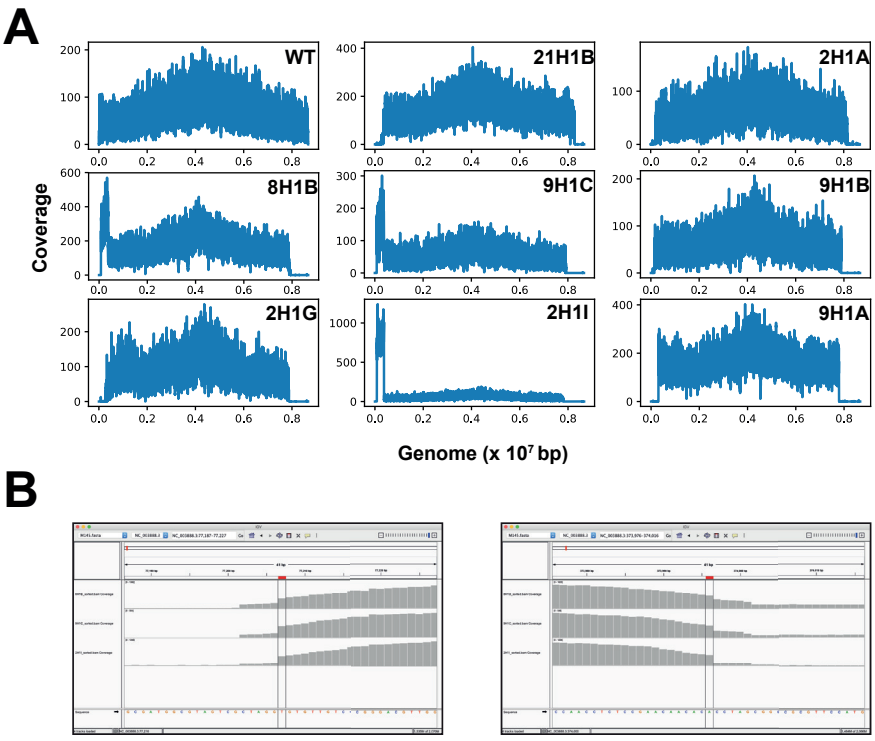

Fig. S2. PacBio sequencing results of nine selected strains. (**A**) PacBio reads mapped to the *S. coelicolor* M145 reference genome indicating coverage and highlighting breakpoints for genome deletions, where coverage declines to 0, as well as the size of the conserved amplified regions on the left chromosomal arms of strains 8H1B, 9H1C and 2H1I. (**B**) IGV snapshots of the left and right borders of amplified regions of strains 8H1B, 9H1C and 2H1I.

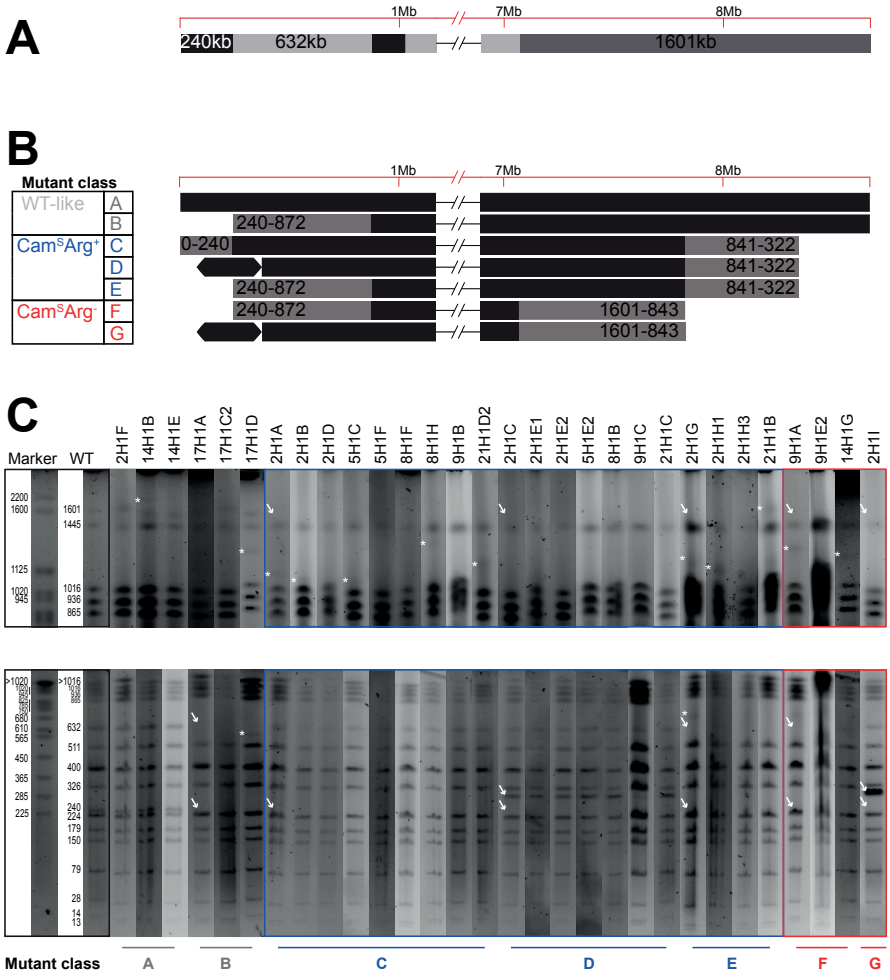

Fig. S3. PFGE results of all sampled strains. (**A**) The schematic of changes to *S. coelicolor* fragments in mutant isolates after Ase I digestion. Two bands (240 kb and 632 kb) and one 1601 kb band can be affected on the left and right arms, respectively. (**B**) Schematic adjusted from Fig. 2B with more detailed mutant classes, designated A-G. (**C**) PFGE results of 30 sampled strains. Two running conditions are used to visualize larger (top panel) or smaller (bottom panel) fragments. (Detailed running conditions are given in the Materials and methods). White arrows indicate missing or newly appearing of the bands for different mutant class. Asterisks indicate the new bands that can be used to estimate precise genome length.

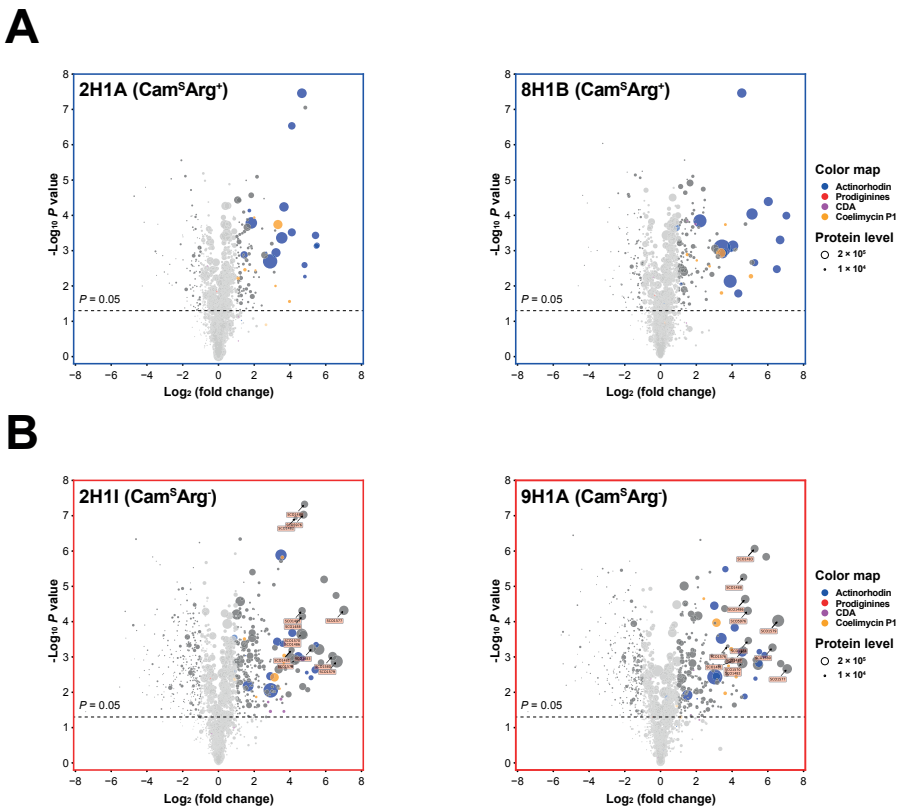

Fig. S4. Volcano plots of proteomics from four mutant strains. Volcano plots of proteomics from two Cam^{S}Arg$^+$ strains (**A**) and two Cam^{S}Arg$^-$ strains with annotated genes from arginine and pyrimidine biosynthesis pathways (**B**).

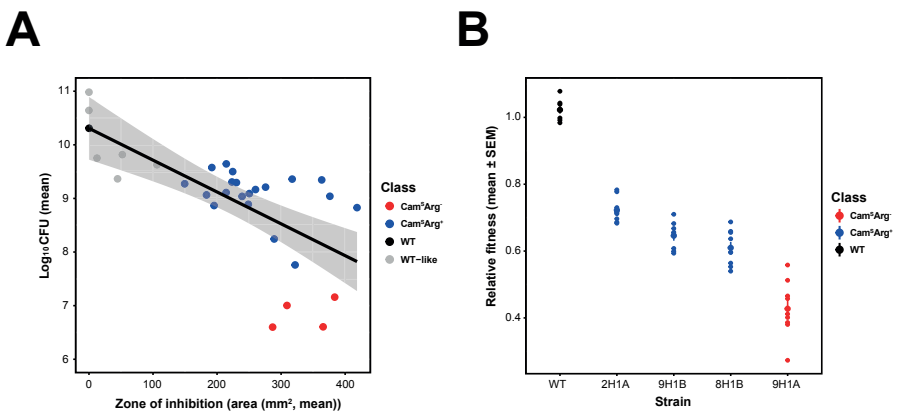

Fig. S5. Trade-off between fitness and antibiotic production. Trade-off between fitness (CFU) and antibiotic production (**A**) and relative fitness of selected strains (**B**). (**A**) The trade-off between antibiotic production and reproductive capacity, partitioned by different mutant classes. (**B**) Relative fitness of four selected strains. Detailed methods are given in the Materials and methods.

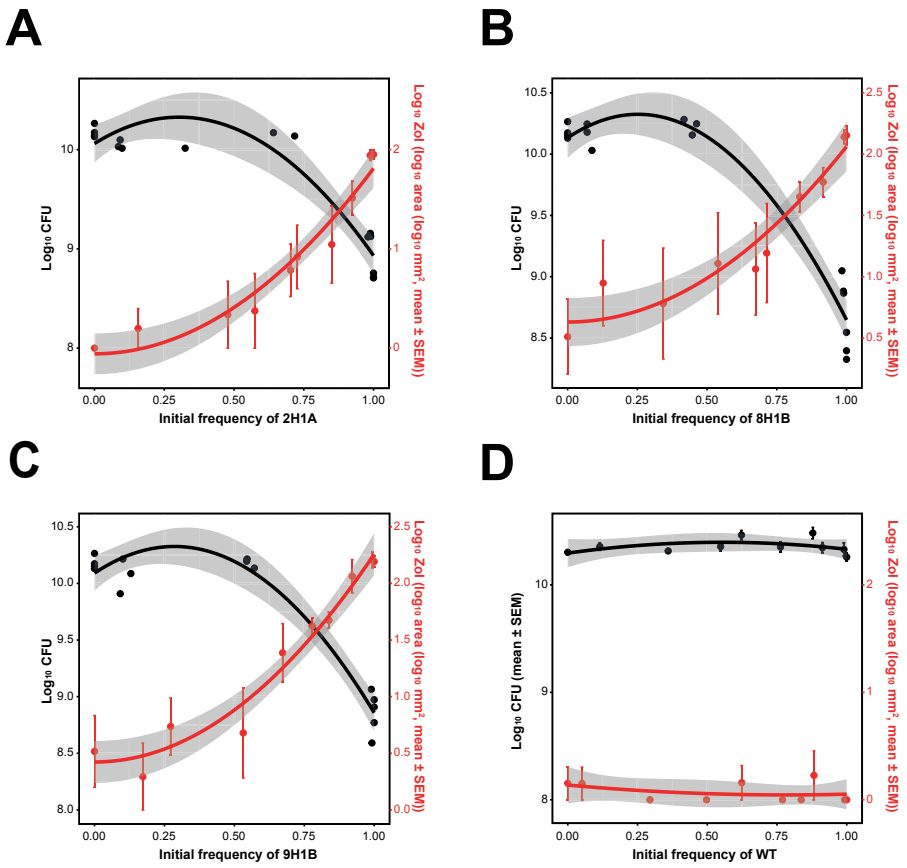

Fig. S6. Extended evidence for division of labor during co-culture of the WT and three mutant strains at different starting frequencies. Increasing frequencies of mutants cause increased antibiotic production (red) for **(A)** 2H1A ($F_{1,8}$ = 170.3, r^2 = 0.955, $P < 0.001$), **(B)** 8H1B ($F_{1,8}$ = 105.3, r^2 = 0.929, $P < 0.001$) and **(C)** 9H1B ($F_{1,8}$ = 201.1, r^2 = 0.962, $P < 0.001$) but not **(D)** WT ($F_{2,7}$ = 0.576, r^2 = 0.141, P = 0.587). Increasing frequencies of mutants only negatively impact colony fitness at frequencies > ~50% (black) for **(A)** 2H1A ($F_{2,13}$ = 59.44, r^2 = 0.901, $P < 0.001$), **(B)** 8H1B ($F_{2,13}$ = 131.7, r^2 = 0.953, $P < 0.001$) and **(C)** 9H1B ($F_{2,12}$ = 101.7, r^2 = 0.944, $P < 0.001$) but not **(D)** WT ($F_{2,7}$ = 1.076, r^2 = 0.235, P = 0.391). Quadratic regression lines include the 95% CI.

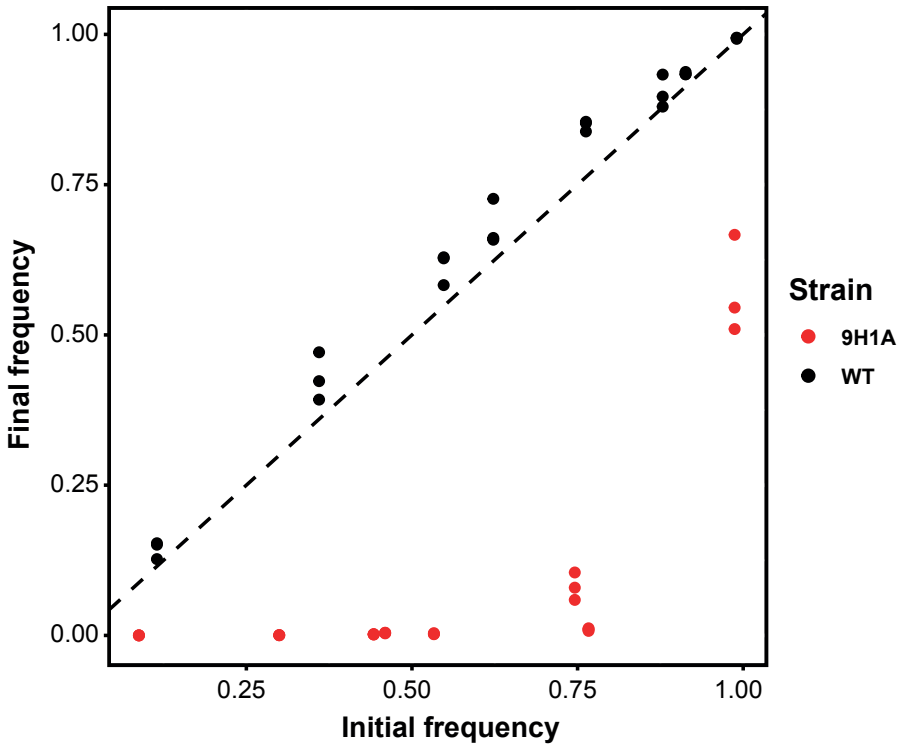

Fig. S7. Competition assays between WT and mutant strain 9H1A at different starting frequencies. Initial and final frequencies of strains during pairwise competition assays between 9H1A (red) and the wild-type or between two differentially marked wild-type strains (black). The dashed line indicates that initial and final frequencies are equal, while values below the line indicate that the competing strain has declined during the competition assay. While the differentially marked wild-type strains have equal fitness, the mutant strain has dramatically reduced fitness at every starting frequency.

Table S1. Filtered proteomics data.

This table presents the data of detected proteins from four antibiotic biosynthetic gene clusters, including CDA (SCO3210-3249), actinorhodin (SCO5071-5092), prodiginines (SCO5877-5898) and coelimycin P1 (SCO6265, 6273-6288). The data of all significantly changed proteins compared to wild type (log_2 fold change ≥ 1 or ≤ -1, *P* value < 0.05) is available online (DOI: 10.1126/sciadv.aay5781).

Protein ID	Mean TOP3 quantification of three replicates						Log_2 fold change compared to WT					-Log_{10} *P* value				
	WT	2H1A	9H1B	8H1B	2H1I	9H1A	2H1A	9H1B	8H1B	2H1I	9H1A	2H1A	9H1B	8H1B	2H1I	9H1A
SCO3222	5238	5386	18335	20320	39237	16984	0.040	1.807	1.956	2.905	1.697	0.043	2.168	3.752	1.459	1.354
SCO3224	2595	5617	7283	7083	32793	4916	1.114	1.489	1.449	3.660	0.922	0.444	3.001	0.951	1.459	2.444
SCO3226	11007	7196	8398	8292	8445	10046	-0.613	-0.390	-0.409	-0.382	-0.132	2.569	2.186	2.246	0.826	0.148
SCO3233	3590	7805	5131	5462	24663	12915	1.120	0.515	0.605	2.780	1.847	1.124	0.527	0.739	1.725	2.627
SCO3244	3010	2039	6691	8480	33941	13551	-0.562	1.153	1.494	3.495	2.171	1.344	1.463	3.840	1.806	1.220
SCO3247	8360	8755	5526	5373	15169	5741	0.067	-0.597	-0.638	0.860	-0.542	0.345	2.318	2.579	1.001	2.379
SCO5071	12725	218414	285335	259024	151877	157058	4.101	4.487	4.347	3.577	3.626	6.534	4.448	1.789	3.375	5.486
SCO5072	27430	347484	459418	453737	267041	238684	3.663	4.066	4.048	3.283	3.121	4.239	5.927	3.135	3.433	2.450
SCO5073	14613	250918	319566	340819	255534	259116	4.102	4.451	4.544	4.128	4.148	3.519	4.654	7.463	3.682	3.832
SCO5074	107232	794355	1154078	1162503	814518	878843	2.889	3.428	3.438	2.925	3.035	2.696	2.932	3.070	2.056	2.435
SCO5075	34881	324428	380959	387924	259833	280376	3.217	3.449	3.475	2.897	3.007	2.942	3.227	2.925	2.457	4.451
SCO5077	143690	513528	669521	662288	453526	406203	1.837	2.220	2.205	1.658	1.499	3.786	3.706	3.844	2.180	1.921
SCO5078	4930	212343	288395	321532	209375	223069	5.429	5.870	6.027	5.408	5.500	3.433	3.442	4.392	2.635	2.822
SCO5079	44032	512344	624487	654573	497369	462411	3.540	3.826	3.894	3.498	3.393	3.363	2.667	2.134	5.885	3.527
SCO5080	14247	361477	488741	494139	314194	338898	4.665	5.100	5.116	4.463	4.572	7.458	3.844	4.039	3.011	3.141

SCO5082	2545	6211	3425	2921	5858	4736	1.287	0.429	0.199	1.203	0.896	1.023	2.638	1.507	1.729	1.033
SCO5083	15813	52380	35585	34969	30664	17691	1.728	1.170	1.145	0.955	0.162	4.136	3.231	2.054	2.589	0.352
SCO5084	77413	209543	145087	145515	144054	100427	1.437	0.906	0.911	0.896	0.375	2.882	3.716	3.645	3.547	1.839
SCO5086	5081	142855	194851	195722	132974	133178	4.813	5.261	5.268	4.710	4.712	2.593	4.015	2.660	2.961	1.883
SCO5087	2689	119967	220260	244142	97361	123837	5.479	6.356	6.504	5.178	5.525	3.131	3.984	2.477	2.413	3.148
SCO5088	2821	128458	266321	290656	124184	158883	5.509	6.561	6.687	5.460	5.816	3.149	5.241	3.304	3.347	3.055
SCO5090	1810	51595	227659	237813	55551	71713	4.833	6.974	7.037	4.939	5.308	2.267	3.244	3.992	2.558	2.387
SCO5092	2432	15492	36268	37739	6984	10060	2.671	3.898	3.956	1.522	2.048	2.944	3.037	3.003	3.038	1.816
SCO5898	10329	9660	10899	8235	7579	7539	-0.097	0.078	-0.327	-0.447	-0.454	1.845	0.371	1.731	2.394	1.295
SCO6265	15638	43561	47953	44168	42820	29433	1.478	1.617	1.498	1.453	0.912	2.459	3.404	2.890	3.511	1.614
SCO6276	5769	23084	20376	23495	24895	30836	2.001	1.821	2.026	2.110	2.418	3.937	3.490	2.724	1.868	4.651
SCO6277	2526	23034	27136	31194	26924	47382	3.189	3.426	3.627	3.414	4.230	2.001	3.568	3.738	2.104	2.442
SCO6278	21579	46320	17870	25945	40549	47058	1.102	-0.272	0.266	0.910	1.125	2.215	0.677	0.954	2.366	1.297
SCO6279	4059	17013	21748	27153	48321	49689	2.067	2.422	2.742	3.574	3.614	2.442	3.300	2.560	5.821	1.972
SCO6281	2318	36403	65025	77063	29072	32348	3.973	4.810	5.055	3.649	3.803	1.562	3.058	2.275	2.819	2.748
SCO6282	32474	325323	299293	336899	285156	283698	3.325	3.204	3.375	3.134	3.127	3.740	3.833	2.933	2.425	3.971
SCO6283	4421	27889	32751	46914	56561	68722	2.657	2.889	3.408	3.677	3.958	0.902	1.101	1.803	3.039	3.223

Table S2. The ^{1}H NMR signals ranked by X and Y weights (w* and c) for PLS component 1. The association with the zone of inhibition (ZoI) on *B. subtilis* suggests their contribution to increased killing. cvSE represents corss-validation standard error.

			Continued			Continued		
Signal (ppm)	**w*c [1]**	**cvSE**	**Signal (ppm)**	**w*c [1]**	**cvSE**	**Signal (ppm)**	**w*c [1]**	**cvSE**
2.56	0.123	0.022	7.72	0.064	0.026	3.84	0.001	0.034
2.80	0.115	0.031	5.20	0.064	0.027	9.84	-0.002	0.024
1.56	0.110	0.021	4.00	0.064	0.035	4.76	-0.002	0.015
7.20	0.109	0.019	7.52	0.063	0.017	9.80	-0.002	0.036
7.40	0.106	0.009	6.04	0.063	0.028	8.92	-0.003	0.046
2.84	0.105	0.013	1.84	0.062	0.033	0.40	-0.004	0.030
ZoI	0.105	0.031	6.52	0.062	0.020	0.48	-0.004	0.032
7.28	0.105	0.015	1.72	0.062	0.029	9.16	-0.004	0.045
7.12	0.103	0.010	6.48	0.061	0.021	8.88	-0.004	0.046
7.24	0.103	0.019	6.08	0.061	0.034	0.52	-0.005	0.033
8.00	0.101	0.017	1.96	0.060	0.034	0.44	-0.006	0.032
7.88	0.099	0.018	7.92	0.059	0.026	5.88	-0.006	0.039
3.20	0.097	0.017	5.04	0.058	0.019	6.44	-0.007	0.031
2.60	0.095	0.018	5.80	0.058	0.018	0.36	-0.007	0.030
7.00	0.095	0.017	5.68	0.056	0.015	0.32	-0.007	0.028
3.16	0.095	0.024	0.96	0.055	0.033	0.28	-0.007	0.027
7.56	0.094	0.011	5.84	0.055	0.017	0.20	-0.007	0.025
5.48	0.093	0.024	4.20	0.055	0.023	0.24	-0.007	0.025
7.68	0.092	0.011	1.24	0.051	0.024	0.64	-0.008	0.029
7.36	0.089	0.013	8.76	0.050	0.031	4.08	-0.009	0.033
2.64	0.089	0.020	3.40	0.050	0.045	4.56	-0.010	0.026
7.32	0.088	0.022	4.60	0.050	0.024	9.28	-0.010	0.038
6.96	0.088	0.042	3.48	0.049	0.022	5.64	-0.010	0.023
6.76	0.088	0.026	2.12	0.049	0.022	9.20	-0.011	0.041
7.44	0.087	0.029	1.48	0.049	0.015	9.04	-0.011	0.039
7.60	0.086	0.018	8.12	0.049	0.012	8.96	-0.012	0.044
6.80	0.086	0.021	4.04	0.048	0.044	9.36	-0.013	0.043
6.64	0.086	0.023	7.16	0.047	0.017	9.96	-0.013	0.032

8.08	0.085	0.014	9.12	0.047	0.026	8.20	-0.014	0.035
2.52	0.085	0.023	6.88	0.047	0.034	9.72	-0.016	0.033
6.60	0.085	0.017	1.04	0.046	0.034	0.76	-0.017	0.025
2.68	0.084	0.025	0.80	0.045	0.028	0.56	-0.018	0.033
1.92	0.084	0.020	1.00	0.044	0.031	10.00	-0.018	0.017
5.44	0.083	0.016	4.32	0.044	0.018	9.68	-0.019	0.028
3.60	0.083	0.026	5.56	0.043	0.024	9.44	-0.020	0.034
1.40	0.083	0.045	3.44	0.041	0.037	9.32	-0.020	0.036
2.48	0.083	0.021	3.24	0.041	0.024	9.40	-0.022	0.034
6.72	0.083	0.025	8.44	0.041	0.038	9.48	-0.024	0.036
2.44	0.082	0.024	4.64	0.039	0.030	7.84	-0.025	0.038
3.12	0.082	0.027	2.24	0.039	0.045	9.60	-0.030	0.032
2.76	0.082	0.023	4.68	0.038	0.034	3.52	-0.032	0.037
2.88	0.082	0.026	3.76	0.037	0.040	5.24	-0.033	0.034
2.92	0.082	0.025	4.72	0.036	0.030	9.64	-0.034	0.035
2.72	0.082	0.024	8.56	0.036	0.032	9.56	-0.038	0.035
2.40	0.081	0.025	6.92	0.035	0.037	1.52	-0.042	0.041
2.96	0.081	0.024	3.28	0.035	0.023	1.64	-0.042	0.027
7.48	0.081	0.017	1.44	0.034	0.026	0.60	-0.044	0.036
1.80	0.080	0.018	3.96	0.033	0.023	0.68	-0.048	0.038
6.68	0.080	0.022	3.88	0.033	0.029	8.24	-0.049	0.022
5.52	0.080	0.025	5.00	0.031	0.047	2.28	-0.055	0.034
7.08	0.079	0.018	2.20	0.031	0.028	1.36	-0.060	0.027
3.08	0.078	0.027	9.76	0.030	0.033	4.16	-0.062	0.037
6.32	0.077	0.024	0.72	0.030	0.025	3.80	-0.063	0.029
7.04	0.076	0.017	3.72	0.024	0.031	8.28	-0.063	0.028
6.40	0.076	0.019	3.36	0.024	0.026	1.12	-0.066	0.020
7.64	0.076	0.021	8.60	0.024	0.045	8.68	-0.068	0.027
3.04	0.076	0.029	3.92	0.024	0.025	8.64	-0.071	0.021
6.20	0.075	0.028	1.28	0.022	0.047	5.28	-0.074	0.036
3.00	0.074	0.026	1.08	0.021	0.027	4.36	-0.075	0.037
6.24	0.074	0.019	8.52	0.020	0.031	2.08	-0.080	0.033
4.24	0.074	0.026	7.80	0.019	0.026	2.32	-0.085	0.035
5.72	0.074	0.024	4.40	0.018	0.037	3.64	-0.088	0.026

8.04	0.074	0.013	0.84	0.017	0.029	1.20	-0.090	0.021
8.40	0.074	0.018	7.96	0.017	0.046	8.36	-0.093	0.022
6.16	0.072	0.030	9.24	0.017	0.031	2.04	-0.097	0.026
6.12	0.072	0.032	4.52	0.016	0.017	5.16	-0.098	0.035
6.56	0.072	0.021	9.92	0.015	0.018	8.32	-0.100	0.020
6.28	0.071	0.027	3.56	0.014	0.039	8.16	-0.102	0.035
4.28	0.071	0.024	5.60	0.011	0.048	5.12	-0.103	0.034
1.76	0.070	0.030	1.68	0.009	0.027	5.96	-0.104	0.018
4.44	0.070	0.018	8.80	0.009	0.046	9.08	-0.105	0.016
6.36	0.070	0.031	8.48	0.008	0.046	1.60	-0.107	0.027
7.76	0.069	0.014	5.92	0.008	0.020	2.36	-0.108	0.031
6.84	0.069	0.023	4.48	0.006	0.025	1.16	-0.110	0.031
2.16	0.068	0.017	8.84	0.006	0.048	5.32	-0.111	0.029
6.00	0.068	0.016	2.00	0.005	0.033	4.12	-0.119	0.037
8.72	0.067	0.023	3.68	0.005	0.036	5.36	-0.120	0.022
5.40	0.066	0.026	9.52	0.005	0.042	5.08	-0.123	0.028
1.88	0.065	0.021	9.88	0.003	0.026	1.32	-0.125	0.015
5.76	0.065	0.015	9.00	0.002	0.041	0.88	-0.127	0.021
0.92	0.065	0.039	Continue on the right					
Continue on the right								

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Table S3. Mutant strains used in this study. This table shows mutant strains that were used in different experiments in this study.

Strain	Experiment							
	CFU production	Antibiotic production	^{1}H NMR profiling	PFGE	Pacbio sequencing	Proteomics	Antibiotic production (against soil isolates)	Fitness estimates (competition assay)
2H1A	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8H1B	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9H1B	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9H1A	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2H1I	Yes	Yes	Yes	Yes	Yes	Yes		
9H1C	Yes	Yes	Yes	Yes	Yes			
2H1G	Yes	Yes	Yes	Yes	Yes			
21H1B	Yes	Yes	Yes	Yes	Yes			
2H1F	Yes	Yes	Yes	Yes				
14H1B	Yes	Yes	Yes	Yes				
14H1E	Yes	Yes	Yes	Yes				
17H1A	Yes	Yes	Yes	Yes				
17H1C2	Yes	Yes	Yes	Yes				
17H1D	Yes	Yes	Yes	Yes				
2H1B	Yes	Yes	Yes	Yes				
2H1D	Yes	Yes	Yes	Yes				
5H1C	Yes	Yes	Yes	Yes				
5H1F	Yes	Yes	Yes	Yes				

8H1F	Yes	Yes	Yes	Yes
8H1H	Yes	Yes	Yes	Yes
21H1D2	Yes	Yes	Yes	Yes
2H1C	Yes	Yes	Yes	Yes
2H1E1	Yes	Yes	Yes	Yes
2H1E2	Yes	Yes	Yes	Yes
5H1E2	Yes	Yes	Yes	Yes
21H1C	Yes	Yes	Yes	Yes
2H1H1	Yes	Yes	Yes	Yes
2H1H3	Yes	Yes	Yes	Yes
9H1E2	Yes	Yes	Yes	Yes
14H1G	Yes	Yes	Yes	Yes

Appendix III

Supplementary materials for Chapter 4

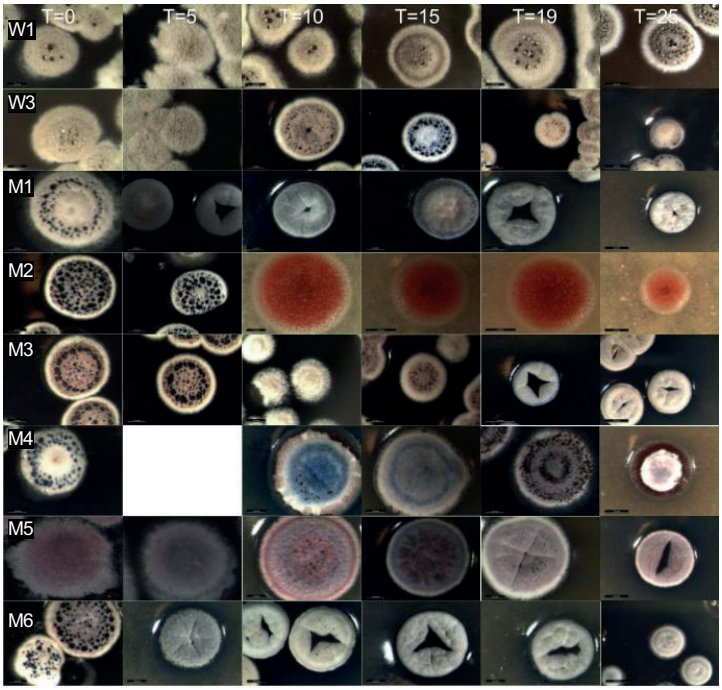

Fig. S1. Morphology of sampled strains. Morphogenesis, including aerial growth, sporulation and pigmentation, varies among and within lineages.

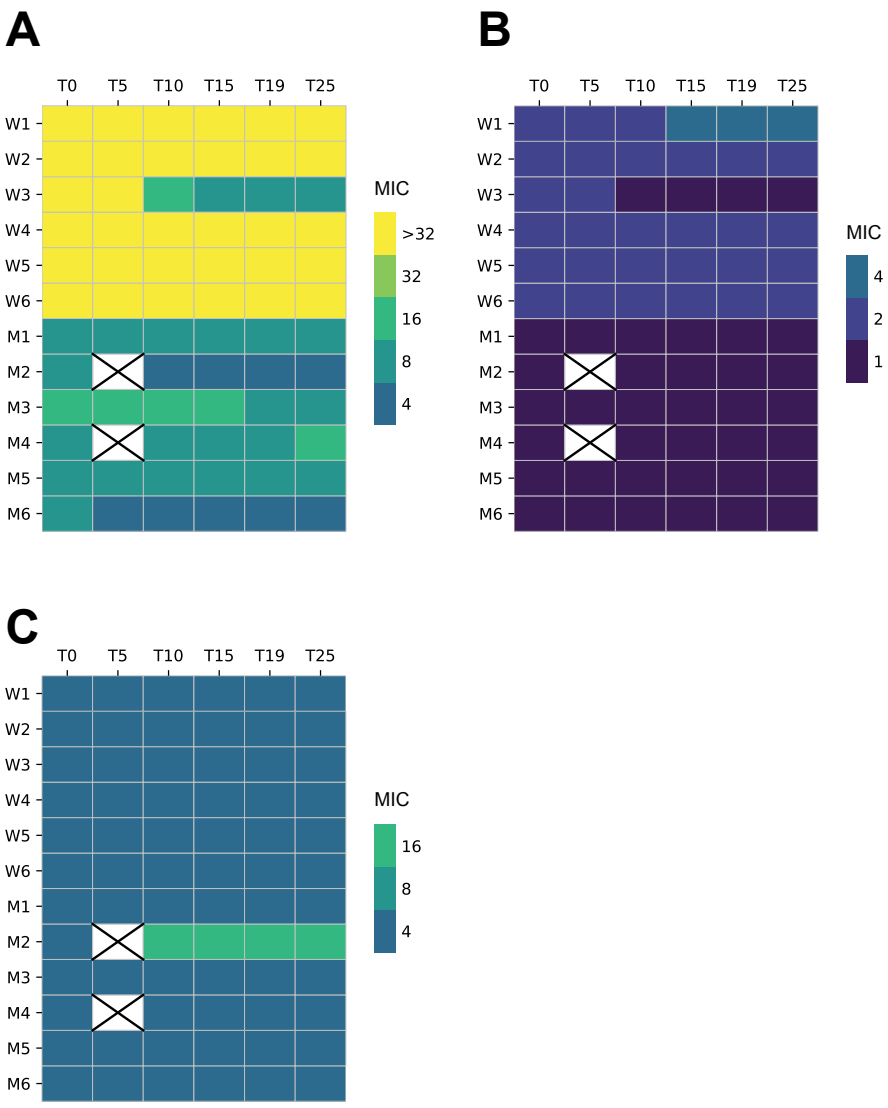

Fig. S2. Antibiotic resistance of sampled strains. MIC of (**A**) oxytetracycline (**B**) streptomycin or (**C**) ciprofloxacin. Unit is shown as µg ml^{-1}.

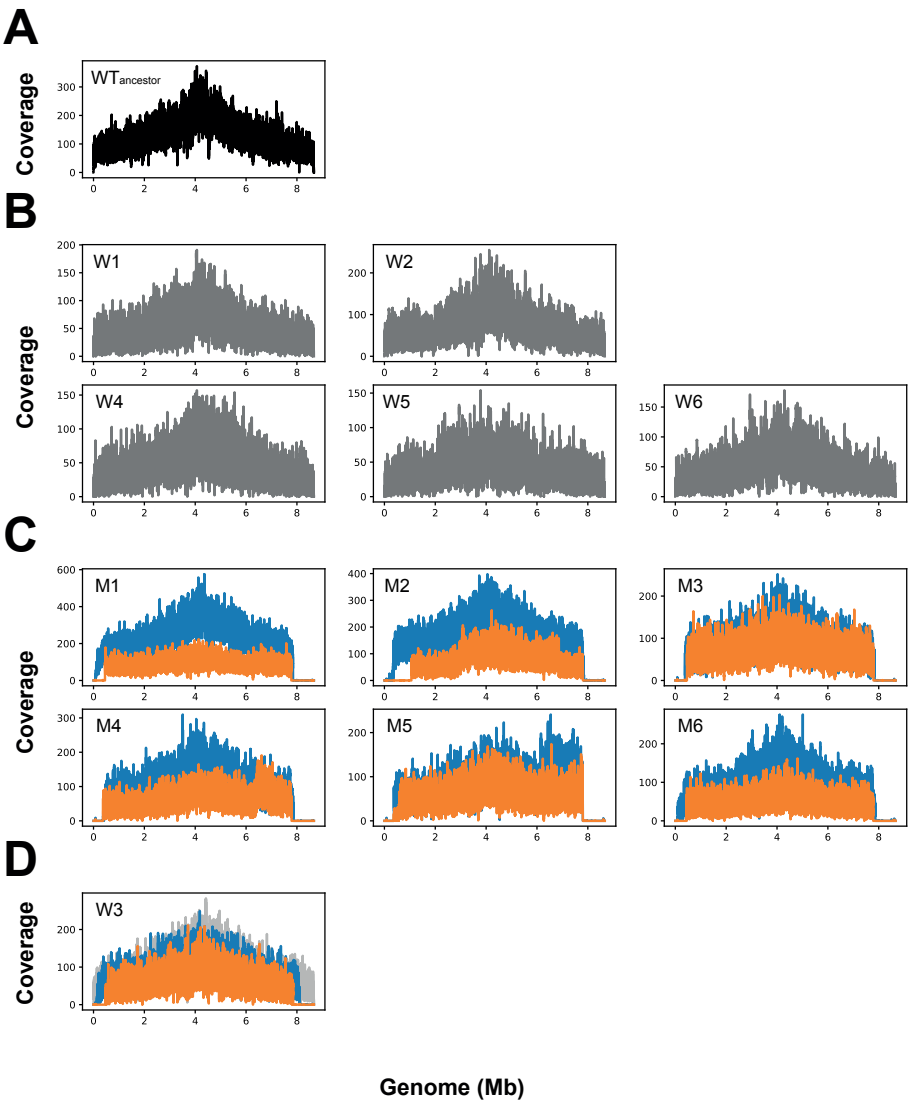

Fig. S3. Pacbio sequencing results of sampled strains. Plots indicate the coverage of reads mapped to the *S. coelicolor* M145 reference genome. (**A**) Ancestor WT. (**B**) T25 strains from WT lineages. (**C**) T0 (blue) and T25 (orange) strains from mutant lineages. (**D**) Strains of lineage W3 at T5 (light gray), T7 (blue) and T25 (orange).

Table S1. Accumulated mutations in wild-type lineages during the experiment.

Lineage	Transfer	Region	Reference	Allele	Amino acid change	Non-synonymous	Mutation type	Gene	Gene product
W1	27	221017	C	G		-	G:C>C:G		
		2208542	G	A	NP_626320.1:p.Ala91Val	Yes	G:C>A:T	SCO2060	integral membrane transport protein
		2349467	A	C	NP_626436.1:p.Glu243Ala	Yes	A:T>C:G	SCO2183	2-oxoacid dehydrogenase subunit E1
		2462887..2462888	GG	-		-	Deletion	*mpB*	
		4148255	G	C		-	G:C>C:G		
		8355042	C	T	NP_631579.1:p.Ser103Leu	Yes	G:C>A:T	SCO7535	lipoprotein
W2	27	221017	C	G		-	G:C>C:G		
		4744885	C	G	NP_628502.1:p.Asp703Glu	Yes	G:C>C:G	SCO4331	hypothetical protein
		5939426	G	C	NP_629591.1:p.Gly51Ala	Yes	G:C>C:G	SCO5454	two-component system sensor kinase
		6176177^6176178	-	G		-	Insertion		
		7313624	C	-		-	Deletion		
W3	7	2458948^2458949	-	C		-	Insertion		
W4	27	221017	C	G		-	G:C>C:G		
		3739268	G	A		-	G:C>A:T		
		7988760	A	G		-	A:T>G:C		
		8613917	C	T		No	G:C>A:T	SCO7788	hypothetical protein

W5	27	373226	G	C	NP_624691.1:p.Ser61Thr	Yes	G:C>C:G	SCO0368	transposase
		1650273	A	G		-	A:T>G:C		
		2458948^2458949	-	C		-	Insertion		
		2462887	G	-		-	Deletion	*mpB*	
		4938336	C	A	NP_628680.1:p.Arg62Leu	Yes	G:C>T:A	SCO4516	hypothetical protein
		5371295	G	T		No	G:C>T:A	SCO4935	hypothetical protein
		8424413	G	A		No	G:C>A:T	SCO7597	hypothetical protein
		8424416	G	A		No	G:C>A:T	SCO7597	hypothetical protein
		8424506	A	G		No	A:T>G:C	SCO7597	hypothetical protein
		8424513	G	-	NP_631639.1:p.Ala122fs	Yes	Deletion	SCO7597	hypothetical protein
W6	27	221017	C	G		-	G:C>C:G		
		6769943	G	C	NP_630271.1:p.Arg179Pro	Yes	G:C>C:G	SCO6167	proline rich protein membrane protein
		7700826	C	G	NP_631001.1:p.Val185Leu	Yes	G:C>C:G	SCO6935	hypothetical protein

Table S2. Accumulated mutations in mutant lineages during the experiment.

Lineage	Transfer	Region	Reference	Allele	Amino acid change	Non-synonymous	Mutation type	Gene	Gene product
M1	25	481270	G	A	NP_624779.1:p.Ala87Thr	Yes	G:C>A:T	SCO0459	hypothetical protein
M1	25	712725	C	T		No	G:C>A:T	SCO0673	hypothetical protein
M1	25	1134186	C	T		No	G:C>A:T	SCO1074	hypothetical protein
M1	25	1467045	G	A		No	G:C>A:T	SCO1388	mannose-1-phosphate guanyltransferase
M1	25	1503926	C	T	NP_625691.1:p.Val132Met	Yes	G:C>A:T	SCO1409	hypothetical protein
M1	25	1615858	C	T	NP_733536.1:p.His187Tyr	Yes	G:C>A:T	SCO1511	hypothetical protein
M1	25	1811018	G	T		No	G:C>T:A	SCO1691	TetR family transcriptional regulator
M1	25	1906862	G	T		No	G:C>T:A	*ppnK*	inorganic polyphosphate/ATP-NAD kinase
M1	25	2119778	G	A	NP_626243.1:p.Val7Ile	Yes	G:C>A:T	SCO1981	hypothetical protein
M1	25	2458948^2458949	-	C		-	Insertion		
M1	25	2489834	C	G	NP_626565.1:p.Gly74Ala	Yes	G:C>C:G	SCO2318	glycosyl transferase
M1	25	2884357	G	C	NP_626888.1:p.Ala92Pro	Yes	G:C>C:G	SCO2652	hypothetical protein
M1	25	2968251	C	A	NP_626956.1:p.Glu646*	Yes	G:C>T:A	SCO2723	ABC transporter ATP-binding protein
M1	25	3345283	C	A		No	G:C>T:A	SCO3052	UDP-glucose 6-dehydrogenase
M1	25	4040725	G	T		No	G:C>T:A	SCO3661	ATP-dependent protease ATP-binding subunit
M1	25	4127767	G	T	NP_733616.1:p.Ala71Asp	Yes	G:C>T:A	SCO3754	ABC transporter
M1	25	4148505	T	C		-	A:T>G:C		
M1	25	4532381	T	C		-	A:T>G:C		

M1	25	4732128	C	G	NP_628492.1:p.Arg849Pro	Yes	G:C>C:G	SCO4321	hypothetical protein
M1	25	5777386	C	T		No	G:C>A:T	SCO5304	sensor-like histidine kinase
M1	25	5840932	C	G		No	G:C>C:G	SCO5371	F0F1 ATP synthase subunit alpha
M1	25	5960922	C	A		-	G:C>T:A		
M1	25	6203952	C	G	NP_629822.1:p.Pro11Ala	Yes	G:C>C:G	SCO5694	1-deoxy-D-xylulose 5-phosphate reductoisomerase
M1	25	6371032	C	A		No	G:C>T:A	SCO5822	DNA topoisomerase IV subunit B
M1	25	6629123	G	C	NP_630149.1:p.Ala170Gly	Yes	G:C>C:G	SCO6038	hypothetical protein
M1	25	6960345	G	C	NP_630397.1:p.Thr489Ser	Yes	G:C>C:G	SCO6300	hydrolase
M1	25	6972333	G	C	NP_630409.1:p.Pro115Ala	Yes	G:C>C:G	SCO6312	transcriptional regulator
M1	25	7009564	G	-	NP_630440.1:p.Ala444fs	Yes	Deletion	SCO6348	hypothetical protein
M1	25	7009566	C	T	NP_630440.1:p.Val443Ile	Yes	G:C>A:T	SCO6348	hypothetical protein
M1	25	7101047	G	T		No	G:C>T:A	SCO6428	hypothetical protein
M1	25	7271648	G	A		No	G:C>A:T	SCO6568	ABC transporter
M1	25	7547616	G	C	NP_630861.1:p.Val132Leu	Yes	G:C>C:G	SCO6789	fatty oxidation protein
M1	25	7559869	G	A		No	G:C>A:T	*tdh*	L-threonine 3-dehydrogenase
M1	25	7566905	G	C	NP_630878.1:p.Ala148Gly	Yes	G:C>C:G	SCO6806	phage integrase
M1	25	7766466	C	G		No	G:C>C:G	SCO6995	protease
M1	25	7776421	G	A	NP_631068.1:p.Ser251Phe	Yes	G:C>A:T	SCO7003	hypothetical protein
M2	25	1088640	C	A		No	G:C>T:A	SCO1031	ABC transporter
M2	25	1126417	G	A		-	G:C>A:T		
M2	25	1166168	G	C	NP_625402.1:p.Glu85Gln	Yes	G:C>C:G	SCO1109	oxidoreductase
M2	25	1404674	C	T		-	G:C>A:T		

M2	25	1432260	G	A		-	G:C>A:T		
M2	25	1690052	C	A	NP_733539.1:p.Ala129Ser	Yes	G:C>T:A	*argJ*	bifunctional ornithine acetyltransferase/N-acetylglutamate synthase
M2	25	1719518	G	T		-	G:C>T:A		
M2	25	1807393	C	T		-	G:C>A:T		
M2	25	1926842	C	T	NP_626068.1:p.Val225Ile	Yes	G:C>A:T	SCO1798	ABC transporter ATP-binding protein
M2	25	2542975	G	A		-	G:C>A:T		
M2	25	2613098	C	A		-	G:C>T:A		
M2	25	2758023	C	T	NP_626796.1:p.Gly147Glu	Yes	G:C>A:T	SCO2558	hypothetical protein
M2	25	2864030	G	A	NP_626871.1:p.Val777Met	Yes	G:C>A:T	SCO2635	aminopeptidase
M2	25	2908625	G	C	NP_626907.1:p.Gly87Ala	Yes	G:C>C:G	SCO2672	hypothetical protein
M2	25	2990314	A	T		-	A:T>T:A		
M2	25	2993995	G	C		No	G:C>C:G	SCO2747	bifunctional carbohydrate binding and transport protein
M2	25	3282758	G	C		-	G:C>C:G		
M2	25	3583178	G	A		No	G:C>A:T	SCO3232	CDA peptide synthetase III
M2	25	3585292	C	T		No	G:C>A:T	SCO3234	phosphotransferase
M2	25	3796098	G	T	NP_733604.1:p.Ala456Ser	Yes	G:C>T:A	SCO3434	DNA polymerase I
M2	25	4313821	T	G		-	A:T>C:G		
M2	25	4486909	G	A		No	G:C>A:T	SCO4092	ATP-dependent helicase
M2	25	4617566	A	G	NP_628383.1:p.Val98Ala	Yes	A:T>G:C	SCO4208	integral membrane transport protein
M2	25	4811070	C	T	NP_628563.1:p.His79Tyr	Yes	G:C>A:T	SCO4394	iron repressor
M2	25	5532591	C	T	NP_629239.1:p.Thr48Ile	Yes	G:C>A:T	SCO5089	actinorhodin polyketide synthase

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M2	25	5775556	G	A		No	G:C>A:T	SCO5302	integral membrane cell-cycle protein
M2	25	6037691	C	G	NP_629674.1:p.Arg355Pro	Yes	G:C>C:G	SCO5540	hypothetical protein
M2	25	7129427	A	T		-	A:T>T:A		
M2	25	7298296	C	G		No	G:C>C:G	SCO6587	dehydrogenase
M2	25	7406743	G	C	NP_630741.1:p.Ser303Arg	Yes	G:C>C:G	SCO6666	hypothetical protein
M2	25	7634733	C	A	NP_630934.1:p.Gly316Cys	Yes	G:C>T:A	SCO6864	hypothetical protein
M2	25	7645276	T	A	NP_630946.1:p.Lys44Met	Yes	A:T>T:A	SCO6876	hypothetical protein
M2	25	7807060	G	T	NP_631085.1:p.Gly14Trp	Yes	G:C>T:A	SCO7021	hypothetical protein
M3	25	1072216..1072217	GA	TC	NP_625312.1:p.Ser110Asp	Yes	G:C>T:A	SCO1016	hypothetical protein
M3	25						A:T>C:G		
M3	25	1252185	C	T	NP_625474.1:p.Arg3Cys	Yes	G:C>A:T	SCO1184	hypothetical protein
M3	25	1344799	A	C	NP_625561.1:p.His218Pro	Yes	A:T>C:G	SCO1274	hypothetical protein
M3	25	1592949	A	C		-	A:T>C:G		
M3	25	1719817	T	G		-	A:T>C:G		
M3	25	1765203	A	G		-	A:T>G:C		
M3	25	1878761	T	G		-	A:T>C:G		
M3	25	1878763	A	G		-	A:T>G:C		
M3	25	1878792	T	C		-	A:T>G:C		
M3	25	2112369	C	T	NP_626236.1:p.Gly248Ser	Yes	G:C>A:T	SCO1973	hypothetical protein
M3	25	2686414	G	T	NP_626736.1:p.Pro407Gln	Yes	G:C>T:A	SCO2494	pyruvate phosphate dikinase
M3	25	2947470	A	T		-	A:T>T:A		
M3	25	4148246	A	C		-	A:T>C:G		

M3	25	4148252	A	C		-	A:T>C:G		
M3	25	4377204^4377205	-	G		-	Insertion		
M3	25	4430625	A	C		-	A:T>C:G		
M3	25	4663287	G	T		No	G:C>T:A	SCO4254	hypothetical protein
M3	25	4770844	A	G	NP_628526.1:p.Leu99Pro	Yes	A:T>G:C	SCO4356	hypothetical protein
M3	25	4880312	C	T		-	G:C>A:T		
M3	25	5255390	A	C		-	A:T>C:G		
M3	25	5612258	A	G		-	A:T>G:C		
M3	25	5612260	T	G		-	A:T>C:G		
M3	25	5612263	C	G		-	G:C>C:G		
M3	25	5830280	T	C		-	A:T>G:C		
M3	25	5871569	C	T		No	G:C>A:T	SCO5399	acetyl-CoA acetyltransferase
M3	25	6105201	C	A	NP_629737.1:p.Leu112Ile	Yes	G:C>T:A	SCO5603	hypothetical protein
M3	25	6113723	G	C	NP_629746.1:p.Val724Leu	Yes	G:C>C:G	SCO5612	ATP binding protein
M3	25	6598610	A	C		-	A:T>C:G		
M3	25	6935489	C	T	NP_630376.1:p.Pro328Leu	Yes	G:C>A:T	SCO6278	integral membrane transport protein
M3	25	7633600	C	G	NP_630933.1:p.Ala74Pro	Yes	G:C>C:G	SCO6863	hypothetical protein
M3	25	7777209	G	C		-	G:C>C:G		
M4	25	527567	G	A	NP_624811.1:p.Pro91Leu	Yes	G:C>A:T	SCO0494	iron-siderophore binding lipoprotein
M4	25	533965	C	T		No	G:C>A:T	SCO0501	hypothetical protein
M4	25	738352^738353	-	G		-	Insertion		
M4	25	5056054	C	G	NP_628793.1:p.Thr76Ser	Yes	G:C>C:G	SCO4632	ATP/GTP binding protein

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M4	25	5257242	G	A		-	G:C>A:T		
M4	25	6370279^6370280	-	G		-	Insertion		
M4	25	7595845	G	A	NP_630898.1:p.Ala904Val	Yes	G:C>A:T	SCO6827	polyketide synthase
M5	25	2462887	G	-		-	Deletion	*mpB*	
M5	25	2961862	G	T		No	G:C>T:A	SCO2718	hypothetical protein
M5	25	5203768	T	C	NP_628940.1:p.Asp10Gly	Yes	A:T>G:C	SCO4782	hypothetical protein
M5	25	6769937	G	C	NP_630271.1:p.Arg177Pro	Yes	G:C>C:G	SCO6167	proline rich protein membrane protein
M6	25	454451^454452	-	C		-	Insertion		
M6	25	489245	G	-	NP_624786.1:p.Arg211fs	Yes	Deletion	SCO0467	hypothetical protein
M6	25	606363	G	T	NP_624876.1:p.Arg65Ser	Yes	G:C>T:A	SCO0563	hypothetical protein
M6	25	681473	C	G	NP_624950.1:p.Glu131Gln	Yes	G:C>C:G	SCO0640	hypothetical protein
M6	25	1251111	G	C		No	G:C>C:G	SCO1183	hypothetical protein
M6	25	1562784	C	G	NP_625746.1:p.Cys246Ser	Yes	G:C>C:G	SCO1465	hypothetical protein
M6	25	1670045	G	T	NP_625836.1:p.Leu210Met	Yes	G:C>T:A	SCO1558	ABC transporter permease
M6	25	1765203^1765204	-	G		-	Insertion		
M6	25	1984265	G	A	NP_626119.1:p.Gly82Arg	Yes	G:C>A:T	SCO1851	cob(I)yrinic acid a,c-diamide adenosyltransferase
M6	25	1988841	A	C	NP_626123.1:p.Thr125Pro	Yes	A:T>C:G	SCO1856	precorrin-6Y C5,15-methyltransferase
M6	25	2295007	G	A	NP_626389.1:p.Gly376Ser	Yes	G:C>A:T	SCO2133	hypothetical protein
M6	25	2646572	A	G		No	A:T>G:C	SCO2460	hypothetical protein
M6	25	3008164	T	A		-	A:T>T:A		
M6	25	4377204^4377205	-	G		-	Insertion		
M6	25	4564696	C	G		No	G:C>C:G	SCO4148	ABC transporter ATP-binding protein

M6	25	4658248	G	T	NP_628424.1:p.Gly450Trp	Yes	G:C>T:A	SCO4250	hypothetical protein
M6	25	4880904	C	T	NP_733638.1:p.Gly24Asp	Yes	G:C>A:T	SCO4458	lipoprotein
M6	25	4940282	A	C	NP_628682.1:p.Trp194Gly	Yes	A:T>C:G	SCO4518	hypothetical protein
M6	25	6503843	G	T	NP_630050.1:p.Pro27His	Yes	G:C>T:A	SCO5933	hypothetical protein
M6	25	6597353	A	T	NP_630127.1:p.Glu104Asp	Yes	A:T>T:A	SCO6014	cationic amino acid transporter
M6	25	6826638	C	T		-	G:C>A:T		
M6	25	6994242	G	C		No	G:C>C:G	SCO6334	transcriptional regulator
M6	25	7163598	G	A		-	G:C>A:T		
M6	25	7198042	C	A	NP_630588.1:p.Asp37Glu	Yes	G:C>T:A	SCO6506	gas vesicle protein
M6	25	7237535	G	T	NP_630625.1:p.Glu108*	Yes	G:C>T:A	SCO6544	hypothetical protein
M6	25	7671689	G	A	NP_630976.1:p.Val119Met	Yes	G:C>A:T	SCO6907	DNA ligase
M6	25	7755660	C	G		No	G:C>C:G	SCO6988	oxidoreductase
M6	25	7783012	G	-	NP_631073.1:p.Val118fs	Yes	Deletion	SCO7008	ABC transporter ATP-binding protein
W3	18	466074	A	C	NP_624768.1:p.Ile29Leu	Yes	A:T>C:G	SCO0447	MarR family regulatory protein
W3	18	515777	C	G		No	G:C>C:G	SCO0492	peptide synthetase
W3	18	1467501	T	A		No	A:T>T:A	SCO1388	mannose-1-phosphate guanyltransferase
W3	18	1765203^1765204	-	G		-	Insertion		
W3	18	1792889	G	A	NP_625946.1:p.Arg682Gln	Yes	G:C>A:T	SCO1671	hypothetical protein
W3	18	2077625	G	A		-	G:C>A:T		
W3	18	2139959	G	C	NP_626262.1:p.Tyr255*	Yes	G:C>C:G	SCO2001	hypothetical protein
W3	18	3137890	G	A	NP_627111.1:p.Arg77Gln	Yes	G:C>A:T	SCO2883	cytochrome P450
W3	18	3155674	G	A		No	G:C>A:T	SCO2902	deoxyribonucleotide triphosphate pyrophosphatase

A

W3	18	3277441	G	C	NP_627227.1:p.Asp760Glu	Yes	G:C>C:G	SCO3005	preprotein translocase subunit SecA
W3	18	3560736	G	C	NP_627443.1:p.Arg-5801Pro	Yes	G:C>C:G	SCO3230	CDA peptide synthetase I
W3	18	3910675	G	A		No	G:C>A:T	SCO3541	DNA polymerase III subunit delta'
W3	18	4377204^4377205	-	G		-	Insertion		
W3	18	4700228	C	A	NP_628456.1:p.Gly106Trp	Yes	G:C>T:A	SCO4284	N-acetylglucosamine-6-phosphate deacetylase
W3	18	5611647	C	T		No	G:C>A:T	SCO5165	hydrolase
W3	18	6052711	C	T		No	G:C>A:T	SCO5553	3-isopropylmalate dehydratase large subunit
W3	18	6613228	C	G		No	G:C>C:G	SCO6026	fatty acid oxidation complex alpha-subunit
W3	18	7224671	C	G	NP_630614.1:p.Gly95Arg	Yes	G:C>C:G	SCO6533	nitrate reductase subunit delta NarJ
W3	18	7799431	A	T		-	A:T>T:A		

Appendix IV

Supplementary materials for Chapter 5

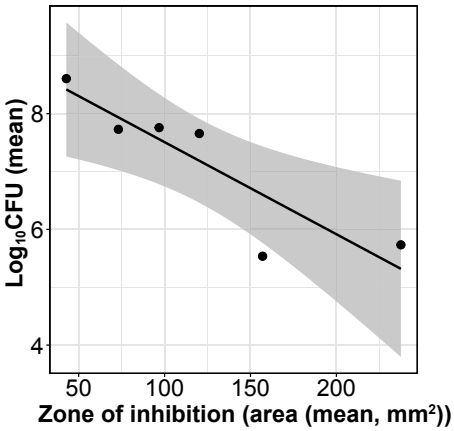

Fig. S1. CFU production and antibacterial activity is negatively correlated. The linear regression line includes the 95% confidence interval.

Strain		WT			2H1A			8H1B			9H1B			2H1I			9H1A		
	R.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
WT	1	1.00	0.99	0.99	0.86	0.87	0.85	0.77	0.76	0.75	0.77	0.76	0.76	0.65	0.64	0.64	0.62	0.60	0.61
	2	0.99	1.00	0.99	0.86	0.87	0.85	0.77	0.76	0.75	0.77	0.76	0.76	0.64	0.63	0.63	0.61	0.60	0.60
	3	0.99	0.99	1.00	0.86	0.86	0.85	0.77	0.77	0.76	0.78	0.76	0.77	0.65	0.64	0.64	0.62	0.60	0.61
2H1A	1	0.86	0.86	0.86	1.00	0.99	0.99	0.96	0.96	0.95	0.96	0.96	0.96	0.78	0.77	0.78	0.76	0.75	0.77
	2	0.87	0.87	0.86	0.99	1.00	1.00	0.96	0.94	0.94	0.95	0.95	0.95	0.76	0.75	0.77	0.74	0.73	0.74
	3	0.85	0.85	0.85	0.99	1.00	1.00	0.96	0.95	0.95	0.95	0.95	0.95	0.77	0.77	0.78	0.76	0.74	0.76
8H1B	1	0.77	0.77	0.77	0.96	0.96	0.96	1.00	0.99	0.99	0.99	0.99	0.99	0.80	0.80	0.82	0.79	0.78	0.81
	2	0.76	0.76	0.77	0.96	0.94	0.95	0.99	1.00	0.99	0.99	0.99	0.99	0.79	0.79	0.80	0.78	0.76	0.79
	3	0.75	0.75	0.76	0.95	0.94	0.95	0.99	0.99	1.00	0.99	0.99	0.99	0.79	0.79	0.81	0.78	0.77	0.80
9H1B	1	0.77	0.77	0.78	0.96	0.95	0.95	0.99	0.99	0.99	1.00	1.00	0.99	0.81	0.81	0.82	0.80	0.78	0.81
	2	0.76	0.76	0.76	0.96	0.95	0.95	0.99	0.99	0.99	1.00	1.00	1.00	0.79	0.79	0.80	0.78	0.77	0.79
	3	0.76	0.76	0.77	0.96	0.95	0.95	0.99	0.99	0.99	0.99	1.00	1.00	0.78	0.78	0.80	0.78	0.76	0.79
2H1I	1	0.65	0.64	0.65	0.78	0.76	0.77	0.80	0.79	0.79	0.81	0.79	0.78	1.00	0.99	0.99	0.96	0.96	0.97
	2	0.64	0.63	0.64	0.77	0.75	0.77	0.80	0.79	0.79	0.81	0.79	0.78	0.99	1.00	0.99	0.97	0.97	0.98
	3	0.64	0.63	0.64	0.78	0.77	0.78	0.82	0.80	0.81	0.82	0.80	0.80	0.99	0.99	1.00	0.96	0.96	0.98
9H1A	1	0.62	0.61	0.62	0.76	0.74	0.76	0.79	0.78	0.78	0.80	0.78	0.78	0.96	0.97	0.96	1.00	0.99	0.99
	2	0.60	0.60	0.60	0.75	0.73	0.74	0.78	0.76	0.77	0.78	0.77	0.76	0.96	0.97	0.96	0.99	1.00	0.99
	3	0.61	0.60	0.61	0.77	0.74	0.76	0.81	0.79	0.80	0.81	0.79	0.79	0.97	0.98	0.98	0.99	0.99	1.00

Fig. S2. Pearson's correlation coefficient between each sample. This heatmap shows reproducibility between replicates of the same strain and distinction among strains similar to what is indicated by genomic rearrangements.

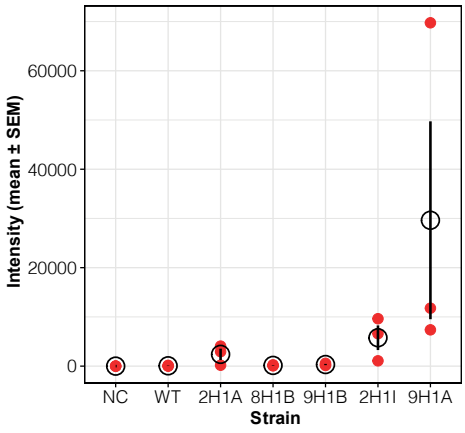

Fig. S3. Undecylprodigiosin production quantified by LC-MS/MS. No significant difference was detected among strains based one-way ANOVA followed by Tukey's tests and multiple two-sample *t* tests followed by Bonferroni correction ($P<0.05$).

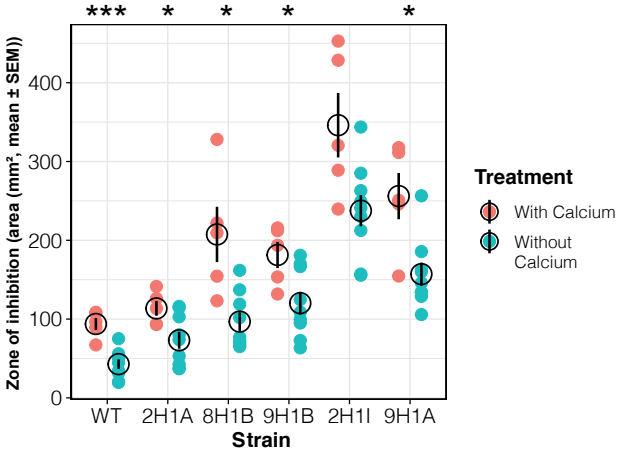

Fig. S4. The zone of inhibition against *B. subtilis* increase in most strains when overlaid media are supplemented with 20mM calcium nitrate. Asterisks indicate the statistical difference between two treatments according to Welch's *t* tests (* $P<0.05$, *** $P<0.001$).

Table S1. List of proteins with a VIP > 1.4 and a regression coefficent > 0 based on the PLS model using antibacterial activity (zone of inhibition) as a Y variable.

Locus tag	Coef.	VIP	Gene	Gene product
SCO0170	0.174	1.533		hypothetical protein
SCO7013	0.171	1.497		sugar-binding lipoprotein
SCO0158	0.171	1.562		oxidoreductase
SCO0299	0.170	1.515		oxidoreductase
SCO3671	0.166	1.423	*dnaK*	molecular chaperone DnaK
SCO0254	0.162	1.476		hypothetical protein
SCO3224	0.160	1.518		ABC transporter ATP-binding protein
SCO0335	0.159	1.521		hypothetical protein
SCO0257	0.158	1.493		hypothetical protein
SCO0259	0.158	1.468		alcohol dehydrogenase
SCO0137	0.157	1.466		sugar-transport protein
SCO0214	0.156	1.471		hypothetical protein
SCO1254	0.154	1.420	*purB*	adenylosuccinate lyase
SCO3132	0.152	1.519		trans-aconitate 2-methyltransferase
SCO4992	0.152	1.401		hypothetical protein
SCO2407	0.147	1.518		aldose 1-epimerase
SCO0203	0.146	1.417		two-component sensor
SCO0315	0.144	1.477		decarboxylase
SCO0361	0.144	1.472		hypothetical protein
SCO4071	0.139	1.553	*purC*	phosphoribosylaminoimidazole-succinocarbox-amide synthase
SCO3236	0.139	1.525		oxygenase
SCO2196	0.139	1.494		hypothetical protein
SCO4739	0.137	1.470		lipoprotein
SCO3222	0.136	1.473		hypothetical protein
SCO1366	0.136	1.497		hypothetical protein
SCO3229	0.136	1.507		4-hydroxyphenylpyruvic acid dioxygenase
SCO4472	0.134	1.442	*resA*	hypothetical protein
SCO2404	0.133	1.455		sugar-binding receptor
SCO3244	0.130	1.510		hypothetical protein
SCO1361	0.128	1.453		hypothetical protein

SCO1906	0.125	1.451		hypothetical protein
SCO1838	0.124	1.426		enoyl-CoA hydratase/isomerase
SCO0723	0.124	1.505		fructose transport system kinase
SCO1903	0.122	1.461		transport associated protein
SCO1138	0.122	1.441		hypothetical protein
SCO5285	0.122	1.475	*lon*	ATP-dependent protease
SCO4919	0.115	1.493		flavoprotein disulfide reductase
SCO2380	0.113	1.450		hypothetical protein
SCO3661	0.112	1.474	*clpB*	ATP-dependent protease ATP-binding subunit
SCO2887	0.111	1.460		hypothetical protein
SCO1530	0.110	1.422		hypothetical protein
SCO5059	0.109	1.463	*ppgK*	polyphosphate glucokinase
SCO4490	0.108	1.436		decarboxylase
SCO0462	0.107	1.447		oxidoreductase
SCO4956	0.105	1.433		methionine sulfoxide reductase A
SCO2262	0.102	1.430		oxidoreductase
SCO4754	0.101	1.428		transcriptional regulator
SCO3092	0.099	1.431		oxidoreductase
SCO6010	0.099	1.438		ABC transporter ATP-binding protein
SCO3945	0.098	1.453	*cydA*	cytochrome oxidase subunit I
SCO3890	0.092	1.426	*trxB*	thioredoxin reductase
SCO6026	0.090	1.404		fatty acid oxidation complex alpha-subunit
SCO5281	0.088	1.403		alpha-ketoglutarate decarboxylase
SCO1081	0.087	1.408		electron transfer flavoprotein subunit alpha

Table S2. List of proteins with a VIP > 1.4 and a regression coefficent < 0 based on the PLS model using antibacterial activity (zone of inhibition) as a Y variable.

Locus tag	Coef.	VIP	Gene	Gene product
SCO5040	-0.149	1.538		hypothetical protein
SCO4654	-0.144	1.501	*rpoB*	DNA-directed RNA polymerase subunit beta
SCO1388	-0.142	1.538		mannose-1-phosphate guanyltransferase
SCO4655	-0.133	1.428	*rpoC*	DNA-directed RNA polymerase subunit beta'
SCO6750	-0.128	1.413		isopentenyl-diphosphate delta-isomerase
SCO3328	-0.120	1.499	*bdtA*	hypothetical protein
SCO1234	-0.109	1.404	*ureC*	urease subunit alpha
SCO3144	-0.104	1.467		two-component system response regulator
SCO6638	-0.097	1.435		hypothetical protein
SCO3677	-0.096	1.419		purine phosphoribosyltransferase
SCO5629	-0.096	1.415		ATP /GTP-binding protein
SCO1908	-0.091	1.404		large hypothetical protein
SCO4568	-0.091	1.406	*nuoG*	NADH dehydrogenase subunit G
SCO7028	-0.090	1.408	*bxlE*	sugar-binding lipoprotein
SCO5176	-0.090	1.410		reductase
SCO2035	-0.090	1.425		hypothetical protein
SCO1141	-0.089	1.419		hypothetical protein
SCO7072	-0.081	1.400		hypothetical protein
SCO0932	-0.080	1.402		hypothetical protein

Table S3. List of proteins with a VIP > 1.4 and a regression coefficent > 0 based on the PLS model using CFU as a Y variable.

Locus tag	Coef.	VIP	Gene	Gene product
SCO5819	1.46E-3	1.423	*whiH*	sporulation transcription factor, WhiH
SCO6375	1.42E-3	1.452		hypothetical protein
SCO1090	1.42E-3	1.433	*ugpQ1*	phosphodiesterase
SCO2520	1.41E-3	1.408		hypothetical protein
SCO6154	1.40E-3	1.446		hypothetical protein
SCO0592	1.40E-3	1.447		hypothetical protein
SCO4159	1.39E-3	1.446	*glnR*	transcriptional regulator
SCO4239	1.39E-3	1.446		small membrane protein
SCO4768	1.38E-3	1.439	*bldM*	two-component regulator
SCO1163	1.38E-3	1.456		hypothetical protein
SCO4042	1.38E-3	1.436		hypothetical protein
SCO7073	1.37E-3	1.446		dihydroxyacetone kinase subunit DhaK
SCO3101	1.37E-3	1.414		lipoprotein
SCO3540	1.36E-3	1.466	*slpD*	proteinase
SCO5444	1.36E-3	1.450	*glgP*	glycogen phosphorylase
SCO5806	1.36E-3	1.401		hypothetical protein
SCO1611	1.36E-3	1.420		short chain dehydrogenase
SCO1612	1.36E-3	1.441		aldehyde dehydrogenase
SCO1590	1.35E-3	1.461		hypothetical protein
SCO1384	1.35E-3	1.443		hypothetical protein
SCO2522	1.35E-3	1.409		hypothetical protein
SCO3845	1.35E-3	1.451		protein phosphatase
SCO6482	1.34E-3	1.439		hypothetical protein
SCO4568	1.34E-3	1.408	*nuoG*	NADH dehydrogenase subunit G
SCO3326	1.34E-3	1.428		epimerase
SCO5028	1.34E-3	1.411		ATP-binding protein
SCO3721	1.34E-3	1.466		carbonic anhydrase
SCO2062	1.34E-3	1.413		hypothetical protein
SCO6437	1.34E-3	1.405		hypothetical protein
SCO5461	1.33E-3	1.429		hypothetical protein
SCO2035	1.33E-3	1.439		hypothetical protein

SCO0780	1.33E-3	1.404		zinc-binding oxidoreductase
SCO2088	1.33E-3	1.416	*murF*	UDP-N-acetylmuramoylalanyl-D-glutamyl-2, 6-diaminopimelate- D-alanyl-alanyl ligase
SCO2473	1.32E-3	1.470		nitrate reductase
SCO5315	1.32E-3	1.429	*whiE,* ORFVI	polyketide cyclase
SCO5803	1.32E-3	1.474	*lexA*	LexA repressor
SCO1639	1.32E-3	1.436	*fkpA*	peptidyl-prolyl cis-trans isomerase
SCO0591	1.32E-3	1.433		lysozyme
SCO1993	1.32E-3	1.443		hypothetical protein
SCO2390	1.32E-3	1.401	*fabF*	3-oxoacyl-ACP synthase
SCO7036	1.31E-3	1.412	*argG*	argininosuccinate synthase
SCO4141	1.31E-3	1.462	*pstC*	phosphate ABC transporter permease
SCO2822	1.31E-3	1.469		decarboxylase
SCO5466	1.31E-3	1.458		hydrolase
SCO2079	1.31E-3	1.400		hypothetical protein
SCO1507	1.31E-3	1.414		hypothetical protein
SCO2253	1.31E-3	1.404		hypothetical protein
SCO4774	1.30E-3	1.459		glycerol phosphate dehydrogenase
SCO5249	1.30E-3	1.456		nucleotide-binding protein
SCO4421	1.30E-3	1.417		TetR family transcriptional regulator
SCO2241	1.30E-3	1.459		glutamine synthetase
SCO4677	1.30E-3	1.468	*rsfA*	regulatory protein
SCO4881	1.29E-3	1.462		polysaccharide biosynthesis-like protein
SCO0597	1.29E-3	1.417		hypothetical protein
SCO2900	1.29E-3	1.421		hypothetical protein
SCO7057	1.28E-3	1.460		esterase
SCO5539	1.28E-3	1.420	*cvnB2*	hypothetical protein
SCO0654	1.28E-3	1.410	*gvpZ2*	hypothetical protein
SCO6714	1.28E-3	1.411		hydroxylase
SCO2813	1.28E-3	1.464		hypothetical protein
SCO4907	1.28E-3	1.428	*afsQ1*	transcriptional regulator
SCO5556	1.28E-3	1.418	*hupS*	histone-like DNA binding protein
SCO7028	1.28E-3	1.409	*bxlE*	sugar-binding lipoprotein
SCO0932	1.28E-3	1.447		hypothetical protein

SCO3887	1.27E-3	1.434	*parB*	partitioning or sporulation protein
SCO1473	1.27E-3	1.418	*fmt*	methionyl-tRNA formyltransferase
SCO5693	1.27E-3	1.416		acyl CoA dehydrogenase
SCO6014	1.27E-3	1.437		cationic amino acid transporter
SCO1141	1.27E-3	1.423		hypothetical protein
SCO1050	1.27E-3	1.426		DNA protection protein
SCO6218	1.27E-3	1.446		phosphatase
SCO4077	1.27E-3	1.425		phosphoribosylformylglycinamidine synthase subunit PurS
SCO5586	1.27E-3	1.453	*ffh*	signal recognition particle protein
SCO2238	1.26E-3	1.449	*nadE*	NAD(+) synthase (glutamine-hydrolysing)
SCO2958	1.26E-3	1.460		bifunctional uroporphyrinogen-III synthetase/response regulator domain-containing protein
SCO4880	1.26E-3	1.414		transferase
SCO6059	1.26E-3	1.407		hypothetical protein
SCO5583	1.26E-3	1.462	*amtB*	ammonium transporter
SCO7072	1.25E-3	1.442		hypothetical protein
SCO3652	1.25E-3	1.404		hypothetical protein
SCO6416	1.25E-3	1.416		oxidoreductase
SCO1643	1.25E-3	1.412	*pcrA*	20S proteasome alpha-subunit
SCO5668	1.25E-3	1.406		polyamine ABC transporter ATP-binding protein
SCO0726	1.25E-3	1.417		oxidoreductase
SCO1996	1.25E-3	1.409	*coaE*	dephospho-CoA kinase
SCO2021	1.25E-3	1.430		hypothetical protein
SCO2232	1.24E-3	1.456	*malR*	maltose operon transcriptional repressor
SCO6476	1.24E-3	1.444		adenylosuccinate lyase
SCO4038	1.24E-3	1.421		deaminase
SCO5533	1.24E-3	1.419		hypothetical protein
SCO1766	1.23E-3	1.447		glycohydrolase
SCO5465	1.22E-3	1.413		hypothetical protein
SCO6637	1.22E-3	1.439		hypothetical protein
SCO0509	1.22E-3	1.410	*glpK2*	glycerol kinase
SCO0648	1.22E-3	1.426		methyltransferase
SCO4675	1.22E-3	1.416		hypothetical protein
SCO4055	1.21E-3	1.406		alcohol dehydrogenase

SCO2529	1.21E-3	1.429		metalloprotease
SCO0398	1.20E-3	1.420		glycosyl transferase
SCO2140	1.19E-3	1.409		transcriptional regulator
SCO0582	1.19E-3	1.410		transcriptional regulator
SCO2385	1.19E-3	1.416		hypothetical protein
SCO1153	1.17E-3	1.424		acyl-CoA thioesterase II
SCO5585	1.17E-3	1.414	*glnD*	PII uridylyl-transferase
SCO5116	1.16E-3	1.406	*bldKE*	peptide transport system ATP-binding subunit
SCO2950	1.14E-3	1.401	*hup, hupA*	DNA-binding protein HU (hs1)

Table S4. List of proteins with a VIP > 1.4 and a regression coefficent < 0 based on the PLS model using CFU as a Y variable.

Locus tag	Coef.	VIP	Gene	Gene product
SCO2164	-1.40E-3	1.426		integral membrane efflux protein
SCO0852	-1.39E-3	1.407		aldolase
SCO2478	-1.38E-3	1.446		reductase
SCO6019	-1.38E-3	1.450		hypothetical protein
SCO6009	-1.37E-3	1.418		solute-binding protein
SCO2162	-1.37E-3	1.440		quinolinate synthetase
SCO6027	-1.36E-3	1.458		acetyl-CoA acetyltransferase
SCO5178	-1.35E-3	1.455	*moeB*	molybdopterin biosynthesis-like protein MoeZ
SCO0960	-1.34E-3	1.451		hydrolase
SCO2614	-1.34E-3	1.457	*fpgS*	folylpolyglutamate synthase
SCO2593	-1.34E-3	1.460		hypothetical protein
SCO2369	-1.33E-3	1.455		thiol-specific antioxidant protein
SCO2532	-1.33E-3	1.463		PhoH-like protein
SCO1594	-1.32E-3	1.442	*pheT*	phenylalanyl-tRNA synthetase subunit beta
SCO2013	-1.32E-3	1.444		two-component system response regulator
SCO1424	-1.31E-3	1.447		hypothetical protein
SCO3420	-1.30E-3	1.409		aldehyde dehydrogenase
SCO1296	-1.30E-3	1.433		hypothetical protein
SCO6445	-1.30E-3	1.446		inositol monophosphatase
SCO5523	-1.30E-3	1.467	*ilvE*	branched-chain amino acid aminotransferase
SCO2065	-1.29E-3	1.400		hypothetical protein
SCO6026	-1.29E-3	1.419		fatty acid oxidation complex alpha-subunit
SCO4827	-1.29E-3	1.442	*mdh*	malate dehydrogenase
SCO6148	-1.29E-3	1.445		hypothetical protein
SCO4709	-1.28E-3	1.404	*rplP*	50S ribosomal protein L16
SCO3947	-1.28E-3	1.409	*cydCD*	ABC transporter
SCO3890	-1.28E-3	1.425	*trxB*	thioredoxin reductase
SCO6740	-1.28E-3	1.437		D-amino acid oxidase
SCO1780	-1.28E-3	1.451		DNA repair protein
SCO5044	-1.28E-3	1.452	*fumB*	fumarate hydratase class I

SCO3119	-1.28E-3	1.454		hypothetical protein
SCO6102	-1.27E-3	1.404		nitrite/sulfite reductase
SCO6097	-1.27E-3	1.414	*cysN*	sulfate adenylyltransferase subunit 1
SCO4474	-1.27E-3	1.437		hypothetical protein
SCO2179	-1.27E-3	1.440		leucyl aminopeptidase
SCO1345	-1.26E-3	1.411	*fabG2*	3-ketoacyl-ACP reductase
SCO5405	-1.26E-3	1.417		transcriptional regulator
SCO4506	-1.26E-3	1.424		hypothetical protein
SCO1081	-1.26E-3	1.426		electron transfer flavoprotein subunit alpha
SCO1905	-1.26E-3	1.426		hypothetical protein
SCO2615	-1.26E-3	1.429	*valS*	valyl-tRNA synthetase
SCO1484	-1.26E-3	1.434	*pyrAA*	carbamoyl phosphate synthase small subunit
SCO1580	-1.26E-3	1.437	*argC*	N-acetyl-gamma-glutamyl-phosphate reductase
SCO5172	-1.26E-3	1.439		hydrolase
SCO2004	-1.26E-3	1.443		formate dehydrogenase
SCO1546	-1.26E-3	1.447		aminotransferase
SCO4963	-1.25E-3	1.404		ABC transporter ATP-binding protein
SCO0909	-1.25E-3	1.405		hypothetical protein
SCO2935	-1.25E-3	1.414	*scrX*	transcriptional regulator
SCO6031	-1.25E-3	1.421	*hemE*	uroporphyrinogen decarboxylase
SCO1557	-1.25E-3	1.422		lipoprotein
SCO5024	-1.25E-3	1.424		oxidoreductase
SCO0408	-1.25E-3	1.427		methyltransferase
SCO1481	-1.25E-3	1.428	*pyrF*	orotidine 5'-phosphate decarboxylase
SCO4209	-1.25E-3	1.433	*pgm*	phosphoglyceromutase
SCO6042	-1.25E-3	1.438		hypothetical protein
SCO6264	-1.25E-3	1.439		reductase
SCO4244	-1.25E-3	1.458		hypothetical protein
SCO6279	-1.24E-3	1.406		diaminobutyrate-pyruvate aminotransferase
SCO2291	-1.24E-3	1.420	*axeA*	acetylxylan esterase
SCO1482	-1.24E-3	1.425	*pyrD*	dihydroorotate dehydrogenase 2
SCO1921	-1.24E-3	1.431		aminotransferase
SCO1661	-1.24E-3	1.442		glycerol-3-phosphate dehydrogenase
SCO6099	-1.23E-3	1.401	*cysC*	adenylylsulfate kinase

SCO3945	-1.23E-3	1.407	*cydA*	cytochrome oxidase subunit I
SCO4587	-1.23E-3	1.407		hypothetical protein
SCO4683	-1.23E-3	1.422	*gdhA*	glutamate dehydrogenase
SCO6090	-1.23E-3	1.422		antibiotic resistance macrolide glycosyltrans-ferase
SCO6819	-1.23E-3	1.431	*aroA*	3-phosphoshikimate 1-carboxyvinyltransferase
SCO6091	-1.23E-3	1.438		hypothetical protein
SCO5520	-1.22E-3	1.405		delta-1-pyrroline-5-carboxylate dehydrogenase
SCO4186	-1.22E-3	1.411		hypothetical protein
SCO0769	-1.22E-3	1.419		aldo/keto reductase
SCO2913	-1.22E-3	1.424		hypothetical protein
SCO1223	-1.22E-3	1.426	*rocD*	ornithine aminotransferase
SCO1487	-1.21E-3	1.412	*pyrB*	aspartate carbamoyltransferase catalytic subunit
SCO4494	-1.21E-3	1.412		hypothetical protein
SCO2640	-1.21E-3	1.414	*asd1*	aspartate-semialdehyde dehydrogenase
SCO1222	-1.21E-3	1.420		hypothetical protein
SCO3877	-1.21E-3	1.424		6-phosphogluconate dehydrogenase
SCO1488	-1.21E-3	1.424	*pyrR*	bifunctional pyrimidine regulatory protein PyrR uracil phosphoribosyltransferase
SCO5976	-1.20E-3	1.408	*arcB*	ornithine carbamoyltransferase
SCO2634	-1.20E-3	1.412		hypothetical protein
SCO1570	-1.20E-3	1.415	*argH*	argininosuccinate lyase
SCO4253	-1.20E-3	1.415		hypothetical protein
SCO1578	-1.20E-3	1.420	*argB*	acetylglutamate kinase
SCO3345	-1.20E-3	1.426		dihydroxy-acid dehydratase
SCO1577	-1.20E-3	1.427	*argD*	acetylornithine aminotransferase
SCO1483	-1.20E-3	1.431	*pyrA*	carbamoyl phosphate synthase large subunit
SCO5389	-1.19E-3	1.403		hypothetical protein
SCO3096	-1.19E-3	1.421	*eno*	phosphopyruvate hydratase
SCO1579	-1.19E-3	1.422	*argJ*	bifunctional ornithine acetyltransferase/N-acetyl-glutamate synthase
SCO1868	-1.19E-3	1.443		hypothetical protein
SCO1523	-1.18E-3	1.413		pyridoxal biosynthesis lyase PdxS
SCO1086	-1.17E-3	1.408		hypothetical protein

SCO1486	-1.16E-3	1.406	*pyrC*	dihydroorotase
SCO3127	-1.16E-3	1.408	*ppc*	phosphoenolpyruvate carboxylase
SCO3889	-1.16E-3	1.409	*trxA*	thioredoxin
SCO5554	-1.15E-3	1.408	*leuD*	isopropylmalate isomerase small subunit
SCO1916	-1.14E-3	1.406		transferase
SCO6220	-1.12E-3	1.403		hypothetical protein

Curriculum vitae

Zheren Zhang was born on 18 August 1991 in Xinyang, China. In 2008, he started a bachelor's program in veterinary medicine at South China Agricultural University in Guangzhou, China. Soon after arriving, he participated and passed one entrance examination and interview, allowing him to be enrolled in Dingying class specializing in animal science. Since 2010, he worked as an intern in the laboratory of Prof. dr. Mei Hong, studying the functions of drug transporter proteins in animals. After spending two years on bench work, he obtained a bachelor's degree with distinction. Later, he decided to move abroad for the master's program in microbial biotechnology and health at Leiden University after receiving the Leiden University Excellence Scholarship. Upon arrival in January 2013, he started an internship under the supervision of Dr. Daniel Rozen and Prof. dr. Dennis Claessen. During the next two years, he completed two research projects where he revisited a known phenomenon in *Streptomyces coelicolor*, genomic instability. According to his preliminary results, he wrote a successful proposal to fund his doctoral studies from the China Scholarship Council. In March 2015, he initiated his Ph.D. under the supervision of Dr. Daniel Rozen, Prof. dr. Dennis Claessen and Prof. dr. Gilles van Wezel and the majority of his Ph.D. work is presented in this thesis. Zheren will soon join the laboratory of Prof. dr. Stuart West at the Department of Zoology, University of Oxford (United Kingdom).

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Zheren Zhang

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