Supplementary Information: Resuscitation-promoting factor (Rpf) terminates dormancy among diverse soil bacteria

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TABLE S1 **Experimental conditions for Rpf mutagenesis.** Upper: primer sets and sequences used for site-directed mutagenesis of the conserved glutamate residue in *rpf* from *Micrococcus* KBS0714. Capitalized bases indicate introduced mutations in the sequence. Lower: reagents for master mix used in PCR for site-directed mutagenesis.

Primers	**Sequence**
5′ Rpf E54A	cctcgccgagtgcGCGtccagcggcacc
3′ Rpf E54A	ggtgccgctggaCGCgcactcggcgagg
5′ Rpf E54Q	cctcgccgagtgcCAGtccagcggcacc
3′ Rpf E54Q	ggtgccgctggaCTGgcactcggcgagg
5′ Rpf E54K	cctcgccgagtgcAAGtccagcggcacc
3′ Rpf E54K	ggtgccgctggaCTTgcactcggcgagg

Reagent	**Volume (μL)**
H_2O	35
10X Phusion HF buffer	5
100% DMSO	4
25 mM dNTPs	1
50 mM $MgCl_2$	1
5′ Rpf mutagenesis primer, 4 μM	1
3′ Rpf mutagenesis primer, 4 μM	1
Rpf template plasmid, 15 ng/μL	1
Phusion enzyme	1
Total	**50**

TABLE S2 **Bacterial taxonomy.** We measured the growth characteristics of different soil bacteria after exposure to recombinant Rpf. More information about the enrichment, isolation, and genomics of these strains can be found elsewhere [1, 2].

Strain	**Cell type**	**Phylum**	**Family**	**Genus**
KBS0701	Gram -	Bacteroidota	Sphingobacteriaceae	*Pedobacter*
KBS0702	Gram +	Actinomycetota	Micrococcaceae	*Arthrobacter*
KBS0703	Gram +	Actinomycetota	Micrococcaceae	*Arthrobacter*
KBS0705	Gram -	Pseudomonadota	Azospirillaceae	*Azospirillum*
KBS0706	Gram +	Actinomycetota	Mycobacteriaceae	*Mycobacterium*
KBS0710	Gram -	Pseudomonadota	Pseudomonadaceae	*Pseudomonas*
KBS0711	Gram -	Pseudomonadota	Oxalobacteraceae	*Janthinobacterium*
KBS0712	Gram -	Pseudomonadota	Comamonadaceae	*Variovorax*
KBS0714	Gram +	Actinomycetota	Micrococcaceae	*Micrococcus*
KBS0715	Gram +	Actinomycetota	Microbacteriaceae	*Curtobacterium*
KBS0724	Gram +	Actinomycetota	Nocardiaceae	*Rhodococcus*
KBS0812	Gram +	Bacillota	Bacillaceae	*Bacillus*

TABLE S3 **Bacterial traits.** Functional traits of soil bacteria that were exposed to recombinant Rpf. In a previous study [1], the moisture niche was constructed for each strain by exposing cells to a soil moisture gradient and measuring rates of respiration (R). From this, we estimated the maximum respiration rate (R_{max}, µg C-CO_2 g^{-1} d^{-1}), the optimum water potential (W_{opt}, MPa), niche breadth (b, MPa), and minimum water potential (W_{min}, MPa). Separately, we measured microaerotolerance (O_2-Tol, proportion of biomass produced under low O_2 [2%] to biomass produced under high O_2 [21.5%]), maximum growth rate (μ_{max}, h^{-1}), lag time (h), biofilm production (a_{550}), and motility (mm/hr).

Strain	R_{max}	W_{opt}	b	W_{min}	**O_2-Tol**	μ_{max}	**lag**	**biofilm**	**motility**
KBS0701	59	-0.0007	0.0007	-0.0659	0.52	0.0777	5.41	0.17	0.21
KBS0702	64	-0.0464	0.0097	-0.3986	0.20	0.1151	4.26	0.11	0.42
KBS0703	70	-0.0093	0.0211	-0.3034	0.37	0.1184	4.38	0.19	0.21
KBS0705	74	-0.0990	0.0225	-0.1198	0.34	0.0318	7.01	0.21	0.25
KBS0706	46	-0.0106	0.0048	-0.0477	0.11	0.0325	18.1	1.99	0.25
KBS0710	59	-0.0010	0.0973	-0.1277	1.02	0.0830	6.26	0.53	2.75
KBS0711	64	-0.0010	0.0058	-0.0093	0.23	0.1062	6.47	1.76	1.04
KBS0712	11	-0.2420	0.4645	-1.6092	0.31	0.0526	29.2	2.64	0.17
KBS0714	108	-0.5180	0.3848	-1.5525	0.31	0.0544	7.09	1.01	0.29
KBS0715	92	-0.2560	0.0866	-0.8515	0.73	0.0742	11.14	1.20	0.33
KBS0724	36	-1.0700	1.1345	-2.3469	0.44	0.0182	1.95	3.06	2.00
KBS0812	59	-0.0007	0.0007	-0.0659	0.52	0.0777	5.41	0.17	0.21

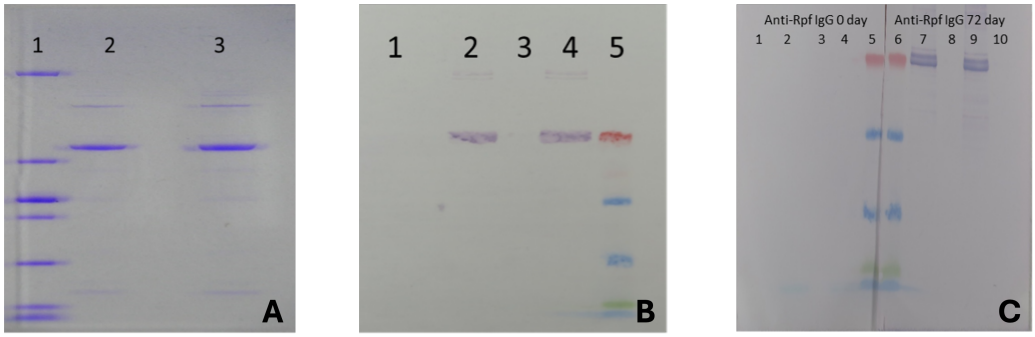

FIG S1 **Rpf over-expression gels. A.** SDS-PAGE of pET15b Rpf at 900 µg/mL confirming protein purity extracted from NI-NA column extraction. First lane is a low-range protein marker (100, 30, 25, 20, 15, 10, 5, 3.4 kDa), second lane is 5 µL of Rpf, third lane is 10 µL of Rpf. **B.** Western blot of 5 and 10 µL of pET15b recombinant Rpf at 900 µg/mL concentration bound with anti-Histidine (1:50000) primary IgG in lane 2 and 4. Lane 1 and 3 are empty. Lane 5 is a low-range western marker (red/40, blue/15, green/10, blue/2.6, blue/1.7 kDa). **C.** Western blot of 5 and 10 µL of pET15b Rpf protein at 900 µg/mL concentrations bound with 72 day Rpf-specific primary IgG antibodies (1:50000) in lanes 7 and 9. This is contrasted with the 0 day antibodies which did not bind to Rpf protein in lane 2 and 4. Lanes 1, 3, 8, and 10 are empty. Lanes 5 and 6 are low-range western marker (red/40, blue/15, green/10, blue/2.6, blue/1.7 kDa)

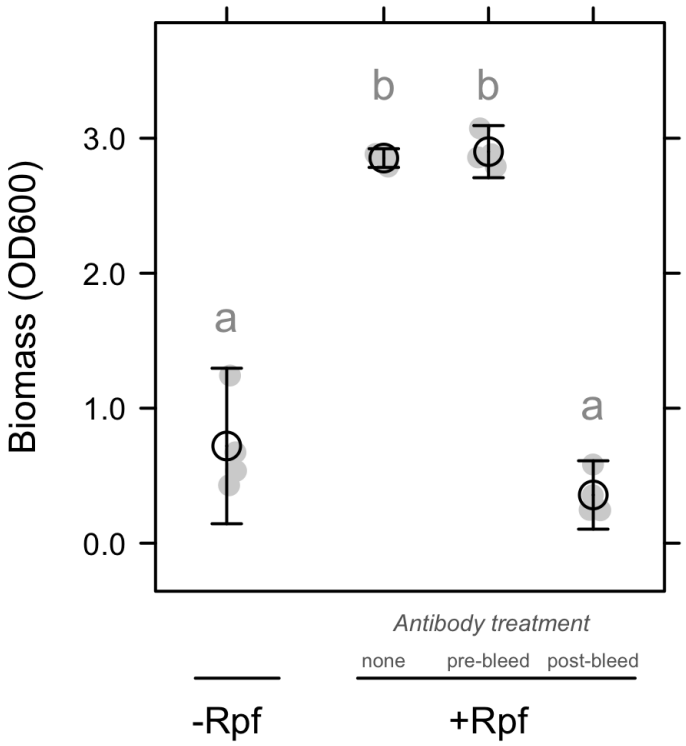

FIG S2 **Assessment of negative controls.** We performed an experiment where we measured the regrowth of dormant *Micrococcus* KBS0714 in R2A broth medium with and without Rpf. Final biomass (OD_{600}) was estimated at 120 h. In the negative control (-Rpf), we only added protein buffer. For the +Rpf treatment, cells were exposed to 1.6 µM of recombinant Rpf. In the +Rpf treatment, cells were assigned to an antibody treatment. They were either left untreated ("none") or exposed to antibodies from rabbit serum (600 µg/mL) collected before (day 0, "pre-bleed") or after (day 28, "post-bleed") the Rpf antigen was administered. Results demonstrate that antibody binding to Rpf active site of muralytic activity is sufficient for eliminating resuscitation effects on *Micrococcus* KBS0714 biomass to levels comparable to protein buffer

control. Treatments sharing the same letter are not significantly different (one-way ANVOA followed by Tukey's HSD, $P > 0.05$).

FIG S3 **Amino acid sequences of Rpf.** Amino acid sequences for resuscitation promoting factors (Rpf) from *Micrococcus luteus* (Fleming) and a soil isolate used in this study, *Micrococcus* KBS7014. Starting at residue 42, there is a conserved glutamic acid (E) residue at position 54 (*), which is within the lysozyme-like domain highlighted in green. We made recombinant Rpf with KBS014 strain that had site-directed substitutions at the conserved site to evaluate effects on resuscitation (see Fig. 3 in main text). The lectin-rich linker region, the length of which is greatly reduced in the Fleming strain (-), is highlighted in blue while the LysM domain is highlighted in yellow. We made a recombinant Rpf from *Micrococcus* KBS0714 where a significant fraction of the lectin-rich linker region and the LysM domain starting at residue 145 (†) were truncated. Other symbols follow UniProt convention where solid vertical lines represent fully conserved residues, colons (:) represent residues with strongly

similar properties, and periods (.) represent residues with weakly similar properties.

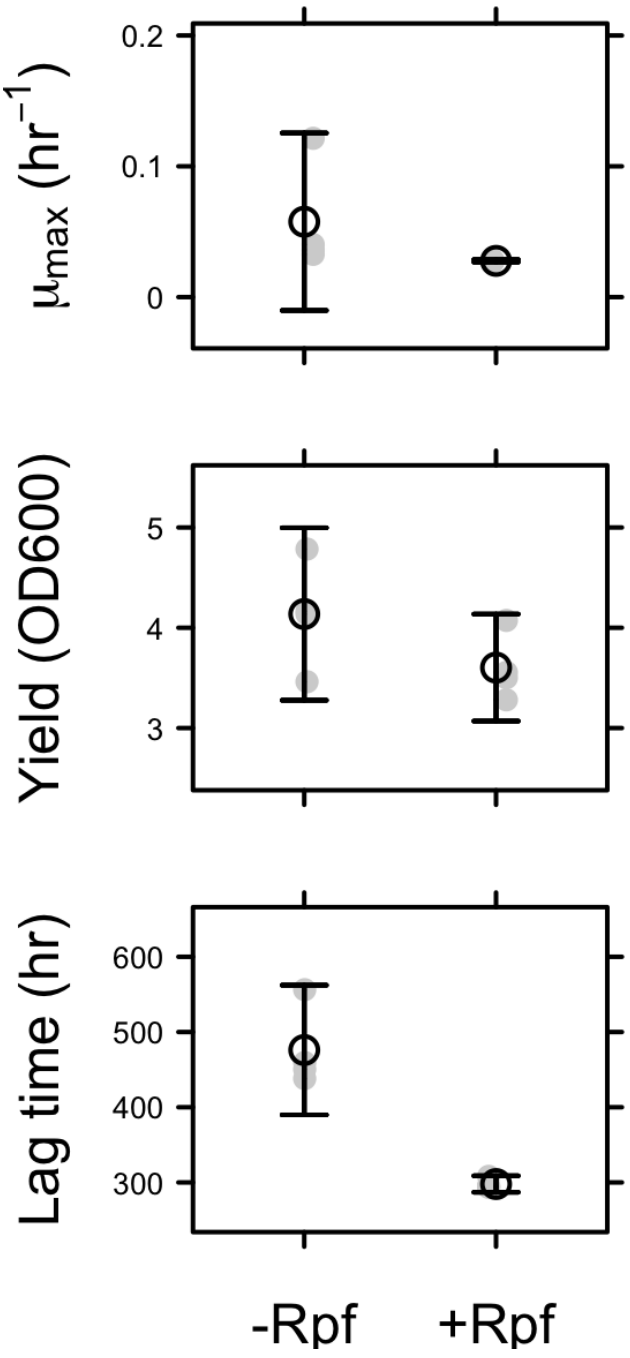

FIG S4 **Growth parameters of *Micrococcus* KBS0714 exposed to Rpf.** We estimated μ_{max}, yield, and lag time after transferring 90-d starved

cells into fresh medium with vs. without recombinant Rpf. Grey symbols represent raw data. Black symbols represent the mean ± SEM (n = 4). See Fig. 4 in main text.

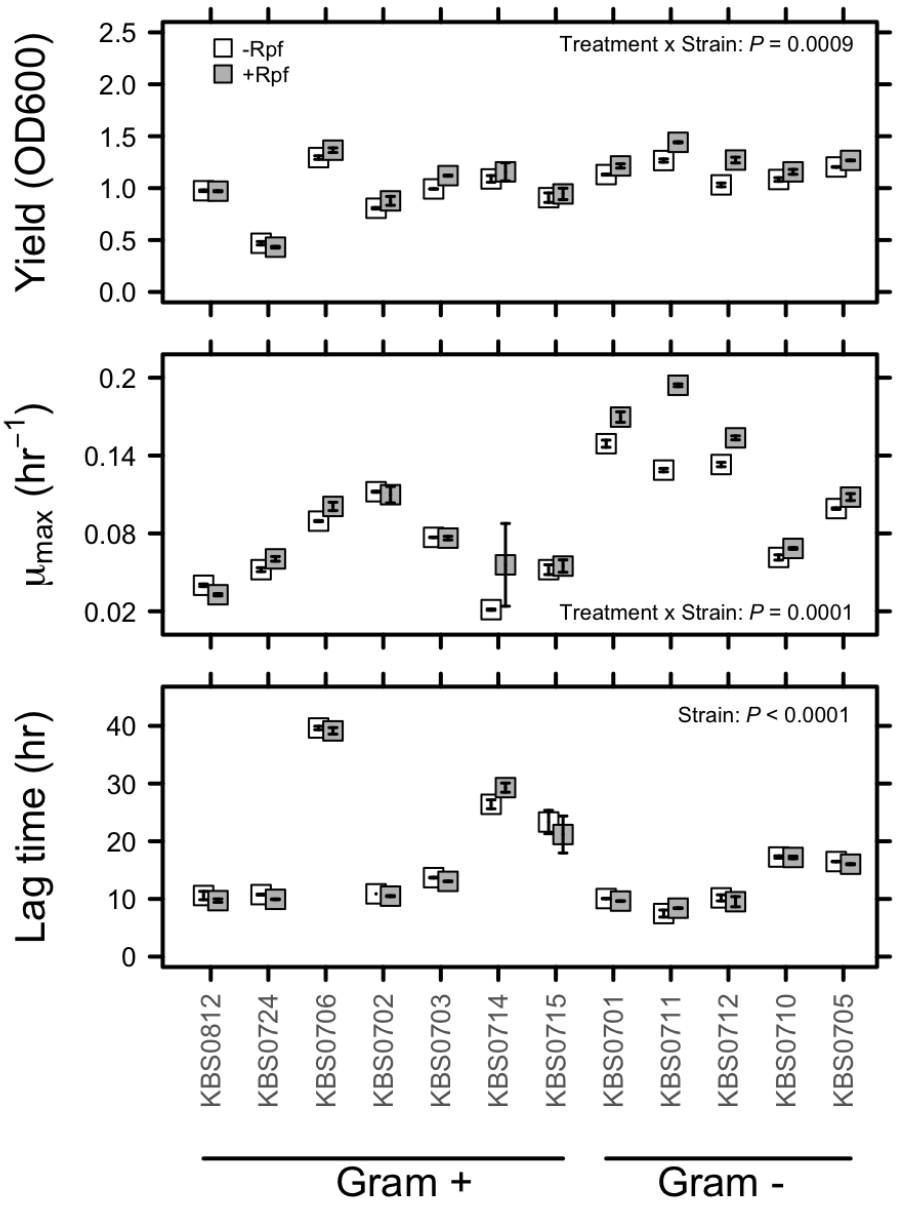

FIG S5 **Growth parameters of diverse bacteria exposed to Rpf.** Growth parameters for different soil bacterial when exposed to recombinant protein (+Rpf) or a negative control (-Rpf). Grey and white symbols represent raw data. Black symbols represent the mean ± SEM (n = 4). Tests of significance were determined using a generalized linear mixed

model (GLMM) where Rpf treatment was a fixed effect and cell type (Gram + vs. Gram -) was a random effect.

REFERENCES

References

[1] **Lennon JT, Aanderud ZT, Lehmkuhl BK, Schoolmaster Jr DR**. 2012. Mapping the niche space of soil microorganisms using taxonomy and traits. *Ecology* 93 (8):1867–1879. doi:https://doi.org/10.1890/11-1745.1.

[2] **Shoemaker WR, Jones SE, Muscarella ME, Behringer MG, Lehmkuhl BK, Lennon JT**. 2021. Microbial population dynamics and evolutionary outcomes under extreme energy-limitation. *Proceedings of the National Academy of Sciences of the United States of America* 118 (e2101691118). doi:10.1073/pnas.2101691118.