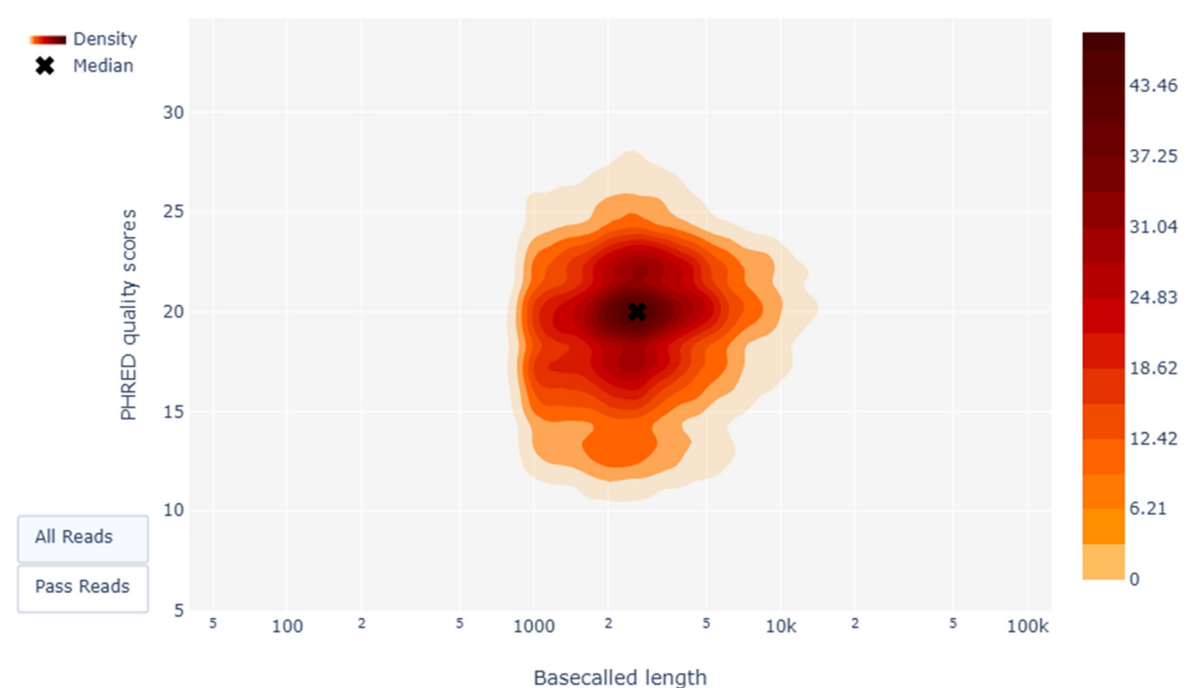


Genomic and functional characterization of the endophytic *Bacillus* strain BACIII with plant growth–promoting and antifungal activity

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Supplementary Figure S1. Density contour plot showing the distribution of basecalled read lengths and PHRED quality scores. Warmer colors indicate regions of higher read density. The black cross marks the median values for both metrics.

Supplementary Table S1. Tetranucleotide (TETRA) frequency analysis of the *Bacillus* sp. strain BACIII genome

Genome	Strain	NCBI Ref Seq assembly	z-score
<i>Bacillus velezensis</i> [T]	NRRL B-41580	GCF_001461825.1	99.904
<i>B. velezensis</i> [T]	KCTC 13012	GCF_001267695.1	99.903
<i>B. velezensis</i>	KACC 18228	GCF_001461835.1	99.905
<i>B. velezensis</i>	NRRL B-4257	GCF_001461845.1	99.914
<i>B. velezensis</i>	NBIF-003	GCF_001440465.1	99.863
<i>B. velezensis</i>	FKM10	GCF_001469675.1	99.911
<i>B. velezensis</i>	JS25R	GCF_000769555.1	99.915

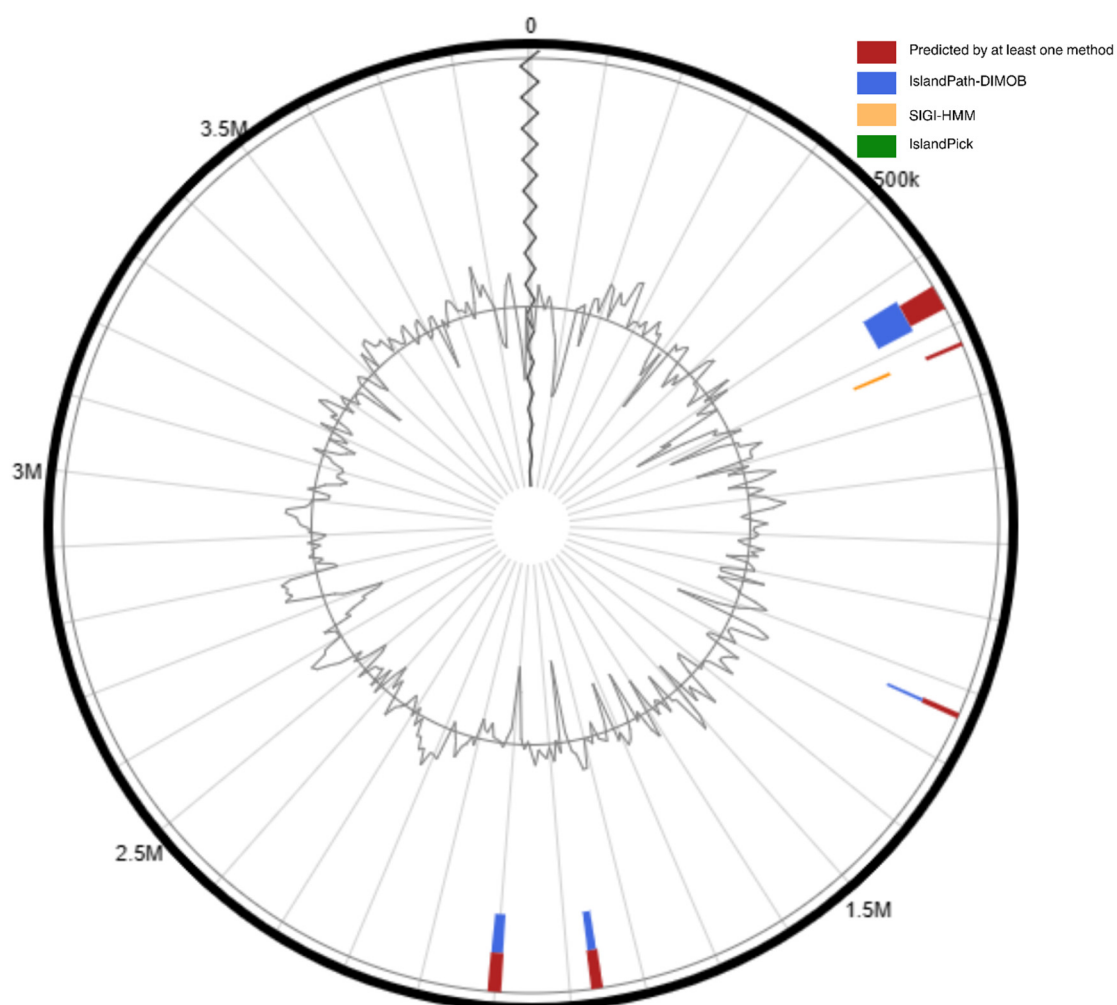
<i>B. velezensis</i>	AS43.3	GCF_000319475.1	99.913
<i>B. velezensis</i>	CAU B946	GCF_000283695.1	99.888
<i>B. velezensis</i>	FZB42	GCF_000015785.1	99.893
<i>B. velezensis</i>	L-H15	GCF_000833005.1	99.889
<i>B. velezensis</i>	L-S60	GCF_000973485.1	99.887
<i>B. velezensis</i>	KACC 13105	GCF_000960265.2	99.888
<i>B. velezensis</i>	NKYL29	GCF_000740715.1	99.889
<i>B. siamensis</i>	KCTC 13613	GCF_000262045.1	99.994
<i>B. siamensis</i>	SRCM100169	GCF_001662915.1	99.892
<i>B. amyloliquefaciens</i> [T]	DSM 7 = ATCC 23350 DSM 7	GCF_000196735.1	99.978
<i>B. amyloliquefaciens</i> [T]	CIP103265T	GCF_965136255.1	99.806
<i>B. amyloliquefaciens</i> [T]	NRRL B-14393	GCF_050473685.1	99.754
<i>B. amyloliquefaciens</i>	X1	GCF_000750045.1	99.91
<i>B. amyloliquefaciens</i>	Bs006	GCF_001278635.1	99.832
<i>B. amyloliquefaciens</i>	JRS5	GCF_001286945.1	99.891
<i>B. amyloliquefaciens</i>	JRS8	GCF_001286965.1	99.853
<i>B. amyloliquefaciens</i>	LL3	GCF_000204275.1	99.783
<i>B. amyloliquefaciens</i>	LFB112	GCF_000508265.1	99.882
<i>B. amyloliquefaciens</i>	KHG19	GCF_000835145.1	99.90
<i>B. amyloliquefaciens</i>	IT-45	GCF_000242855.2	99.870

[T] Strain type

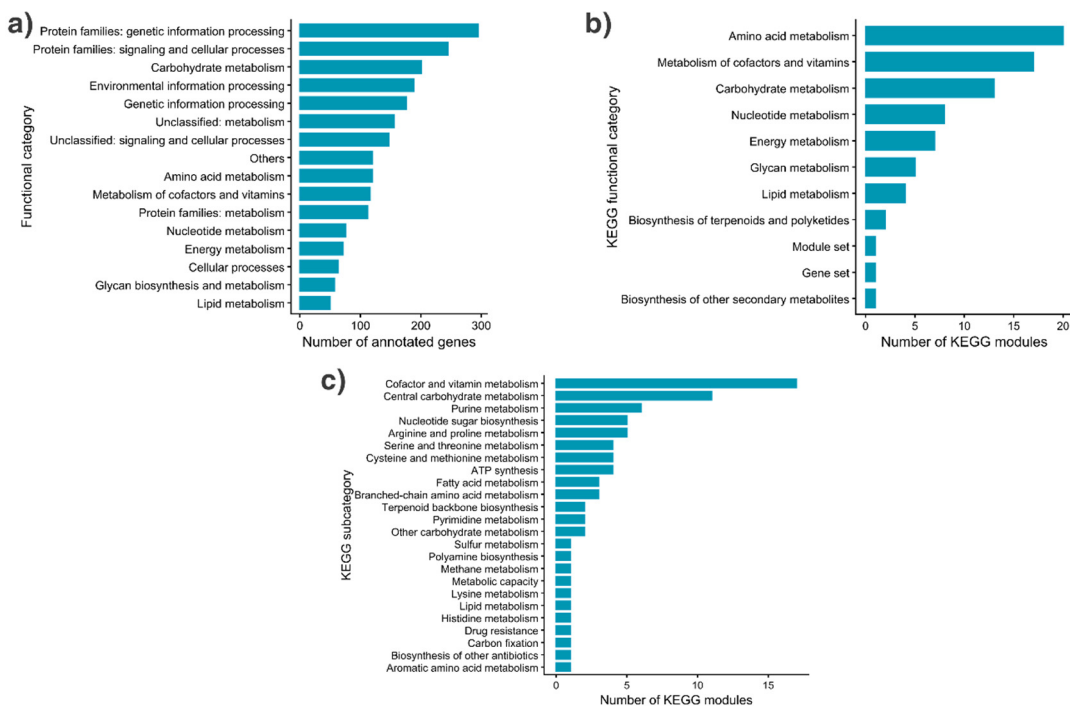
Supplementary Table S2. Biosynthetic gene clusters identified in the *Bacillus* sp. strain BACIII genome and their closest known matches according to AntiSMASH (v8.0.4)

Region	Type	From	To	Similarity Confidence	Most similar known cluster	
Region 1	Terpene-precursor	588,083	608,973			
Region 2	TransAT-PKS	630,794	736,953	High	Difficidin	PKS
Region 3	T3PKS	854,485	895,585			
Region 4	Terpene	944,376	966,259			
Region 5	NRPS,betalactone,transAT-PKS	991,654	1,129,495	High	Fengycin	NRPS:Type I

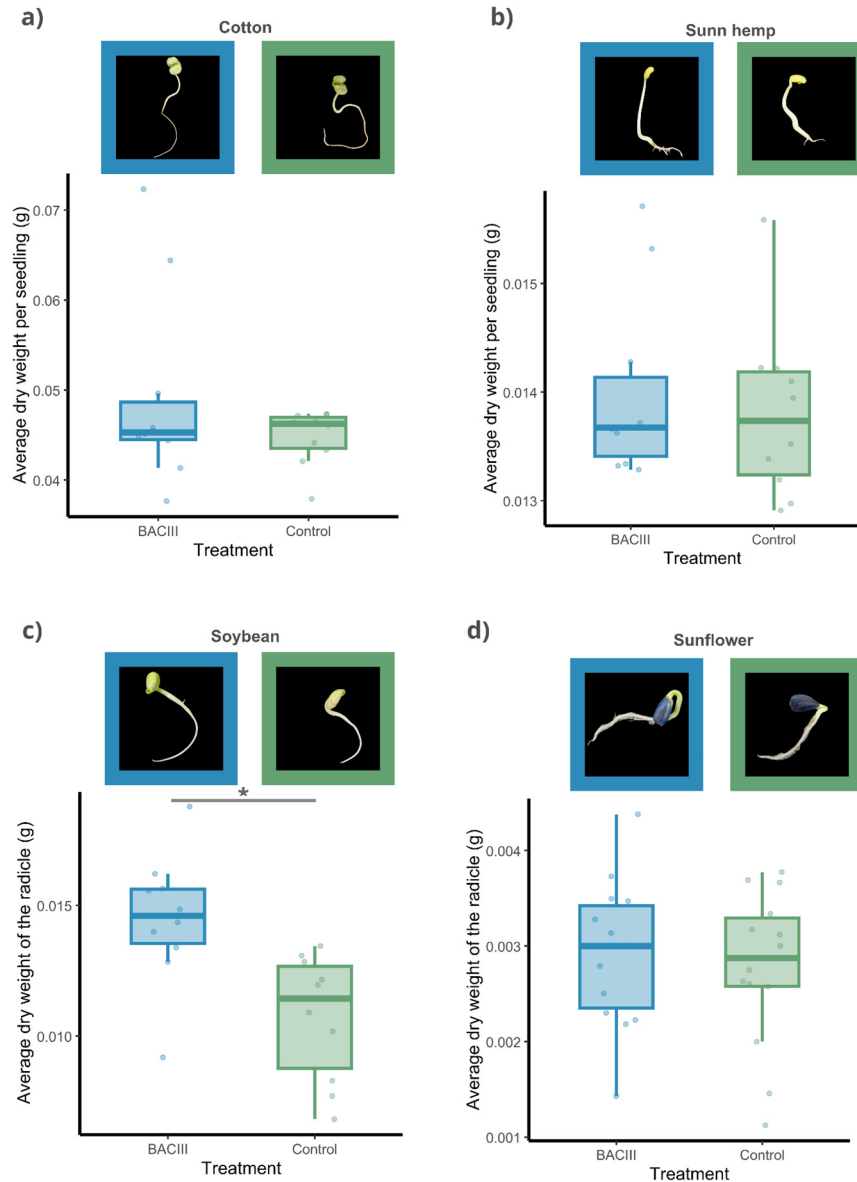
Region 6	TransAT- PKS,NRPS,T3P KS	1,181,496	1,291,496	High	Bacillaene	NRPS:Type I+PKS:Type I
Region 7	TransAT-PKS	1,518,867	1,607,085	High	Macrolactin H	PKS
Region 8	Terpene	1,902,730	1,923,473			
Region 9	PKS-like	2,005,120	2,046,364			
Region 10	NRPS	2,600,593	2,666,000	High	Surfactin	NRPS:Type I
Region 11	Terpene- precursor,NRP- metallophore,NR PS,RiPP-like	2,943,983	3,009,321	High	Bacillibactin	NRPS:Type I
Region 12	Other	3,536,852	3,578,270	High	Bacilysin	other:other



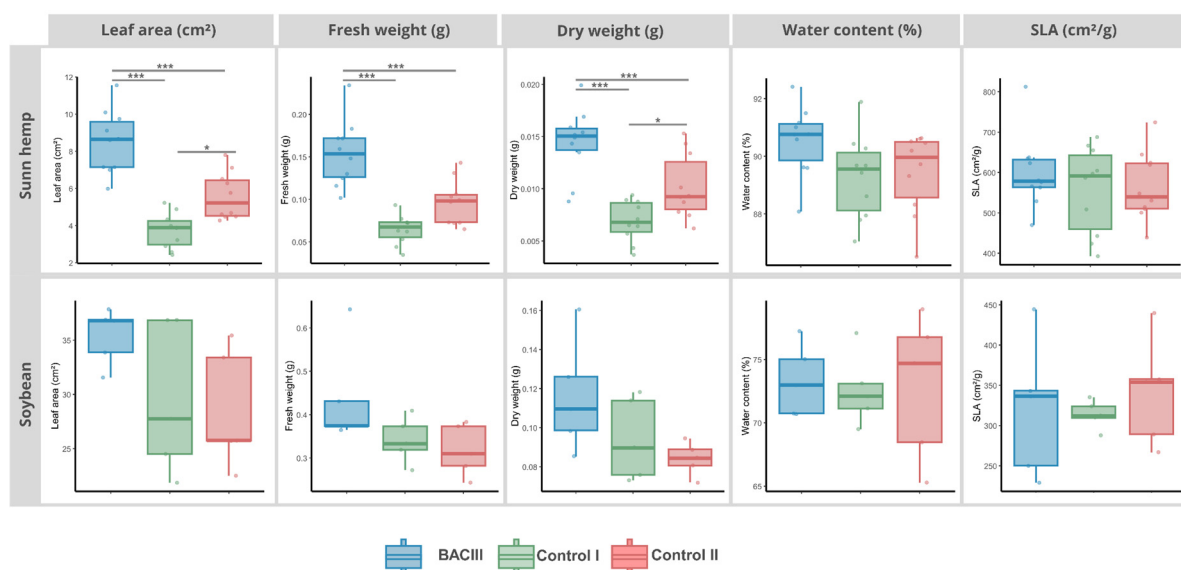
Supplementary Figure S2. Circular map generated by IslandViewer4 representing the locations of genomic islands within the genome of *Bacillus* sp. strain BACIII.



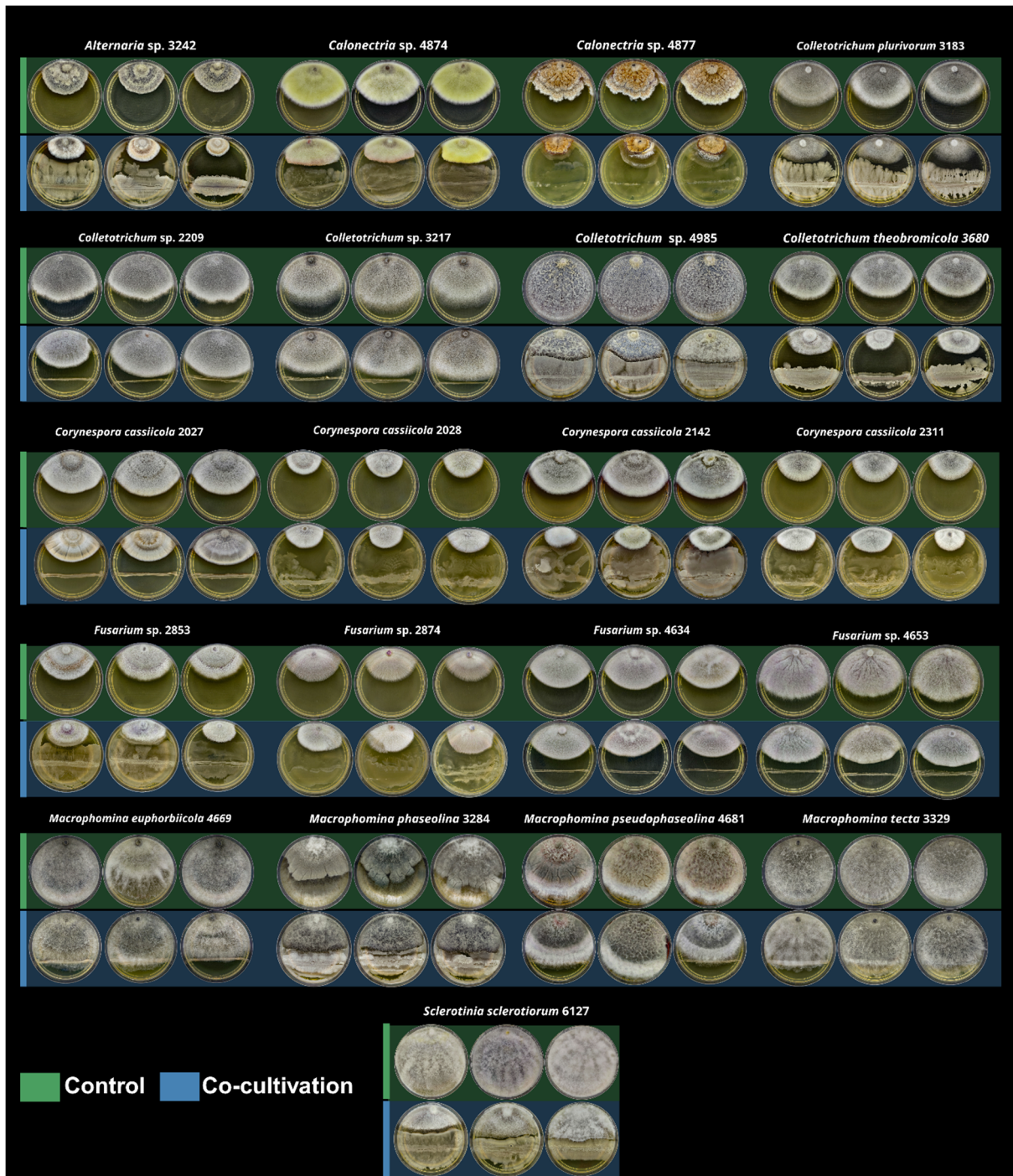
Supplementary Figure S3. Prediction of functional characteristics encoded in the *Bacillus* sp. BACIII genome. In a-c) functional annotation obtained with BlastKOALA. a) Distribution of annotated genes in the main functional categories of KEGG. b) Number of KEGG modules assigned to each functional category. c) Distribution of KEGG subcategories based on module annotation.



Supplementary Figure S4. Effect of *Bacillus* sp. strain BACIII treatment on seedling dry weight of four agricultural plant species. Panels show the average dry weight per seedling (a– cotton and b– sunn hemp) and the average dry weight of the radicle (c– soybean and d– sunflower) under two treatments: BACIII (bacterial inoculation) and Control (2% sucrose broth). Asterisks indicate statistically significant differences between treatments within the same species and parameter ($p \leq 0.05$), based on Student’s t–test or Wilcoxon–Mann–Whitney test: “*” $p = 0.05–0.02$; “***” $p = 0.01$; “****” $p = 0.001$. Sample size per treatment: $n = 10$ for cotton; $n = 10$ for sunn hemp; $n = 5$ for soybean; $n = 14$ for sunflower.



Supplementary Figure S5. Effect of *Bacillus* sp. strain BACIII treatment on leaf morphological traits of two plant species. Leaf area (cm²), fresh and dry weight (g), water content (%), and specific leaf area (SLA, cm²·g⁻¹) of sunn hemp (*Crotalaria juncea*, cv. C52333-C) and soybean (*Glycine max*, cv. Brasmax Olimpo IPRO 80I82RSF) under three treatments: BACIII (bacterial inoculation), Control I (water only), and Control II (culture medium without bacteria). Asterisks denote statistically significant differences between treatments within the same species and parameter ($p \leq 0.05$), based on ANOVA followed by Tukey's HSD test or Kruskal-Wallis followed by Dunn's multiple comparison test with Bonferroni correction: "*" $p = 0.05-0.02$; "***" $p = 0.01$; "****" $p = 0.001$. Sample size per treatment: $n = 10$ for sunn hemp; $n = 5$ for soybean.



Supplementary Figure S6. Double culture assay of the *Bacillus* sp. BACIII strain against phytopathogenic fungi.

Supplementary Table S3. Results of statistical tests for the co-culture assay of the bacterial strain *Bacillus* sp. BACIII against different phytopathogenic fungi.

Genus	Specie	.y.	Group 1	Group 2	N1	N2	Statistic	DF	p	Test	Shapiro_ctrl	Shapiro_bac	Levene_p	p.adj	p.adj.sig nif
<i>Alternaria</i>	<i>Alternaria</i> sp. 3242	Growth (mm)	BACIII	Control	10	10	-11,87550362	18	0,00000000 0597	t-test	0,609663374	0,949972759	0,34049320 27	0,00000000 0597	****
<i>Calonectria</i>	<i>Calonectria</i> sp. 4874	Growth (mm)	BACIII	Control	10	10	-15,08915272	18	0	t-test	0,762494854 6	0,748068358 8	0,53159310 13	0	****
<i>Calonectria</i>	<i>Calonectria</i> sp. 4877	Growth (mm)	BACIII	Control	10	10	11		0,0034	wilcox	0,001390148 093	0,317814711 9	0,89141293 4	0,0034	**
<i>Colletotrichum</i>	<i>C. plurivorum</i> 3183	Growth (mm)	BACIII	Control	10	10	-14,82063247	18	0	t-test	0,095352675 9	0,535167371 9	0,43998842 86	0	****
<i>Colletotrichum</i>	<i>Colletotrichum</i> sp. 2209	Growth (mm)	BACIII	Control	10	10	36		0,304	wilcox	0,006794147 626	0,614617045 4	0,37482806 91	0,304	ns
<i>Colletotrichum</i>	<i>Colletotrichum</i> sp. 3217	Growth (mm)	BACIII	Control	10	10	-4,254892301	18	0,000476	t-test	0,890473509 7	0,137113282 1	0,65817126 24	0,000476	***
<i>Colletotrichum</i>	<i>Colletotrichum</i> sp. 4985	Growth (mm)	BACIII	Control	10	10	0		0,000145	wilcox	0,001171076 256	0,246692648 1	0,11495396 33	0,000145	***
<i>Colletotrichum</i>	<i>C. theobromicola</i> 3680	Growth (mm)	BACIII	Control	10	10	-12,12315445	18	0,00000000 0428	t-test	0,319453873 2	0,717366211 9	0,29403810 96	0,00000000 0428	****
<i>Corynespora</i>	<i>C. cassicola</i> 2027	Growth (mm)	BACIII	Control	10	10	27,5		0,0948	wilcox	0,668697761	0,012901148 18	0,31773696 84	0,0948	ns
<i>Corynespora</i>	<i>C. cassicola</i> 2028	Growth (mm)	BACIII	Control	10	10	-3,275847762	18	0,0042	t-test	0,947629966 5	0,437618279 3	0,93151531 43	0,0042	**
<i>Corynespora</i>	<i>C. cassicola</i> 2142	Growth (mm)	BACIII	Control	10	10	-8,858315353	18	0,00000005 57	t-test	0,649851797 3	0,584744295 4	0,53643753 74	0,00000005 57	****
<i>Corynespora</i>	<i>Corynespora</i> <i>cassicola</i> 2311	Growth (mm)	BACIII	Control	10	10	-5,443996397	18	0,0000359	t-test	0,531596217 6	0,752101832 2	0,16477532 05	0,0000359	****
<i>Fusarium</i>	<i>Fusarium</i> sp. 2853	Growth (mm)	BACIII	Control	10	10	-7,817993908	18	0,00000034	t-test	0,685190729 8	0,051008127 38	0,47561251 3	0,00000034	****
<i>Fusarium</i>	<i>Fusarium</i> sp. 2874	Growth (mm)	BACIII	Control	10	10	-5,947207096	18	0,0000126	t-test	0,872445156	0,086571526 45	0,19169276 18	0,0000126	****
<i>Fusarium</i>	<i>Fusarium</i> sp. 4634	Growth (mm)	BACIII	Control	10	10	-8,907912635	18	0,00000005 13	t-test	0,141542807 6	0,528391827 5	0,44073313 69	0,00000005 13	****
<i>Fusarium</i>	<i>Fusarium</i> sp. 4653	Growth (mm)	BACIII	Control	10	10	-11,42249416	18	0,00000000 111	t-test	0,140422642 8	0,628955294 7	0,45868976 39	0,00000000 111	****

<i>Macrophomina</i>	<i>M. euphorbiicola</i> 4669	Growth (mm)	BACIII	Control	10	10	22,5		0,0386	wilcox	0,033137642 06	0,676238662 8	0,10096836 59	0,0386	*
<i>Macrophomina</i>	<i>M. phaseolina</i> 3284	Growth (mm)	BACIII	Control	10	10	0		0,000122	wilcox	0,000199575 8122	0,171757302 9	0,53782124 6	0,000122	***
<i>Macrophomina</i>	<i>M. pseudophaseolina</i> 4681	Growth (mm)	BACIII	Control	10	10	- 0,5514881433	18	0,588	t-test	0,104550252 4	0,134190595 9	0,47621287 07	0,588	ns
<i>Macrophomina</i>	<i>M. tecta</i> 3329	Growth (mm)	BACIII	Control	10	10	18,5		0,015	wilcox	0,000386846 6429	0,523180342	0,00708202 8505	0,015	*
<i>Sclerotinia</i>	<i>S. sclerotiorum</i> 6127	Growth (mm)	BACIII	Control	10	10	0		0,000146	wilcox	0,000112248 4505	0,218533716 8	0,29141457 43	0,000146	***

y: Observed value of the response variable;

Group1: First group compared in the statistical test (BACIII);

Group2: Second group compared in the statistical test (Control);

N1: Number of observations in Group1;

N2: Number of observations in Group2;

Statistic: Test statistic calculated for the comparison;

DF: Degrees of freedom associated with the test;

p: *p*-value obtained from the comparison before multiple-testing correction;

Test: Type of statistical test applied;

Shapiro_ctrl: *p*-value of the Shapiro–Wilk test for the control group;

Shapiro_bac: *p*-value of the Shapiro–Wilk test for the treated group;

Levene_p: *p*-value of Levene’s test assessing variance homogeneity between groups;

p.adj: Adjusted *p*-value after multiple-comparison correction;

p.adj.signif: Categorical significance of the adjusted *p*-value (e.g., *, **, ***, ns). Asterisks indicate statistically significant differences between treatments within the same species and parameter ($p \leq 0.05$), based on Student’s *t*-test or Wilcoxon–Mann–Whitney test: “*” $p = 0.05$ – 0.02 ; “**” $p = 0.01$; “***” $p = 0$.